Received: 17 June 2020 | Revised: 9 September 2020 | Accepted: 28 September 2020

DOI: 10.1002/cam4.3546

ORIGINAL RESEARCH

Cancer Medicine Open Access WILEY

An in silico approach to identify and prioritize miRNAs target sites polymorphisms in colorectal cancer and obesity

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Funding information
National Institute for Medical Research Development Grant Number: 971211.

Abstract

Colorectal cancer (CRC) and obesity are linked clinical entities with a series of complex processes being engaged in their development. MicroRNAs (miRNAs) participate in these processes through regulating CRC and obesity-related genes. This study aimed to develop an in silico approach to systematically identify and prioritize miRNAs target sites polymorphisms in obesity and CRC. Data from genome-wide association studies (GWASs) were used to retrieve CRC and obesity-associated variants. The polymorphisms that were resided in experimentally verified or computationally predicted miRNA target sites were retrieved and prioritized using a range of bioinformatics analyses. We found 6284 CRC and 38931 obesity unique variants. For CRC 33 haplotypes variants in 134 interactions were in miRNA targetome, while for obesity we found more than 935 unique interactions. Functionally prioritized SNPs revealed that, SNPs in 153 obesity and 50 CRC unique interactions were have disruptive effects on miRNA:mRNA integration by changing on target RNA secondary structure. Structural accessibility of target sites were decreased in 418 and 103 unique interactions and increased in 516 and 79 interactions, for obesity and CRC, respectively. The miRNA:mRNA hybrid stability was increased in 127 and 17 unique interactions and decreased in 33 and 24 interactions for the effect of obesity and CRC SNPs, respectively. In this study, seven SNPs with 15 interactions and three SNPs with four interactions were prioritized for obesity and CRC, respectively. These SNPs could be used for future studies for finding potential biomarkers for diagnoses, prognosis, or treatment of CRC and obesity.

KEYWORDS

bioinformatics, biomarkers, colorectal cancer, polymorphisms

1 | INTRODUCTION

The previous studies have witnessed growing concerns about two related clinical entities: cancer, as the second cause of death, and obesity, as a chronic inflammatory disease in the recent years. According to a report by the world health organization (WHO), obesity effects more than 1/8 of adults worldwide.[1] A growing body of research provided

evidence that functionally links obesity to the risk of several cancers including CRC.[2-5] A range of genetic and environmental factors contributes to the risk of CRC and obesity. Experimentations have documented a range of pathological molecular alterations contributing to CRC and obesity, among which dysregulation of miRNAs is specially highlighted.

MiRNAs are small noncoding RNAs that regulate tumorigenesis through functioning as either tumor suppressors or oncogenes.[6] They participate in transcriptome regulation by binding to 3'-untranslated region (3'-UTR) of target mRNAs and either suppressing translation or inducing mRNA degradation. It has been shown that the intricate miRNA:mRNA network may be influence by the presence of single nucleotide polymorphism (SNP) within or near miRNA binding site. Such miRNA binding site polymorphism may influence miRNA:mRNA interaction through altering miRNA:mRNA hybrid stability, secondary structure of local RNA, structural accessibility of target sites, or even creating novel biding sites. Several recent genetic association studies have highlighted the contribution of miRNA binding site polymorphisms to the risk of complex disease specially CRC.[7,8] In this study, we leveraged data from genome-wide association studies (GWASs) on CRC, obesity, and obesity-related traits to explore putative disease-associated polymorphisms that influence miRNA target sites. A range of bioinformatics analyses was also performed to predict functional consequences of these polymorphisms and provide a list of prioritized variants for future experimentations.

2 | METHOD AND ANALYSIS

The bioinformatics methods applied in this study are depicted in Figure 1. Variants from GWA studies on CRC risk, CRC survival, obesity risk, and obesity-related traits were retrieved from NHGRI-EBI GWAS catalog (gwas_catalog_v1.0.1-associations_e90_r2017-10-10), available at (https://www.ebi.ac.uk/gwas/). The HaploReg (version 4.1) database was used to construct population-specific association blocks for each GWAS lead variant based on 1000 Genome project Phase I populations and a defined linkage disequilibrium threshold (i.e., r^2 of at least 0.6). The obtained association blocks, containing all putative disease-associated variants were intersected with a miRNA targetome data set to identify putative disease-associated variants that are resided within or in flanks of an experimentally verified or a computationally predicted miRNAs target site. The functional effects of these polymorphism on different aspects of miRNA:mRNA interactions, including local RNA secondary structures, structural accessibility of target sites, and miRNA:mRNA hybrid stability, were analyzed.

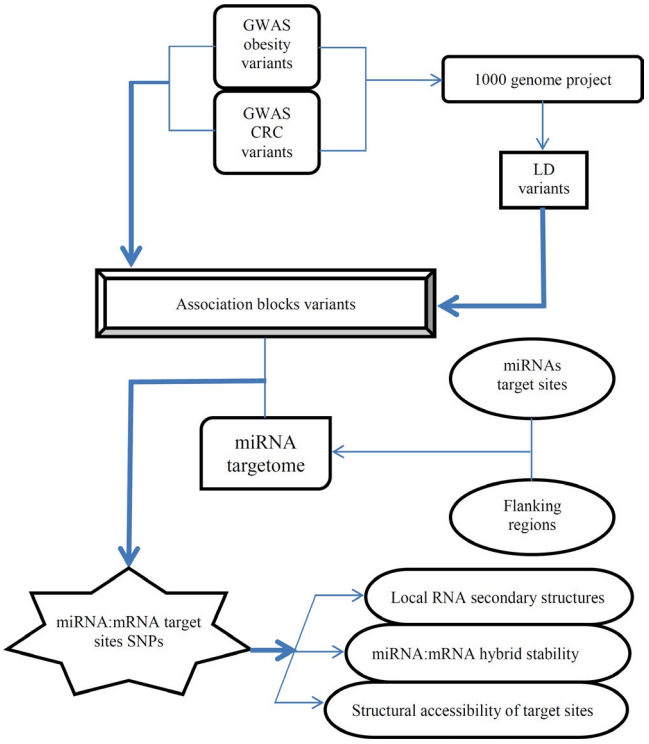

FIGURE 1 The study's workflow. See method for details

2.1 | The MIRNA targetome data set

A comprehensive data set of experimentally verified and computationally predicted miRNA target sites were obtained by combining miRNA:mRNA interactions from the StarBase (version 2, available at http://starbase.sysu.edu.cn/starbase2/index.php), the targetScan (version 7.1, available at http://www.targetscan.org/vert_71/), and the microRNA.org (available at http://www.microrna.org/microrna/home.do) databases. The data set includes both computationally predicted target sites and those that are validated using a range of methods such as CLIP-Seq.[9] The 25 nucleotides upstream and downstream of target sites were considered as flanking regions.

