

Asthma MicroRNA Regulome Development Using Validated miRNA-Target Interaction Visualization

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Abstract

Asthma is a common multifactorial complex disease caused by an interaction of genetic and environmental factors. There are no robust biomarkers or molecular diagnostics for asthma or its detailed phenotypic stratification in the clinic. Regulatory and epigenomic factors are priority candidates for asthma biomarker discovery and translational research because this common disease emerges in association with host/environment interactions. In this context, epigenomic molecular events such as microRNA (miRNA) silencing affect asthma susceptibility and severity. We report here an analysis of the miRNAs in the literature, their targets associated with asthma, and present the findings organized as an miRNA-target network, an miRNA regulome of asthma. The miRNA-target interactions in asthma were extracted from the PubMed and the Web of Science databases, while the miRNA-target network was visualized with the Cytoscape tool. Genomic locations of miRNA and target genes were displayed using the Ensembl Whole Genome tool. We cataloged miRNAs associated with asthma and their experimentally validated targets, retrieving 48 miRNAs associated with asthma, and 54 experimentally validated miRNA targets. Four central molecules involved in 34.5% of all interactions were identified in the network. The miRNA-target pairs were constructed as an asthma-associated miRNA-target regulatory network. The network revealed subnetworks pointing toward potential asthma biomarker candidates. The asthma miRNA regulome reported here offers a strong foundation for future translational research and systems medicine applications for asthma diagnostic and therapeutic innovation. Developed protocol for constructing miRNA regulome could now be used for biomarker development in multifactorial diseases.

Keywords: asthma, epigenomics, biomarkers, microRNA, regulome

Introduction

APPROXIMATELY ONE TO EIGHT PERCENT OF THE WORLD'S POPULATION suffer from chronic inflammation in the airways of the lungs, reversible airflow obstruction, and bronchospasm, representing the recurring symptoms of asthma. Other clinical symptoms include wheezing, coughing, chest tightness, and shortness of breath. For a long time, asthma was considered a single disease, but recent studies have begun to highlight its molecular and clinical heterogeneity (McCracken et al., 2017).

Asthma is caused by an interaction of genetic and environmental contributors as with most common complex diseases (Bunyavanich and Schadt, 2015). Studies have defined asthma subgroups by phenotypes, through which lung function, serum IgE antibodies, allergen skin test responses, and other indications are observed. Genetic studies of such de-

fined subgroups provide valuable data on differential gene expression between subgroups. A recent study has used a multiomics approach to prioritize asthma candidate biomarkers apparent in two or more omics levels (Pecak et al., 2018).

Still, no robust biomarkers or molecular diagnostics for asthma or its detailed phenotypic stratification in the clinic exist. As a complex disease, regulatory and epigenomic factors are priority candidates for asthma biomarker discovery and translational research. In this context, epigenomic molecular events such as microRNA (miRNA) silencing affect asthma susceptibility and severity. Studies have reported differentially expressed miRNA levels in patients with asthma, which could potentially be used as biomarkers for discriminations between asthma phenotypes (Rijavec et al., 2014).

Due to asthma's clinical and molecular heterogeneity, it is difficult to make phenotypic stratification. Therefore, biomarkers such as miRNA molecules could be useful for deep

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TABLE 1. THE LIST OF MICRORNA-TARGET PAIRS ASSOCIATED WITH ASTHMA IN PUBLISHED LITERATURE

