Identification of Genetic Variants for Prioritized miRNA-targeted Genes Associated with Complex Traits

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ABSTRACT

Genome-wide association studies, or GWAS, have reported associations between SNPs and specific diseases/traits. GWAS results contain variants located in different genomic regions, including variants in the 3'UTR. MicroRNAs, or miRNAs, are small noncoding RNAs that bind to the 3'UTRs of genes to regulate gene expression. However, variant(s) that are located in the 3'UTR could impact miRNA binding, thus affecting expression of its targeted gene(s). To specifically elucidate miRNA targeting pairs and binding site variants associated with a specific trait, well-designed downstream analysis along with careful experimental design are necessary. Currently, there is no available state-of-the-art methodology for identifying miRNA targeting pairs and associated variants that could contribute to phenotypes using GWAS. Moreover, it is unrealistic to conduct experiments for elucidating all possible miRNA targeting pairs and binding site variants across the entire genome. In this project, we developed a bioinformatic pipeline to computationally identify genes and their targeting miRNA pairs that are enriched over the miRNA-gene tissue expression network for the studied genetic traits and examined the binding site variants' impact on Body Mass Index (BMI).

1. Introduction

GWAS is a powerful approach to identify common variants associated with common diseases/traits in studied populations [1, 2]. Traits may be activated due to genetics or changes in environment, or both.

MicroRNAs (miRNAs), noncoding RNAs with a length between 17-22 nucleotides, exert their impact on gene regulation through targeting mRNAs. To achieve their function, miRNAs bind to the 3' untranslated regions (3'UTRs) of target mRNAs which can often result in potential suppression of mRNA translation and/or gene expression.

Single-nucleotide polymorphisms (or SNPs) are the most common mutations found in the human genome [3, 4]. SNPs located in the 3'UTRs can potentially disrupt miRNA regulation [5]. A DNA motif is a conserved DNA sequence segment with biological consequences. The identification of a motif located in the 3'UTR could suggest how miRNAs potentially target genes in that region. Analysis of GWAS data by identifying genes or miRNAs that harbor genetic variants could elucidate trait-associated genetic variants [6].

In our experiment, we analyzed multiple traits compiled from GWAS and found BMI to be the most significant trait in the context of the miRNA-gene tissue expression target network. We further investigated several genes located within the region(s) associated with BMI, *FADS1* and *FADS2* due to their significant relevance to obesity and related conditions [7]. The overarching goal of this study is to identify how the interaction between genes and their targeting miRNAs change due to genetic variations and reveal any underlying biological mechanisms that contribute to the development of complex traits for humans.

2. Experimental Methods

To perform our experiment, we proposed the following approach:

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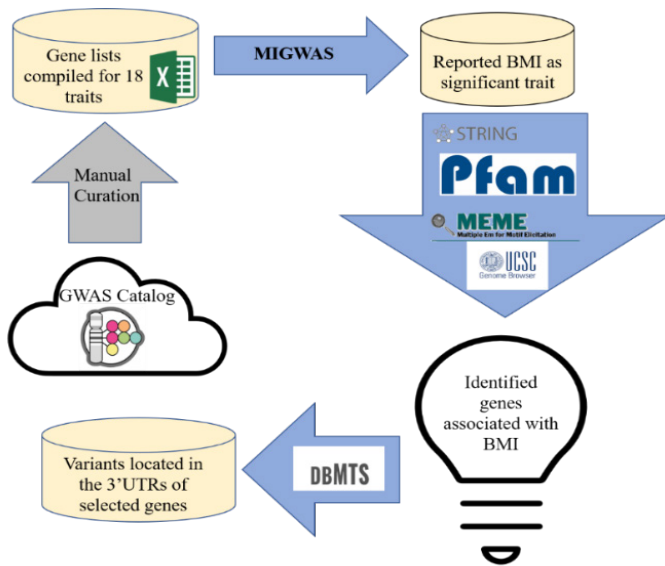
Email: bioinformaticsresearchtomorrow@gmail.com

Figure 1: Workflow of variant identification for complex trait BMI

2.1. GWAS Traits Selection

We downloaded the annotation data of GWAS results from 18 traits, all of which had at least 100 associated genes as compiled by PheGenI [6]. All traits were previously described [8]. These traits ranged from autoimmune disorders such as Rheumatoid Arthritis and Asthma to behavior traits such as smoking.