2.2 | Putative CRC/obesity-associated variants and association blocks

The lead SNP of GWA studies with a p-value $\leq 1.0 \times 10^{-6}$ were retrieved. The HaploReg (version 4.1, available at https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) was used to retrieve all proxy SNPs (defined as $r^2 \geq 0.6$) of GWAS lead variants based on 1000 Genomes project super-populations.[10] Five categories of obesity-related traits were considered (Table 1). Moreover, we obtained GWAS index variants from GWA studies associated with CRC risk and CRC survival.

TABLE 1 Categories of obesity-associated traits obtained from GWAS catalog

Abbreviation	Categories	Covered traits
Ob	Obesity	Adiposity, Obesity, Overweight, Extreme obesity, Early onset extreme obesity
W-B	Weight & BMI	body mass index (BMI), body weight, Childhood BMI, Weight z-score, Birth weight
WHR	Waist/hip circumference(ratio)	Waist circumference, Hip circumference, Waist-hip ratio
AT	Adipose tissue	Visceral adipose tissue (VAT), Subcutaneous adipose tissue (SAT), AT/SAT ratio, Visceral fat
FM	Fat mass	Body fat mass, Trunk fat mass, Body fat percentage, newborns Fat mass

2.3 | Effect of targetome SNPS on local RNA secondary structures

The RNAsnp program (version 1.2, available athttps://rth.dk/resources/rnasnp/)[11] used to assess impacts of CRC or obesity-associated SNPs in target sites and flanking regions on secondary structures of local RNA. RNA sequences and SNPs were inputted in RNAsnp to generate probability matrix associated to wild-type (WT) and mutant (ALT) alleles. RNAsnp shows the structural difference between WT and ALT alleles with the Euclidean distance measure (d) for all sequence intervals and reports the polymorphism with the maximum base pair distances (d_{max}) and the corresponding p-value. A p-value less than 0.2 is considered significant.[11]

2.4 | Structural accessibility of target sites

As described previously, changes in structural accessibility of target sites may interfere with miRNA binding. RNAplfold program was used to calculate structural accessibility for 3′-UTRs with the ALT and WT alleles. The difference between the target site accessibility of WT and ALT allele was computed using ΔPu.[12,13]

2.5 | MIRNA:mRNA hybrid stability

A target site polymorphism may also interfere with miRNA binding through altering the stability miRNA:mRNA hybrid structure. The RNA hybrid v2.1.2 (available at https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/)[14] was employed to identify free energy of hybridization (ΔGhybrid) for ALT and WT alleles. ΔΔGhybrid for each miRNA target site polymorphism (calculated as ΔΔGhybrid = ΔGhybrid assassinate to ALT–ΔGhybrid assassinate to WT), is a measure of the SNP effect on the stability of hybridization. A positive ΔΔGhybrid is an indication of a decreased stability that is imposed by the ALT allele.

3 | RESULTS

3.1 | Putative obesity/CRC-associated SNPs in miRNAs targetome

We obtained 159 GWAS index SNPs from 32 studies on CRC risk and 45 GWAS index SNPs from three studies on CRC survival by mining GWAS catalog. After extending polymorphisms to association haplotypes, 5163 and 1121 putative disease variants were retrieved for CRC risk and survival, respectively. The CRC-associated index and haplotypes variants were reported in a range of populations including Europeans (n = 4464), Asians (n = 2216), Africans (n = 53), and Americans (n = 130), with some variants being reported in multiple populations. After mapping putative CRC-associated variants to the miRNA targetome data set, 134 and 48 unique miRNA:mRNA:SNP were identified for CRC risk and survival, respectively. In other words, 33 the CRC-associated index and haplotypes variants were identified to reside within or near miRNA binding sites, potentially influencing 134 miRNA:mRNA:SNP interactions (Table 2). Moreover, 11 unique CRC survival-associated variants were found to reside within or near miRNA binding sites, influencing 48 miRNA:mRNA:SNP interactions (Table 3).

We retrieved 1079 GWAS index variants from 75 studies pertaining to five obesity-related categories. After extending to association blocks, 38931 unique putative obesity-related variants were obtained. Since the most of included GWA studies were related to European populations, the more of putative obesity-related variants belonging to the European. Table 4 summarized number of variants in each super-population. Variants from our results were intersected which targetome intervals. Finally, we find 935

TABLE 2 CRC risk-related haplotype variants of GWA studies raised on miRNAs targetome

Databases	Targetome SNP	miRNA	Gene	GWAS SNP	r^2 [a]	Population
StarBase	rs10318 C > T	miR−124-3p	GREM1	rs2293582 G > A, rs73376930 A > C	0.79, 0.82	EUR, EUR
Microrna.org	rs10318 C > T	miR−96, miR−182, miR−1271	GREM1	rs2293582 G > A, rs73376930 A > C	0.79, 0.82	EUR, EUR
Microrna.org	rs1046097 A > G	miR−382	TUBG2	rs9901225 C > G	0.85	EUR
StarBase	rs1048165 A > C	miR−142-3p, miR−155-5p, miR−185-5p	SNRNP27	rs4853036 G > A	0.7	EUR
Microrna.org	rs1048165 A > C	miR−185, miR−155	SNRNP27	rs4853036 G > A	0.7	EUR
StarBase	rs1051473 T > C	miR−433-3p, miR−203a	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs1051473 T > C	miR−203	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs1062044 A > G	miR−96	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
StarBase	rs11064467 C > T	miR−7-5p, miR−328-3p, miR−330-3p, miR−128-3p, miR−335-5p	ENO2	rs11064437 C > T	0.6	ASN
Microrna.org	rs11064467 C > T	miR−7, miR−128, miR−328, miR−335	ENO2	rs11064437 C > T	0.6	ASN
Microrna.org	rs11085537 G > C	miR−140-5p, miR−125a−3p, miR−136	LOC440518	rs11671104 A > C	0.6	EUR
Microrna.org	rs11571475 A > G	miR−17, miR−20a, miR−93, miR−106a/b, miR−20b, miR−519d	RAD52	rs12309274 T > C, rs12309274 T > C	0.82, 0.71	EUR, ASN
StarBase	rs1547715 A > G	miR−511-5p, miR−16-5p, miR−15a−5p, miR−431-5p, miR−195-5p, miR−423-3p, miR−150-5p, miR−15b−5p, miR−424-5p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs1547715 A > G	miR−362-3p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
StarBase	rs174545 C > A	miR−452-5p	FADS1	rs174537 G > C, rs174537 G > C	0.98, 1	EUR, ASN
StarBase	rs174546 C > T	miR−496	FADS1	rs174537 G > C, rs174537 G > C	0.98, 1	EUR, ASN
Microrna.org	rs174546 C > T	miR−496	FADS1	rs174537 G > C, rs174537 G > C	0.98, 1	EUR, ASN
Microrna.org	rs2715761 C > T	miR−203	MORC1	rs2593957 T > C	0.74	EUR