<i>miRNA name (used in article)</i>	<i>miRNA HGNC symbol</i>	<i>miRNA expression; upregulated (↑)/ downregulated (↓)</i>	<i>Target gene (synonym)</i>	<i>Sample type</i>	<i>References</i>
hsa-let-7a	MIRLET7A1 MIRLET7A2 MIRLET7A3	↓	IL13 (IL-13)	Mouse model or cells	Kumar et al. (2011)
hsa-let-7b	MIRLET7B				
hsa-let-7c	MIRLET7C				
hsa-let-7d	MIRLET7D				
hsa-let-7e	MIRLET7E				
hsa-let-7f	MIRLET7F1 MIRLET7F2				
hsa-let-7g	MIRLET7G				
hsa-let-7i	MIRLET7I				
miR-9	MIR9-1 MIR9-2 MIR9-3	↑	PPP2R5D	Mouse model or cells	Li et al. (2015)
miR-15a	MIR15A	↓	VEGFA	Human samples	Nakano et al. (2013)
miR-17	MIR17	↑	CREB1 CRTC1 CRTC2 CRTC3	Mouse model or cells	Bartel et al. (2017)
miR-18a	MIR18A	↓	SMAD2	Human samples	Martinez-Nunez et al. (2014)
miR-19a	MIR19A	↑	TGFBR2	Human samples	Haj-Salem et al. (2015)
miR-21	MIR21	↑	PTEN IL12A (IL-12A)	Human samples Mouse model or cells	Simpson et al. (2014) Lu et al. (2009a)
			PDCD4 CCL26 POSTN CREB1 CRTC1 CRTC2 CRTC3	Mouse model or cells Human samples Mouse model or cells	Sheedy et al. (2010) ^a Lu et al. (2012) Bartel et al. (2017)
miR-22-3p	MIR22	↓	AKT1 CBL PPARGC1B ESR1	Mouse model or cells Human samples	Kim et al. (2017) Dong et al. (2016)
miR-26a	MIR26A1 MIR26A2	↑	GSK3B	Human cell cultures	Mohamed et al. (2010)
miR-27a	MIR27A	↓	SMAD2	Human samples	Martinez-Nunez et al. (2014)
miR-34	MIR34C	↓	IGFBP3	Human samples	Yin et al. (2017)
miR-125b	MIR125B1 MIR125B2	↓	SPDEF	Human samples	Liu et al. (2016)
miR-126	MIR126	↑	POU2AF1	Mouse model or cells	Mattes et al. (2009)
miR-128	MIR128-1 MIR128-2	↓	SMAD2	Human samples	Martinez-Nunez et al. (2014)
miR-133a-3p	MIR133A1 MIR133A2	/	RHOA	Human cell cultures and mouse model	Chiba et al. (2017)
miR-140-3p	MIR140	/	CD38	Human cell cultures	Jude et al. (2012)
miR-142-3p	MIR142	↑	LRRC32	Human cell cultures	Zhou et al. (2013)
miR-143	MIR143	↓	IL13RA1 NFATc1	Human samples Human samples	Teng et al. (2015) Cheng et al. (2016)
miR-144	MIR144	↑	CREB1 CRTC1 CRTC2 CRTC3	Mouse model or cells	Bartel et al. (2017)
miR-145	MIR145	↑	RUNX3 KLF4	Human samples Human cell cultures	Fan et al. (2016) Liu et al. (2015)
miR-146a	MIR146A	/	STAT1 IRAK1 TRAF6 PTGS2	Mouse model or cells Human cell cultures Human cell cultures	Lu et al. (2010) Taganov et al. (2006)
				Human cell cultures	Comer et al. (2014)

(continued)

TABLE 1. (CONTINUED)

<i>miRNA name (used in article)</i>	<i>miRNA HGNC symbol</i>	<i>miRNA expression; upregulated (↑)/ downregulated (↓)</i>	<i>Target gene (synonym)</i>	<i>Sample type</i>	<i>References</i>
miR-146b	MIR146B	/	STAT1 IRAK1 TRAF6	Mouse model or cells Human cell cultures	Lu et al. (2010) Taganov et al. (2006)
miR-148a	MIR148A	/	HLA-G	Human samples	Nicodemus-Johnson et al. (2013)
miR-148b	MIR148B	/			
miR-152	MIR152	/			
hsa-mir-155	MIR155	↑	SP11 IFNG (IFN-γ) CTLA4 IL13RA1	Mouse model or cells Mouse model or cells Human patients Human cell culture	Vigorito et al. (2007) Banerjee et al. (2010) Sonkoly et al. (2010) Martinez-Nunez et al. (2011)
			SMAD2	Human cell culture	Martinez-Nunez et al. (2014)
			SOCS1 INPP5D MAF SPP1	Mouse model or cells Mouse model or cells Mouse model or cells Human samples	Lu et al. (2009b) O'Connell et al. (2009) Rodriguez et al. (2007) Huo et al. (2016)
miR-181b-5p	MIR181B1 MIR181B2	↓	AQP4	Human samples	Jardim et al. (2012)
miR-203	MIR203A	↓	SPRED2	Human samples	Liu et al. (2012)
miR-221	MIR221	↑	CDKN1B KIT	Human cell cultures	Davis et al. (2009) ^a
miR-222	MIR222	/	CDKN1A CDKN1B KIT	Human samples Human cell cultures	Perry et al. (2014) Davis et al. (2009) ^a
miR-223	MIR223	↑	IGF1R PARP1 MEF2C	Mouse model or cells Human samples Mouse model or cells	Lu et al. (2013) Maes et al. (2016) Johnnidis et al. (2008)
hsa-miR-296-5p	MIR296	/	IKBKE	Human samples	Davis et al. (2017)
miR-323-3p	MIR323A	↑	SMAD2 SMAD3	Human samples	Kärner et al. (2017)
miR-328	MIR328	↑	TGFB2	Human samples	Narožna et al. (2017)
miR-449	MIR449B	↓	IGFBP3	Human samples	Yin et al. (2017)
miR-485-3p	MIR485	↓	SPRED2	Human samples	Liu et al. (2012)
miR-570-3p	MIR570	↓	ELAVL1	Human samples	Roff et al. (2014)
miR-513a-5p	MIR513A1	↓	CBL PPARGC1B	Human samples	Dong et al. (2016)
miR-625-5p	MIR625	↓	ESR1		
miR-708	MIR708	↑	CD38	Human samples	Dileepan et al. (2014)
miR-1248	MIR1248	↑	IL5 (IL-5)	Human samples	Panganiban et al. (2012)