2.2. Trait Relevant miRNA-gene Pairs Identification

We employed MIGWAS software [9, 10] using default running parameters (2018 Github version: https://github.com/saorisakaue/MIGWAS) to strengthen our analysis regarding all 18 traits and identify significant traits and miRNA-gene pair candidates over the gene tissue expression network. In order to run MIGWAS, we converted the variant genomic coordinate annotations (hg38) provided by PheGenI to the hg19 version. Figure 2 shows the Python pipeline of running MIGWAS.

```
Step 1:

python3    minimgnt.py    hg19_BMI.txt    --out
hg19_BMI_sumstats_trans --cpus 10 --not-remove-
HLA --remove-NA --no-rsid

Step 2:

python3       migwas.py       --phenotype
hg19_BMI_sumstats_trans              --out
miRA_hg19_BMI_sumstats_trans   --cpus   10   --
iterations 500 --output-candidate
```

Figure 2: Python code of running MIGWAS

2.3. Protein-Protein Interaction Examination and Domain Elucidation

We ran STRING database to look for evidence of interaction between the ten candidate genes associated with BMI at the protein level [11]. We also used Pfam to identify if there were any possible domains associated with BMI [12].

2.4. Motif Discovery for miRNA-targeted Genes Relevant to BMI Trait

We applied MEME software (version 5.3) to identify enriched motifs for the 3'UTR sequences retrieved from Ensembl Biomart Database [13, 14]. To run MEME, we stipulated the software parameter for the searched motif width to be between six and twenty base pairs. Also, the number of searched motifs was restricted to report the top six motifs. All other running parameters were kept as default.

2.5. Variant Identification for Selected Genes Associated with BMI

We checked the dbMTS database for reported candidate genes associated with BMI through MIGWAS and pinpointed SNPs located inside each gene's respective 3'UTRs [5].

3. Results

3.1. Selection of GWAS Results

We acquired the GWAS results of 18 traits with more than 100 associated genes that were reported in our recent study [8]. All selected traits are displayed in Table 1 below.

Table 1: Selected 18 traits as reported by PheGeni. All traits listed were associated with at least 100 genes.

Trait	# SNPs	# Genes
Alcoholism	802	657
Alzheimer's	873	1037
Arthritis, Rheumatoid	374	276
Asthma	712	818
Autistic Disorder	982	505
BMI	3601	1971
Coronary Artery Disease	493	471
Coronary Disease	440	345
Crohn's	633	403
T1D	309	235
T2D	542	416
Hypertension	314	270
NSCLC	129	131
Obesity	426	434
Osteoporosis	77	102
Psoriasis	425	292
Schizophrenia	1530	1453
Smoking	781	938

3.2. MIGWAS Analysis

We identified in particular that one out of the 18 traits, BMI, has a significant association with all tissues (p-value of .0313). There are only three traits which have a p-value reported for trait

and tissue association, including BMI, Crohn's, and Type 1 Diabetes (T1D) (Figure 3).

We have identified ten genes that are targeted by five miRNAs as candidate biomarkers in the BMI trait. For Crohn's, there was only one pair while T1D had two pairs (Table 2).
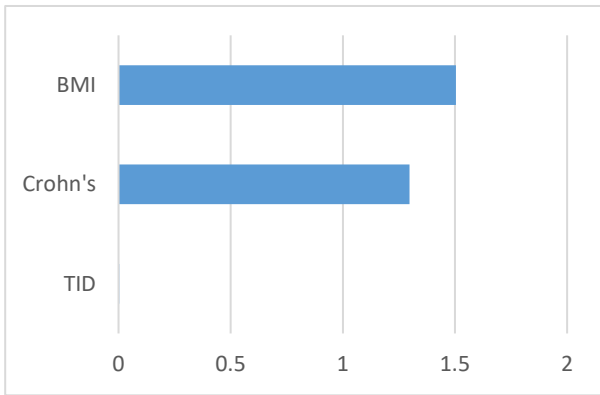

Figure 3: MIGWAS reported p-values (-log base 10 scale) for BMI, Crohn's disease, and Type 1 Diabetes.