(Continues)

TABLE 2 (Continued)

Databases	Targetome SNP	miRNA	Gene	GWAS SNP	r^2 [a]	Population
StarBase	rs295458 C > A	miR−128-3p, miR−96-5p	KLHL18	rs8180040 T > A	0.98	EUR
TargetScan	rs295458 C > A	miR−128-3p, miR−1193, miR−493-3p	KLHL18	rs8180040 T > A	0.98	EUR
Microrna.org	rs3087967 T > A	miR−9, miR−186, miR−362-3p, miR−329	C11orf53	rs3802842 C > A, rs3802842 C > A	0.94, 0.98	EUR, ASN
Microrna.org	rs3088140 T > A	miR−494	UTP23	rs6469656 G > A, rs6469656 G > A	0.78, 0.74	EUR, ASN
StarBase	rs3184122 A > G	miR−21-5p, miR−590-5p	LIMA1	rs34245511 G > C	0.87	EUR
Microrna.org	rs3184122 A > G	miR−21, miR−590-5p	LIMA1	rs34245511 G > C	0.87	EUR
StarBase	rs3359 G > C	miR−29c−3p, miR−29b−3p, miR−129-5p, miR−16-5p, miR−15a−5p, miR−422a, miR−195-5p, miR−497-5p, miR−122-5p, miR−15b−5p, miR−424-5p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
TargetScan	rs3359 G > C	miR−15-5p, miR−16-5p, miR−195-5p, miR−424-5p, miR−497-5p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs3359 G > C	miR−15a, miR−16, miR−15b, miR−195, miR−422a, miR−424, miR−497	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs3741698 C > A	miR−488	TBX3	rs59336 T > A	0.63	ASN
Microrna.org	rs5445 C > T	miR−183, miR−124, miR−150, miR−506	GNB3	rs11064437 C > T	0.6	ASN
StarBase	rs58194764 T > C	miR−300	TPI1	rs11064437 C > T	0.88	ASN
Microrna.org	rs58194764 T > C	miR−300	TPI1	rs11064437 C > T	0.88	ASN
StarBase	rs6424890 A > G	miR−320a/b/c/d	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
TargetScan	rs6424890 A > G	miR−140-5p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs6424890 A > G	miR−140-5p, miR−320a, miR−590-3p, miR−320b, miR−320c	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
StarBase	rs679 C > A	miR−218-5p	MLX	rs9901225 C > G	0.72	EUR
Microrna.org	rs679 C > A	miR−103, miR−107	MLX	rs9901225 C > G	0.72	EUR

(Continues)

TABLE 2 (Continued)

Databases	Targetome SNP	miRNA	Gene	GWAS SNP	r^2 [a]	Population
Microrna.org	rs709206 T > A	miR−137, miR−371-5p	CABLES2	rs6061231 C > A	0.95	ASN
StarBase	rs7473 G > A	miR−33b−5p, miR−153-3p, miR−124-3p, miR−33a−5p, miR−448, miR−506-3p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
TargetScan	rs7473 G > A	miR−124-3p.1, miR−124-3p.2, miR−506-3p, miR−33-5p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs747949 G > A	miR−218	CABLES2	rs6061231 C > A	0.95	ASN
StarBase	rs8079855 A > C	let−7c−5p	FAM134C	rs9901225 C > G	0.72	EUR
StarBase	rs8668 A > G	miR−342-3p, miR−216a−5p, miR−155-5p	CABLES2	rs2427308 C > T, rs6061231 C > A	0.7, 0.91	EUR, ASN
Microrna.org	rs8668 A > G	miR−216a, miR−218, miR−155, miR−342-3p	CABLES2	rs2427308 C > T, rs6061231 C > A	0.7, 0.91	EUR, ASN
StarBase	rs8853 T > C	miR−203a, miR−211-5p, miR−204-5p,	TBX3	rs59336 T > A, rs59336 T > A	0.82, 0.94	EUR, ASN
Microrna.org	rs8853 T > C	miR−204, miR−211	TBX3	rs59336 T > A, rs59336 T > A	0.82, 0.94	EUR, ASN
TargetScan	rs888208 A > G	miR−205-5p	NKX2-3	rs12412391 A > G, rs12412391 A > G	0.99, 0.95	EUR, ASN
Microrna.org	rs888208 A > G	miR−205	NKX2-3	rs12412391 A > G, rs12412391 A > G	0.99, 0.95	EUR, ASN
StarBase	rs9364 G > A	miR−346	LIMA1	rs34245511 G > C	0.92	EUR
Microrna.org	rs9364 G > A	miR−194	LIMA1	rs34245511 G > C	0.92	EUR
Microrna.org	rs9375 C > T	miR−141, miR−142-3p, miR−9, miR−200a, miR−494, miR−495, miR−539	RBM16	rs7740797 G > C	0.85	EUR
StarBase	rs944970 T > C	miR−216a−5p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs944970 T > C	miR−25, miR−92a, miR−216a, miR−367	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
StarBase	rs944971 T > C	miR−24-3p, miR−506-3p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
TargetScan	rs944971 T > C	miR−24-3p	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN
Microrna.org	rs944971 T > C	miR−24	LAMC1	rs10752881 A > G, rs10752881 A > G	0.7, 0.77	EUR, ASN

Abbreviations: AMR, American; ASN, Asian; EUR, European.

[a] r^2 (squared Pearson's correlation). SNPs in interactions related to under lined miRNAs located in miRNA:miRNA:SNP target site, others located in flanking region in targetome.