^aReference not mentioning miRNA regulation.
HGNC, HUGO Gene Nomenclature Committee.

phenotyping. Differentially regulated (dysregulated) miRNAs could hold future potential asthma diagnostics and/or as biomarkers to discriminate between different asthma phenotypes (Milger et al., 2017). Furthermore, miRNAs are regarded as therapeutic targets, offering the possibility for development of miRNA-based medicines for asthma-related inflammation and other symptoms (Greene and Gaughan, 2013). We report here a study of the miRNAs in the literature, their targets associated with asthma, and present the findings organized as an miRNA target network, an miRNA regulome of asthma.

Materials and Methods

To retrieve asthma-associated miRNAs and their targets, we searched four online databases, specialized for miRNA research: Human miRNA Disease Database (HMDD, www

.cuilab.cn/hmdd) (Li et al., 2014), miR2Disease (<http://watson.compbio.iupui.edu:8080/miR2Disease/index.jsp>), miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/index.php>) (Chou et al., 2016), and miRdSNP (<http://mirdsnpp.ccr.buffalo.edu/search.php>).

Publications were retrieved from literature databases Web of Science and PubMed, using keywords such as asthma, human, miRNA, target, and epigenetics. The search included original and review articles. Time span of the publication search was from January 2005 to April 2018. The obtained data were organized in a table that included asthma-related miRNAs and their targets in separate columns. HUGO Gene Nomenclature Committee (HGNC) names for miRNA genes were retrieved from miRBase (www.mirbase.org). Symbols indicating up- or downregulation of miRNA were added. A visual representation of the network