Table 2: MIGWAS results for candidate genes/miRNAs identified for three traits

Trait name	Gene	miRNA
BMI	*ADAMTS9*	*hsa-mir-330*
BMI	*AMH*	*hsa-mir-615*
BMI	*CELSR2*	*hsa-mir-615*
BMI	*DNM3*	*hsa-mir-330*
BMI	*FADS1*	*hsa-mir-615*
BMI	*FADS2*	*hsa-mir-615*
BMI	*HOXC5*	*hsa-mir-615*
BMI	*MED17*	*hsa-mir-4531*
BMI	*NEGR1*	*hsa-mir-4721*
BMI	*OTUD7B*	*hsa-mir-642a*
Crohn's	*SYNGR1*	*hsa-mir-4728*
T1D	*HIPK1*	*hsa-mir-1236*
T1D	*TMEM116*	*hsa-mir-4646*

3.3. String Database Results

Out of the ten genes researched, there are three genes—*FADS2, FADS1,* and *AMH*—that interact with each other as reported by the STRING database, as shown in Figure 4. These three genes are also targeted by the same miRNA: *hsa-mir-615*. The STRING database also reported BMI's significant associations (FDR < .01) with fatty acid metabolism (hsa01212) and biosynthesis of unsaturated fatty acids (hsa01040) under KEGG pathways. Likewise, the BMI-related GO terms—linoleic acid metabolic process (GO:0043651), alpha-linolenic acid metabolic process (GO:0036109), and unsaturated fatty acid biosynthetic process (GO:0006636)—are also reported by the STRING database.

3.4. Pfam Database Results

The Pfam database reported two domains associated with BMI: Fatty Acid Desaturase (PF00487) and Cytochrome b5-like heme/steroid binding domain (PF00173), as shown in Figure 5.
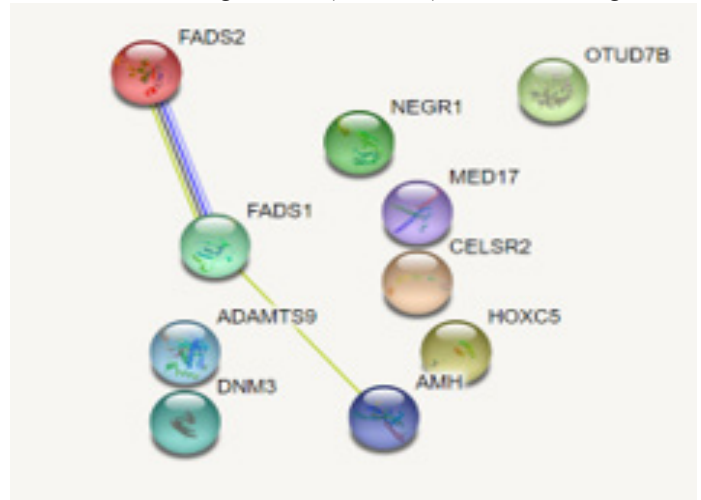

Figure 4: Protein-protein interaction results for all ten genes associated with BMI. Only three genes were found to have interactions with each other (*FADS1, FADS2, AMH*).

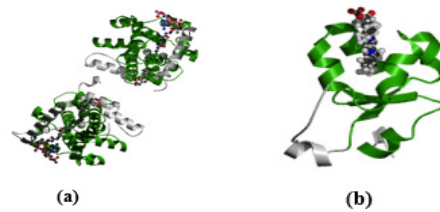These results were consistent with the ones reported by the INTERPRO database.

Figure 5: Protein structures of two BMI-associated domains. (a) displays the Fatty Acid Desaturase Domain. (b) displays the Cytochrome b5-like heme/steroid binding domain.

3.5. Motif Identification

Using MEME, we ran all ten of the genes associated with BMI. A motif was discovered containing nine out of these ten genes (*ADAMTS9, CELSR2, DNM3, FADS1, FADS2, HOXC5, MED17, NEGR1, OTUD7B*), with an e-value of .047 (Figure 6).