TABLE 3 CRC survival-associated variants raised on miRNAs targetome

Databases	Targetome SNP	miRNA	Gene	GWAS SNP	r^2 [a]	Population
Microrna.org	rs1127466 G > C	miR−338-3p	SLC22A23	rs4959799 G > C	0.78	EUR
StarBase	rs1127470 T > A	miR−302e, miR−183-5p	SLC22A23	rs4959799 G > C	0.75	EUR
Microrna.org	rs2076472 T > C	miR−431	APOBEC2	rs2073016 T > C	1	EUR
StarBase, Microrna.org	rs4229 A > C	miR−125a−3p	LARP4B	rs1555895 A > C	0.62	EUR
StarBase	rs45629235 A > G	miR−432-3p, miR−27a−3p, miR−27b−3p	SLC22A23	rs4959799 G > C	0.78	EUR
StarBase	rs7703 C > G	miR−124-3p	LARP4B	rs1555895 A > C	0.69	EUR
Microrna.org	rs9501973 T > C	miR−203	SLC22A23	rs4959799 G > C	0.78	EUR
StarBase	rs9501974 T > C	miR−181b−5p, miR−1297, miR−181c−5p, miR−182-5p, miR−96-5p	SLC22A23	rs4959799 G > C	0.78	EUR
Microrna.org	rs9501974 T > C	miR−181b, miR−181c	SLC22A23	rs4959799 G > C	0.78	EUR
StarBase	rs9501975 C > A	miR−181b−5p, miR−1297, miR−17-5p, miR−20a−5p, miR−203a, miR−181c−5p, miR−106b−5p, miR−182-5p, miR−96-5p, miR−20b−5p, miR−106a−5p	SLC22A23	rs4959799 G > C	0.78	EUR
Microrna.org	rs9501975 C > A	miR−181b, miR−181c	SLC22A23	rs4959799 G > C	0.78	EUR
StarBase	rs9503516 G > A	miR−137,miR−9-5p, let−7a−5p, let−7i−5p, miR−1297, miR−4500, let−7e−5p, let−7c−5p, let−7b−5p, let−7 g−5p, miR−4458, let−7f−5p, let−7d−5p, miR−98-5p	SLC22A23	rs4959799 G > C	0.78	EUR
TargetScan	rs9503516 G > A	miR−9-5p, miR−137	SLC22A23	rs4959799 G > C	0.78	EUR
Microrna.org	rs9503516 G > A	miR−137	SLC22A23	rs4959799 G > C	0.78	EUR
StarBase	rs9503517 C > T	miR−137, miR−1297	C6orf85	rs4959799 G > C	0.78	EUR
Microrna.org	rs9503517 C > T	miR−137	SLC22A23	rs4959799 G > C	0.78	EUR

Abbreviation: EUR, European.

[a] r^2(squared Pearson's correlation).SNPs in interactions related to under lined miRNAs located in miRNA:miRNA:SNP target site, others located in flanking region in targetome.

miRNA:mRNA:SNP unique interactions, in which 198 of them were in miRNAs binding sites and others were in its up or down stream. In other words, 196 putative obesity-associated variants were identified to reside within or near miRNA binding sites. All interactions, GWAS SNPs, and populations are shown in supplementary file 1. Seventeen and 125 unique miRNA:mRNA:SNP interactions (seven and 16 unique SNPs) were find for GWAS obesity-related lead variants in miRNAs binding sites and its flanking regions, respectively, which are presented in Tables 5 and 6.

3.2 | Effect of targetome SNPS on local RNA secondary structures

Previous studies showed that miRNA targetome variants can impose local structural changes[15-17] that may not be quantified using ΔΔGhybrid. The RNAsnp outputs a p-value that measures the significance of the observed the maximum base pair distances (d_{max}) imposed by the variant. The analysis showed that 33 (17%) obesity related and 10 (23%) CRC-associated variants residing within or near target site had local disruptive effects on target RNA secondary structure, which including 153 from 935 obesity related, 41 from 134 CRC risk related, and 9 from 48 CRC survival unique-associated interactions. Data are shown in Figure 2 (d_{max} p-value distribution of CRC and obesity) and supplementary file 2 (d_{max} p-value for each SNPs).

3.3 | Structural accessibility of target sites

Accessibility of target site plays an important role in miRNA-mediated regulation of gene expression.[16,18] The impact of

TABLE 4 Number of obesity-related variants included in this study based on 1000 genomes project super-populations

Categories	Population	Index variants	Proxy variants	Combined
Ob	EUR	123	6074	6197
	ASN	12	610	622
W-B	EUR	534	28323	28857
	ASN	417	20378	20795
	AFR	396	7099	7495
	AMR	334	13071	13405
WHR	EUR	163	7106	7269
	ASN	155	6762	6917
	AFR	149	1991	2140
	AMR	29	384	413
AT	EUR	138	3758	3896
	AFR	6	146	152
FM	EUR	26	1595	1621
	ASN	15	1046	1061
	AFR	15	491	506
	AMR	50	1841	1891
Overall	All	2562	100675	103237
Overall without duplicates[a]	All	1079	37852	38931

Abbreviations: AFR, African; AMR, American; ASN, Asian; AT, Adipose tissue; EUR, European; FM, Fat mass; Ob, Obesity; W-B, Weight & BMI; WHR, Waist/hip circumference (ratio).

[a]Number of unique index and proxy variants in five obesity-related categories.

TABLE 5 Obesity-related GWAS lead variants raised on miRNAs target sites

Categories	SNPs	Gene	miRNA(s)	Population(s)	Database
W-B	rs4395360 C > T	PQLC2L	miR−205	EUR, ASN, AMR, AFR	Microrna.org
W-B	rs715 T > A	CPS1	miR−432-5p	EUR, ASN, AMR, AFR	StarBase
WHR	rs2179129 A > G	ZNRF3	miR−411-5p	EUR, ASN, AFR	StarBase, TargetScan
WHR	rs2179129 A > G	ZNRF3	miR−379, miR−411,	EUR, ASN, AFR	Microrna.org
AT	rs1048497 G > A	ZNF664	miR−17-3p, miR−487b−3p, miR−124-3p	EUR	StarBase
AT	rs1048497 G > A	ZNF664	miR−139-5p, miR−487b	EUR	Microrna.org
Ob, W-B	rs7132908 G > A	FAIM2	miR−326	EUR, EUR, AMR	TargetScan
Ob, W-B	rs9299 C > A	HOXB-AS3	miR−222-3p	EUR, EUR	StarBase
Ob, W-B	rs9299 C > A	HOXB5	miR−7	EUR, EUR	Microrna.org
Ob, FM	rs6857 C > T	NECTIN2	miR−339-5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org

Abbreviations: AFR, African; AMR, American; ASN, Asian; AT, Adipose tissue; EUR, European; Ob, Obesity; FM, Fat mass; W-B, Weight & BMI; WHR, Waist/hip circumference (ratio).

CRC/obesity-associated variants residing within or near miRNA binding sites on accessibility of target sites is measure by ΔPu. For obesity, we found a decreasing accessibility effect 45% (up to −37%) and increasing effect for 55% (up to 30%) of target sites, which 418 unique interactions decreased and 516 interactions increased accessibility effect. While the results for CRC were 67% (up to −39%) and 43% (form up to 9%), respectively. In another word, for CRC survival 24 unique interactions shows decreased and 24 interactions shows increased accessibility effect, and for CRC risk 79 interactions decreased while 55 interactions increased accessibility effect. These results shown that a significant numbers of target site SNPs could effect on the role of miRNA on target RNA and consequently on the role of these genes on CRC and obesity. Detailed results are shown in Figure 3 and supplementary file 3.

TABLE 6 Obesity-related GWAS lead variants raised on flanking regions of miRNA target sites

Categories	SNPs	Gene	miRNA(s)	Population(s)	Database
W-B	rs715 T > A	CPS1	miR−190b	EUR, ASN, AMR, AFR	StarBase, Microrna.org
Ob	rs1048466 G > A	CCDC77	miR−184	EUR	StarBase, Microrna.org
Ob	rs1048466 G > A	CCDC77	miR−542-3p	EUR	Microrna.org
AT	rs1056053 C > A	TBXT	miR−106a, miR−17, miR−218	EUR	Microrna.org
AT	rs1048497 G > A	ZNF664	miR−758	EUR	Microrna.org
AT	rs1048497 G > A	ZNF664	miR−758-3p	EUR	StarBase
Ob, W-B	rs9299 C > A	HOXB5	miR−185	EUR	Microrna.org
Ob, W-B	rs9299 C > A	HOXB5	miR−185-5p, miR−7-5p	EUR	StarBase
Ob, W-B	rs7132908 G > A	FAIM2	miR−185	EUR	Microrna.org
Ob, W-B, WHR	rs2531995 C > T	ADCY9	miR−193a−3p	EUR, ASN, AFR	StarBase
Ob, W-B, WHR	rs2531995 C > T	ADCY9	miR−24-3p	EUR, ASN, AFR	StarBase, TargetScan
Ob, W-B, WHR	rs879620 C > T	ADCY9	miR−506-3p	EUR, ASN, AFR	StarBase
W-B	rs10540 G > A	RNH1	miR−15a−5p, miR−15b−5p, miR−16-5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org
W-B	rs715 T > A	CPS1	miR−190a−5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org
W-B	rs10540 G > A	RNH1	miR−195-5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org
W-B	rs4395360 C > T	C3orf55	miR−199a−5p, miR−199b−5p, miR−300, miR−381	EUR, ASN, AMR, AFR	Microrna.org
W-B	rs10540 G > A	RNH1	miR−424-5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org
W-B	rs715 T > A	CPS1	miR−450a	EUR, ASN, AMR, AFR	Microrna.org
W-B	rs715 T > A	CPS1	miR−496, miR−450a−5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org
W-B	rs10540 G > A	RNH1	miR−497-5p	EUR, ASN, AMR, AFR	StarBase, Microrna.org
W-B, FM	rs6857 C > T	PVRL2	miR−124	EUR, ASN, AMR, AFR	Microrna.org
W-B	rs6857 C > T	PVRL2	miR−506	EUR, ASN, AMR, AFR	Microrna.org
W-B, WHR	rs1042725 C > T	HMGA2	let−7a−5p	EUR	StarBase, TargetScan, Microrna.org

(Continues)

TABLE 6 (Continued)

Categories	SNPs	Gene	miRNA(s)	Population(s)	Database
W-B, WHR	rs1042725 C > T	HMGA2	let−7b−5p, let−7c−5p, let−7d−5p, let−7e−5p, let−7f−5p, let−7 g−5p, let−7i−5p, miR−181a−5p, miR−181b−5p, miR−181c−5p, miR−181d−5p, miR−196a−5p, miR−196b−5p, miR−98-5p	EUR	StarBase, Microrna.org
W-B, WHR	rs1042725 C > T	HMGA2	let−98-5p	EUR	TargetScan
W-B, WHR	rs1042725 C > T	HMGA2	miR−103, miR−107, miR−15a, miR−15b, miR−16, miR−181a	EUR	Microrna.org
W-B, WHR	rs1042725 C > T	HMGA2	miR−196-5p	EUR	TargetScan, Microrna.org
W-B, WHR	rs1042725 C > T	HMGA2	miR−202-3p, miR−445, miR−4500	EUR	StarBase
W-B, WHR	rs1042725 C > T	HMGA2	miR−424, miR−497, miR−503	EUR	Microrna.org
W-B, WHR	rs1042725 C > T	HMGA2	miR−543	EUR	StarBase, TargetScan
WHR	rs1045241 C > T	TNFAIP8	miR−1271-5p, miR−182-5p	EUR, ASN, AFR	StarBase, TargetScan, Microrna.org
WHR	rs3087591 A > C	EVI2B	miR−190, miR−190b, miR−197, miR−590-3p	EUR, ASN, AFR	Microrna.org
WHR	rs1045241 C > T	TNFAIP8	miR−96-5p	EUR, ASN, AFR	StarBase, TargetScan, Microrna.org
WHR	rs2179129 A > G	ZNRF3	let−7a−5p, let−7f−5p, miR−130a−3p, miR−130b−3p, miR−152-3p, miR−301a−3p, miR−301b, miR−3666, miR−379-5p, miR−4295, miR−4500	EUR, ASN, AFR	StarBase
WHR	rs2179129 A > G	ZNRF3	miR−130-3p, miR−301-3p	EUR, ASN, AFR	TargetScan
WHR	rs2179129 A > G	ZNRF3	miR−454-3p	EUR, ASN, AFR	StarBase, TargetScan
FM	rs28381552 T > C	ORC3L	miR−106a−5p, miR−106b−5p, miR−122-5p, miR−144-3p, miR−18a−5p, miR−20a−5p, miR−302a−3p, miR−373-3p, miR−512-3p, miR−519a−3p, miR−519b−3p, miR−520b, miR−520c−3p, miR−520d−3p, miR−520f−3p, miR−93-5p	AMR	StarBase
FM	rs28381552 T > C	ORC3L	miR−144	AMR	Microrna.org

Abbreviations: AFR, African; AMR, American; ASN,Asian; EUR, European; FM, Fat mass; Ob, Obesity; W-B, Weight & BMI; WHR, Waist/hip circumference (ratio).