Figure 6: Motif conservation across nine (out of ten) genes associated with BMI

3.6. Variant Identification Results

To report known common variants contained in miRNA-targeted genes associated with BMI, we checked the UCSC Genome Browser [15]. This region contains multiple known variants (dbSNP 153 Track) that are located inside both *FADS1* and *FADS2* (see Figure 7 below). Interestingly, *FADS1*, *FADS2*, and *FEN1* (a neighboring gene) are highly expressed in the adrenal gland. As indicated by GTEx data, *CELSR2*, *FADS1*, and *FADS2* all are expressed with low amounts in both types of adipose tissue. Since all three genes are targeted by the same miRNA, *hsa-miR-615*, low expression of these genes in adipose tissues could be due to the repression effect of this miRNA.
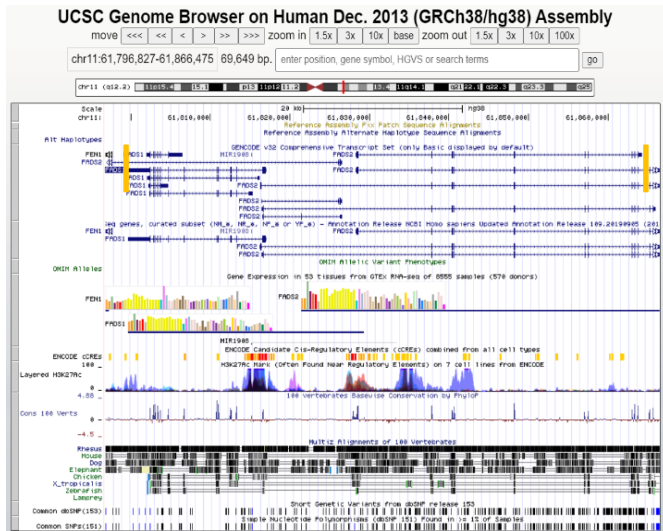

Figure 7: UCSC Genome Browser view for variants located in the genomic regions of *FADS1/FADS2*. The orange vertical bars placed within the view pinpoint the general motif coordinates defined by MEME. The left bar represents the identified motif region for *FADS1* while the right bar represents *FADS2's* identified motif region.

3.7. dbMTS Results

To identify miRNA binding-site variants, we checked the dbMTS database [5]. Table 3 shows that multiple variants, which could alter the binding relationship of miRNAs and their targets, are often present in the 3'UTRs of miRNA-targeted genes associated with BMI.

4. Discussion and Interpretation

In this study, we applied cutting-edge bioinformatics tools and databases to select candidate genes/miRNAs associated with complex genetic trait(s), such as BMI.

Using MIGWAS, we analyzed three traits: BMI, Crohn's, and T1D. While both BMI and Crohn's had significant p-values in the gene expression network, Crohn's yielded only one gene/miRNA pair. On the contrary, T1D had a non-significant p-value but yielded 2 gene/miRNA pairs (see Figure 3 and Table 2). We concluded that a significant p-value does not necessarily indicate the existence of more miRNA-gene targeting pairs for specific traits. As a result of this MIGWAS analysis, we focused on investigating BMI as it had many gene/miRNA pairs in comparison to the other two traits mentioned. When performing

Table 3: Variant results reported in dbMTS

rs_dbSNP150	chr	pos	ref	alt	VEP_ensembl_Gene_Name
rs7528419	1	109274570	A	G	CELSR2
rs11102967	1	109274623	C	G	CELSR2
rs1277917	1	109274857	G	C	CELSR2
rs12740374	1	109274968	G	T	CELSR2
rs660240	1	109275216	T	C	CELSR2
rs57677983	1	109275536	C	A	CELSR2
rs658435	1	109275648	C	T	CELSR2
rs629301	1	109275684	G	T	CELSR2
rs10410	1	109279782	G	A	CELSR2
rs14000	1	109279887	T	C	CELSR2
rs1061955	1	149942458	G	A	OTUD7B
rs2757500	1	172309881	G	A	DNM3
rs2586422	1	172309937	A	G	DNM3
rs2586423	1	172310272	G	A	DNM3
rs2421994	1	172310769	G	A	DNM3
rs10489282	1	172407906	G	A	DNM3
rs4916255	1	172411735	C	A	DNM3
rs7528296	1	172412243	T	C	DNM3
rs11714364	3	64650182	C	A	ADAMTS9
rs9851598	3	64650452	G	A	ADAMTS9
rs4246215	11	61796827	G	T	FADS1, FADS2
rs174544	11	61800281	C	A	FADS1, FADS2
rs174545	11	61801834	C	A	FADS1, FADS2
rs174545	11	61801834	C	G	FADS1, FADS2
rs174546	11	61802358	C	T	FADS1
rs174568	11	61826344	C	T	FADS1, FADS2
rs482548	11	61865710	C	A	FADS2
rs17764288	11	61866264	G	A	FADS2
rs11539526	11	61866298	C	G	FADS2
rs77167250	11	61866475	C	T	FADS2
rs676586	11	93788239	C	T	MED17
rs606087	11	93789476	A	G	MED17
rs17652258	11	93814518	G	C	MED17
rs677019	11	93818291	T	C	MED17
rs654896	11	93818330	A	T	MED17
rs17573753	11	93818630	C	T	MED17
rs679241	11	93818748	A	G	MED17
rs585768	11	93819689	C	T	MED17
rs2071450	12	54034748	C	T	HOXC5
rs104894666	19	2250667	C	T	AMH

this study, we noticed that different tools/databases can analyze data with complementary results from different aspects.