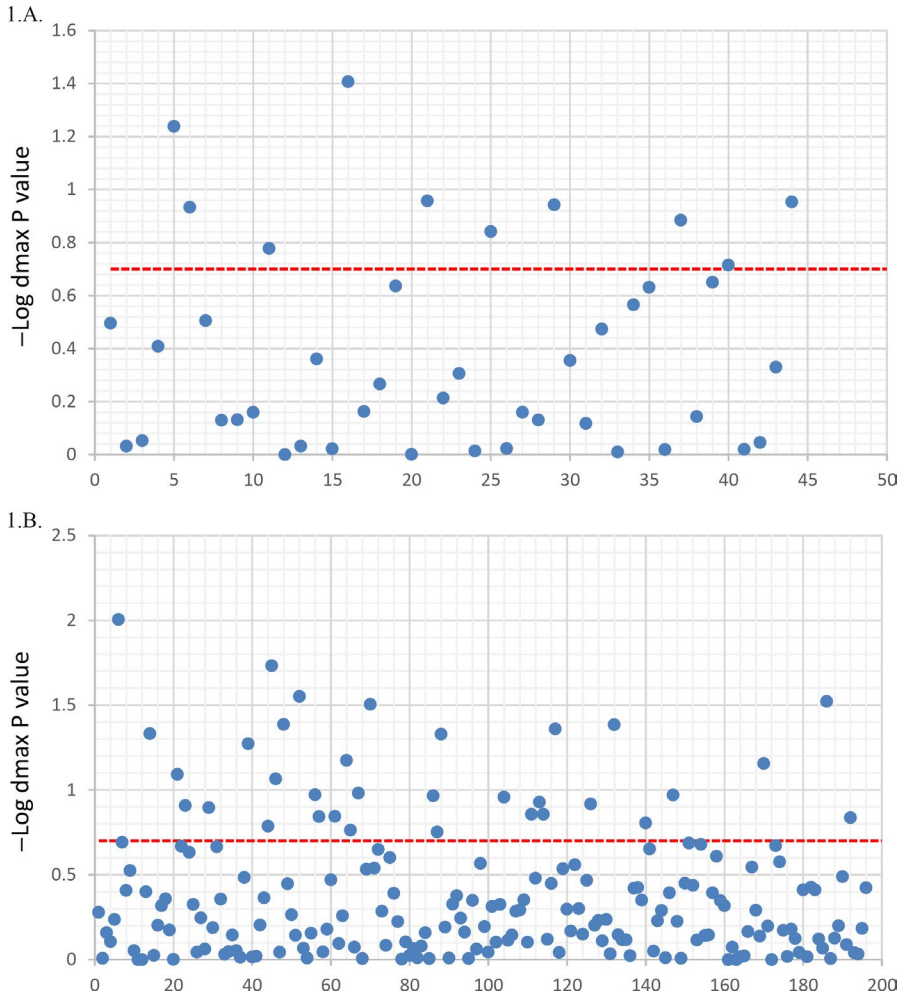

FIGURE 2 Effect of targetome SNPs on local RNA secondary structures. d_{max} p-value distribution for CRC 1.A. and for obesity 1.B. The numbers in X-axis are related to SNPs names and rsID (more detailed supplementary 2, sheet 3)

3.4 | MIRNA:mRNA hybrid stability

The effects on target site variant on interaction between miRNA and its target gene can be measured by change in hybrid stability. This alteration is based on base pair creation or disruption.[16] Here, we applied ΔΔGhybrid to measure target-SNPs effects on included miRNA–mRNA interactions. The miRNA:mRNA hybrid stability was increased in 127 interactions (up to 6.6 kcal/mol) and 17 interactions (up to 4.5 kcal/mol) for the effect of obesity and CRC SNPs, respectively, while decreased in 33 interactions (up to −2.4 kcal/mol) and 24 interactions (up to −4.7 kcal/mol) for them. The results are shown in Figure 4.

Finally, we prioritize interactions for CRC and obesity based on effect of targetome SNPs on local RNA secondary structures, structural accessibility of target sites, miRNA:mRNA hybrid stability, and annotation of polymorphisms with expression quantitative trait loci (eQTL). To validate the prioritized genes, as well as to investigate the association between the obesity genes with CRC, we analyzed the genes in TCGA data (available at https://portal.gdc.cancer.gov/).[19] The obtained results are presented in Table 7.

4 | DISCUSSION

Obesity is one of the most common complex diseases in the world and CRC is a common obesity-related cancer. Recently many GWA studies focused on discovering the variants related to these diseases and found many novel polymorphisms. According to these data several genes appears to be involved in these diseases pathogenesis while different miRNAs have a role in their regulation. Thus, variants in miRNA:mRNA binding sites may play important role in CRC and obesity. Many of the newly identified associations in GWAS are related to noncoding regions variants.[20] The role of these variants in tumor development and cancers risk were previously investigated in many studies.[21-23] These variants can be prioritized by bioinformatics analyses without any cost and laboratory works which could be used in future experimental

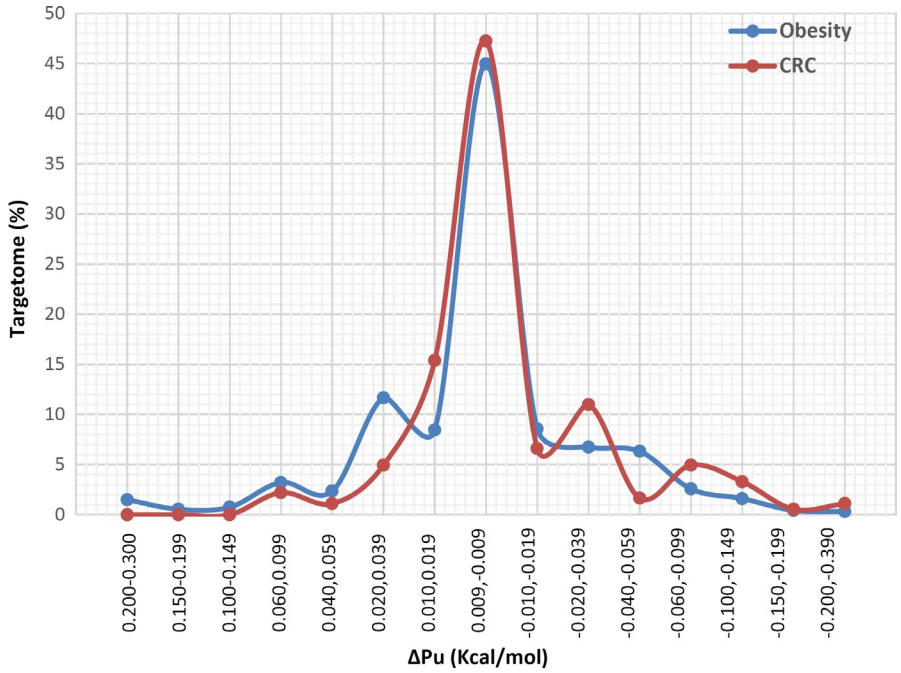

FIGURE 3 The plots show the effect of targetome SNPs on local RNA secondary structures, ΔPu for CRC and obesity shows changes in target site accessibility (Kcal/mol)