For example, MIGWAS identified *CELSR2* containing 3'UTR variants also reported by the PheGenI database. MIGWAS identified two genes (out of nine)—*DNM3* and *FADS1*—that don't contain 3'UTR variants in the data provided by PheGenI. The other seven genes were not reported by PheGenI [6, 9].

After comparison, dbMTS reported results on nine genes total. Six genes (*OTUD7B, DNM3, ADAMTS9, MED17, FADS1,* and *FADS2*) contain 3'UTR variants also reported by MIGWAS. The remaining three genes (*CELSR2, HOXC5,* and *AMH*) do not contain 3'UTR variants as dbMTS states.

Using DIANA Tools, we identified two genes (*CELSR2* and *ADAMTS9*) that are targeted by *hsa-miR-615* [16]. Although MIGWAS stated that *ADAMTS9* was targeted by *hsa-miR-330*, this is likely due to this gene being targeted by multiple miRNAs. However, wet-lab experiments are needed to validate this conclusion. In addition, the targeting outcome of multiple genes associated with BMI by *hsa-miR-615* is significant. Studies show that increased levels of *hsa-miR-615* may help inhibit palmitate-induced hepatocyte apoptosis [17]. If saturation levels of palmitate, a type of fatty acid, are increased, this increases the likelihood of developing diseases such as non-alcoholic fatty liver disease, which is correlated with obesity (or a high BMI) [18]. Indeed, *CELSR2* was recently discovered to have significant high expression in cancerous tissue than normal tissue. Specifically, hepatocellular carcinoma, a primary liver cancer whose major risk factors include non-alcoholic fatty liver disease [19].

Current literature evidence supports that *FADS2* has been discovered to be a drug target gene as well as an miRNA-target gene for rheumatoid arthritis (RA) [10]. However, such evidence reports *hsa-miR-4728* as *FADS2's* target miRNA instead of *hsa-miR-615*. This suggests the involvement of both miRNAs in the development of BMI-related illnesses. In addition, multi-omics analysis proves *FADS2* is a potential biomarker for BMI. This is consistent with the conclusion that a high BMI leads to increased risk of developing RA, even though the biological mechanism responsible has yet to be identified [20, 21]. In addition, a recent study confirms that the *FADS1/2* cluster are significantly associated with fatty acid levels (see Figure 4). This study also reported *FADS1* alongside rs174546, matching our corresponding row in Table 3. Unlike our experiment, however, the mentioned study did not employ dbMTS to generate this match [22]. The conservation of both *FADS1* and *FADS2* in the identified motif (see Figures 6 and 7) further emphasizes the potential importance of these two genes in the association with BMI/development of obesity.

In a recent study, Kuryłowicz and colleagues report that two miRNAs—*hsa-miR-615* and *hsa-miR-330*—and their target genes (of which none are listed in Table 2) are involved in the oncogenesis pathway, otherwise known as the process where healthy cells become cancerous [23]. Shared target miRNAs between a genetic trait (BMI) and cancer suggests that the development of illness as a result of either share similar causal/biological factors. In addition, *hsa-miR-615* was found to be highly expressed in the subcutaneous adipose tissue (SAT) of obese patients after surgery-induced weight loss than the SAT of patients with a normal weight, suggesting that there is a difference in molecular pathways/miRNA expression between losing weight and consistently maintaining a healthy weight [23]. This is consistent with the finding that *hsa-miR-615* facilitates palmitate production which is correlated with a higher likelihood of developing obesity.

In our previous study, we found a statistically significant association between BMI and the PCDHA10 pathway [8]. PCDHA10 is a gene which codes for different protocadherins (Pcdhs), which play an important role in neural generation [24]. As neuronal development and brain structure have been linked to BMI through existing literature, this could be a reason why the PCDHA10 pathway is associated so strongly with BMI [25]. Although obesity is not identified by MIGWAS as a significant trait in our study, it has been reported to have been strongly associated with BMI and has some significant associations with body temperature [4, 26]. For future research, we would like to examine the popularity of variants located in the binding sites of 3'UTR for the miRNA-gene pairs of additional traits to further investigate the underlying biological mechanisms and causal factors of other genetic diseases, traits, and even cancers.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

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