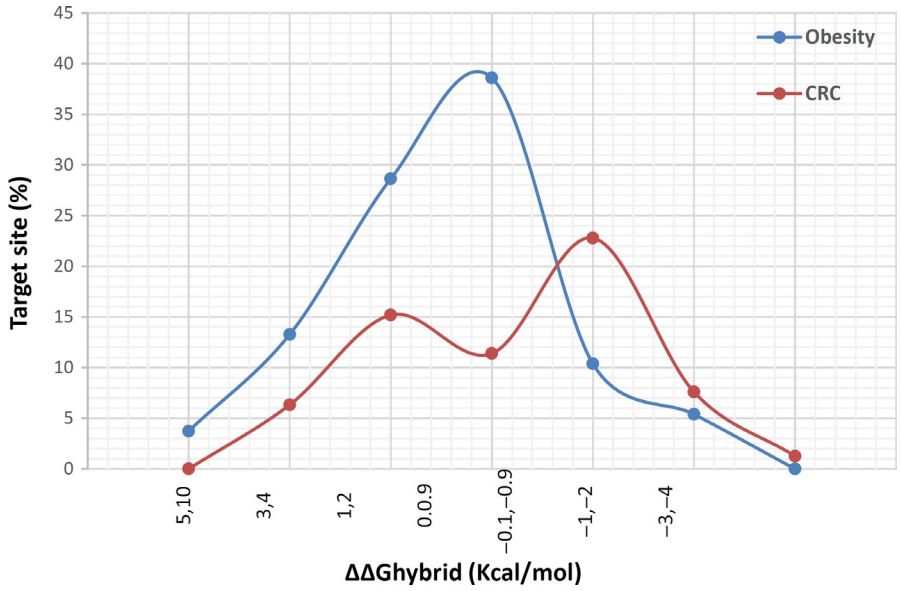

FIGURE 4 The plots show miRNA:mRNA hybrid stability based on ΔΔGhybrid of target sites

studies. Here, we used a bioinformatics approach to find polymorphisms which could potentially effect these diseases, and finally, we prioritized the most important miRNA:mRNA interactions based on using different bioinformatics tools to find the functional significance of these variants. The effect of miRNAs binding site polymorphisms on the binding sites structural changes have been studied previously[16,24] which may influence on miRNA regulatory effect. In our study, 10 and 33 miRNA binding site SNPs significantly changed on local RNA secondary structures in obesity and CRC, respectively. According to the miRNA:mRNA hybrid stability plot (Figure 4) for obesity most of SNPs in target sites do not have any effect on hybrid stability but for CRC most of miRNA binding site SNPs were effective on hybrid stability. While about the accessibility of target sites most of CRC and obesity-related SNPs do not have any significant effect. Finally, seven SNPs with 15 interactions and three SNPs with four interactions were identified for obesity and CRC, respectively.

TABLE 7 Prioritized SNPs in target site associated to obesity or CRC

Databases	Categories	SNP	Reference-GWAS SNP	Is SNP eQTL?	GERP[a]	SiPhy[a]	RNA secondary structures		Structural accessibility	miRNA:mRNA hybrid stability	Interaction	Gene survival effect (according to TCGA data)	
							d_max	*p*-value	ΔPu%	ΔΔGhybrid		*p*-value[b]	*p*-value[c]
StarBase, Microrna.org	W-B	rs16912239 A>C	rs16912238 A>G	No	+	+	0.3988	0.01	2.76	3.9	hsa-miR−30a−5p: PDCL:rs16912239	0.099	0.186
									2.59	3.1	hsa-miR−30c−5p: PDCL:rs16912239		
									2.76	3.9	hsa-miR−30d−5p: PDCL:rs16912239		
									2.47	3.9	hsa-miR−30b−5p: PDCL:rs16912239		
									2.76	3.9	hsa-miR−30e−5p: PDCL:rs16912239		
StarBase, Microrna.org	W-B	rs1044390 T > A	rs1878931 G>C	Yes	−	−	0.2875	0.03	−8.78	0.7	hsa-miR−504-5p: ZNF434:rs1044390	**<0.001**	**0.001**
Microrna.org	W-B	rs11755593 G>A	rs943466 G>A	Yes	−	+	0.3066	0.04	1.76	2.7	hsa-miR−34c−5p: LEMD2:rs11755593	0.283	0.066
StarBase, Microrna.org	W-B	rs5872A>C	rs2844479 A>C	Yes	−	+	0.2219	0.07	9.96	1.7	hsa-miR−488-3p: CSNK2B:rs5872	0.801	0.503
Microrna.org	W-B	rs1055816 G>A	rs492400 C>T	Yes	−	−	0.1652	0.10	0.20	6	hsa-miR−15b−5p: PLCD4:rs1055816	**0.042**	0.086
									0.30	4.2	hsa-miR−195-5p: PLCD4:rs1055816		
									0.30	6	hsa-miR−497-5p: PLCD4:rs1055816		
									0.24	4.3	hsa-miR−15a−5p: PLCD4:rs1055816		
									0.20	2.1	hsa-miR−16-5p: PLCD4:rs1055816		
StarBase, Microrna.org	WHR	rs4759058 C>A	rs1443512 A>C	Yes	−	−	0.1976	0.11	−8.47	6.6	hsa-miR−503-5p:HOXC13: rs4759058	**<0.001**	**<0.001**

(Continues)

TABLE 7 (Continued)

Databases	Categories	SNP	Reference-GWAS SNP	Is SNP eQTL?	GERP[a]	SiPhy[a]	RNA secondary structures		Structural accessibility	miRNA:mRNA hybrid stability	Interaction	Gene survival effect (according to TCGA data)	
							d_max	*p*-value	ΔPu%	ΔΔGhybrid		*p*-value[b]	*p*-value[c]
StarBase, Microrna.org	WHR	rs1045475 A>G	rs2531992 A>G	Yes	−	+	0.1696	0.14	−1.09	0.8	hsa-miR−141-3p: ADCY9:rs1045475	**0.005**	**0.008**
StarBase	CRC risk	rs9364G>A	rs34245511 G>C	Yes	−	−	0.1531	0.11	−11.19	1.9	hsa-miR−346:LIMA1: rs9364	**0.009**	**˂0.001**
StarBase	CRC survival	rs9501974 T > C	rs4959799 G>C	Yes	−	+	0.1541	0.17	1.99	1.4	hsa-miR−182-5p:SLC22A23: rs9501974	0.133	**0.014**
									1.99	1.4	hsa-miR−96-5p: SLC22A23: rs9501974		
Microrna.org	CRC risk	rs11085537 G>C	rs11671104 A>C	Yes	−	−	0.1129	0.19	−7.68	2.3	hsa-miR−136:LOC440518: rs11085537	0.849	0.222

Abbreviation: eQTL, Expression quantitative trait loci.

[a]GERP and SiPhy are measures of evolutionary conservation calculated by PhastCons algorithm.

[b]*p*-value related to survival rate of mutated CRC cases (PDCL n = 24, ZNF434 n = 50, LEMD2 n = 26, CSNK2B n = 31, PLCD4 n = 33, HOXC13 n = 24, ADCY9 n = 66, LIMA1 n = 37, SLC22A23 n = 38, LOC440518 n = 39) compared to unmuted (n = 606) CRC cases, form TCGA data.

[c]*p*-value related to survival rate of mutated CRC cases compared to overall mutated CRC case in 10 genes (n = 182).

These results revealed that miRNA:mRNA hybrid stability, structural accessibility, and RNA secondary structures could be influenced by obesity or CRC-associated variants in miRNA target sites. We used different common algorithms for miRNA:mRNA interaction prediction with highly conserved target site to manage balance between specificity and sensitivity of bioinformatics algorithms.

According to our knowledge, all prioritized SNPs (10 SNPs in Table 7) were not studied for the association with obesity or CRC. About the association of these SNPs with obesity or CRC, only rs4759058 was predicted which to be related to waist-hip ratio.[25] From them rs16912239 and rs11085537 were not eQTL SNPs. We finally found that two and six miRNA binding site SNPs have related to obesity and CRC, respectively.

The TCGA data analysis validated prioritized CRC-related genes and displayed that the obesity prioritized genes were also effective on CRC. Based on the obtained results, the ZSCAN32, PLCD4, HOXC13, and ADCY9 from obesity predicted genes, as well as LIMA1, and SLC22A23 from CRC predicted genes were significantly related to CRC survival rate. Furthermore, there are several lines of evidence on the role of prioritized genes in pathogenesis of CRC or obesity. The GWA studies confirm that, the genes in prioritized interactions for CRC were associated to the cancer.[26-28] The expression of these genes have been considered in the recent studies, for example, the GOLGA2 newly identified as novel differentially expressed proteins in CRC,[29] the expression of LIMA1 changed in Cholangiocarcinoma,[30] and the expression of SLC22A23 expression increased in subjects with Laryngeal squamous cell carcinoma.[31] Besides, the CRC prioritized genes were also associated to obesity for instance, the LIMA1,[32] SLC22A23,[33] and LOC440518[34] related to body fat distribution, obesity-related traits, and BMI, respectively.

The association of predicted obesity genes with obesity approved in the GWA studies.[33,35-39] For instance, LEMD2 is associated with body weight,[38] HOXC13 and ADCY9 polymorphisms are associated with waist-to-hip ratio and BMI, respectively.[39-41] The GWAS and recent published studies significantly proved the genes and miRNAs in obesity prioritized interaction were also associated to cancer. For instance, the ADCY9,[40] and HOXC13[41] were related to cancer, and adverse response to chemotherapy, respectively. The ADCY9 expression remarkably increased in colon tumor tissue and can be a poor prognostic factor for colon survival.[42] The HOXC13 and CSNK2B expression increase in breast cancer and play a key role in the progression of breast cancer.[43,44] The expression of cyclins regulated by HOXC13 and knockdown of this gene resulted to cell cycle arrest and apoptosis, in the colon cancer cell lines.[45] The roles of identified miRNAs in cancer were also determined. The miRNAs in prioritized interactions were related to CRC and other type of cancers. In this case, the miR-30 family, miR-504, miR-34c, miR-488, miR-15b, miR-195, miR-497, miR-15a, miR-503, miR-141, miR-182, miR-96, and miR-136 were also related to invasion, proliferation, metastasis and tumor growth, or survival of several cancers including CRC.[46-65] However, there are few studies on the role of included prioritized miRNAs for interactions with obesity. For obesity there are some studies on mice, for instance, miR-16-5p decrease with high weight and a mutation in miR-16 gene lead to increasing in body weight.[66] The upregulation of miR-16 was observed in calorie-restricted mice with lower body weight.[67] The miR-30e-5p and miR-141-3p were upregulated in high-fat diet mice.[68,69] The miR-30b, miR-16, miR-15b, and miR-15a were downregulated in diet-induced obesity mice.[70] According to our results and above described documents, the miRNAs and mRNAs in obesity prioritized interactions played significant roles in CRC, this represented a strong genetic linkage on the mentioned diseases. Therefore, prioritized polymorphisms with miRNA:mRNA interactions identified in this study could be important for future investigation on the role of miRNAs and their targeted genes on CRC and obesity.

5 | IN CONCLUSION

This was the first comprehensive systematic and bioinformatics approach for identification and prioritization of variants in miRNA binding sites of genes related to obesity or CRC as two most common complex and related diseases. The results of our study will be valuable for future association studies and functional studies to examine the role of these miRNA target site polymorphisms and genes and their association based on these identified interactions. These SNPs and interactions could be used for future studies for finding potential markers for diagnoses, prognosis, or treatment of CRC and obesity.

ACKNOWLEDGMENTS

This study was supported by *National Institute for Medical Research Development Grant No. 971211*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Gholami M, Zoughi M, Larijani B, M. Amoli M, Bastami M. An in silico approach to identify and prioritize miRNAs target sites polymorphisms in colorectal cancer and obesity. *Cancer Med*. 2020;9:9511–9528. https://doi.org/10.1002/cam4.3546