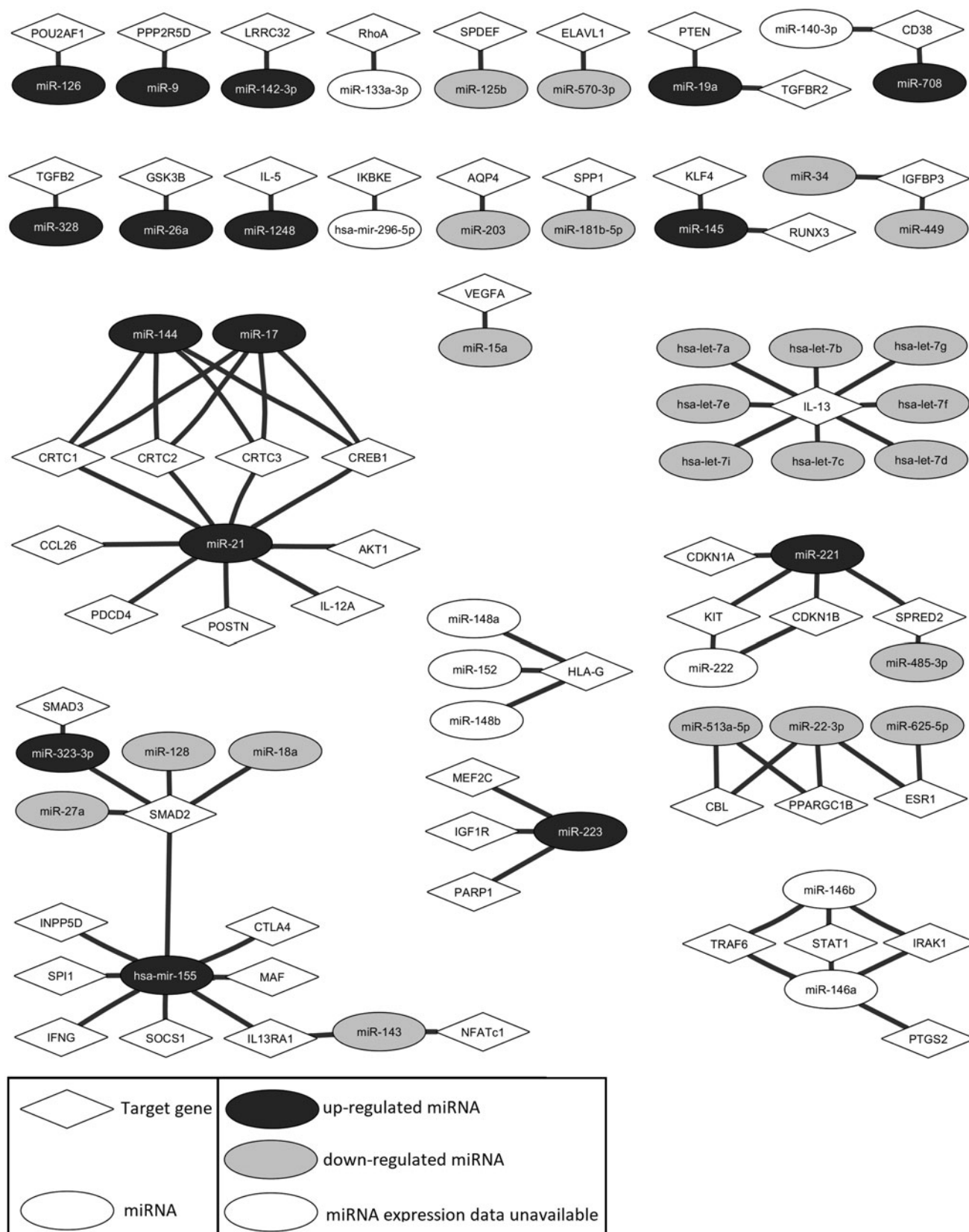


FIG. 1. Presentation of a network of miRNA-target interaction pairs and subnetworks, associated with asthma. Rhombus shapes represent miRNA target genes, while circles represent miRNA, with miRNA expression in asthma visualized where available. White circles represent where miRNA expression data were unavailable, black circles represent upregulated miRNA, and gray circles downregulated miRNA. miRNA, microRNA.

of miRNA-target interactions (MTIs) was made using the Cytoscape tool (www.cytoscape.org) (Shannon et al., 2003).

In addition, a visual representation of all genomic locations of obtained genes was constructed using Ensembl Whole Genome tool (Ensembl, release 92) (www.ensembl.org/Homo_sapiens/Location/Genome) (Aken et al., 2016). The distances between the closest gene pairs were calculated based on genomic locations according to Ensembl, release 92. ConsensusPathDB (CPDB) (<http://cpdb.molgen.mpg.de>) (Kamburov et al., 2011) was used to analyze common pathways for obtained central molecules.

Results

We extracted 48 miRNAs associated with asthma and 54 experimentally validated miRNA targets from four miRNA databases and 48 scientific publications. In addition, some miRNAs were also reported to be either up- or down-regulated. The results are presented in Table 1, showing the miRNA name, miRNA gene HGNC symbol, symbol for up- or downregulation of miRNA, target name, and the references. The integrated data revealed that several miRNAs were paired only with one target, while some were paired with several targets. Majority of studies were performed on human samples, for example (Maes et al., 2016), some studies used murine models, (Kim et al., 2017) or both, murine models and human cell cultures (Bartel et al., 2017).

Cataloged miRNA-target pairs were used to construct a network of asthma-associated miRNA-target pairs. The graphical representation of MTIs and miRNA expression in asthma, where available, is shown in Figure 1. Inside the MTI network,

eight subnetworks, which included at least four interacting partners, have been identified. Of these eight subnetworks, four subnetworks included seven or more interacting partners. The largest subnetwork includes 16 interacting partners: six miRNAs and ten targets. The network includes a total of 87 MTIs, with two miRNAs, miR-155 and miR-21, and two targets, interleukin-13 (IL-13) and SMAD2, together being directly involved with 34.5% (30/87) of all interactions. miR-21 and miR-155 also directly affect 31.5% (17/54) of all targets.

The highest number of targets has been reported for miR-21; it has nine validated targets. miR-155 has eight validated targets. Other miRNAs with multiple targets are as follows: miR-17, miR-144, miR-146a, and miR-221 with four targets. Targets with the highest number of validated miRNA interactions are IL-13 and SMAD2. Eight miRNAs have been reported to target IL-13 and five miRNAs target SMAD2. CAMP responsive element binding protein 1 (CREB1), CREB regulated transcription coactivator 1–3 (CRTC1-3), and human leukocyte antigen G (HLA-G) have been each reported to be targeted by three miRNAs. Our results revealed that IL-13, SMAD2, miR-21, and miR-155 are central molecules in the regulome, having the highest number of interactions.

Asthma critical regions were determined using two approaches. First, we determined genomic distances between the gene pairs less than 1 Mb apart, with the genomic locations of miRNA and target genes shown in Figure 2. The closest 17 pairs are presented in Table 2. Six pairs are less than 1 kb apart. Six of the genes are found on chromosome 5, and four genes on chromosomes 11 and 14. And second, by determining regions consisting of at least three genes located less than 1 Mb apart. Two genomic hotspots with three

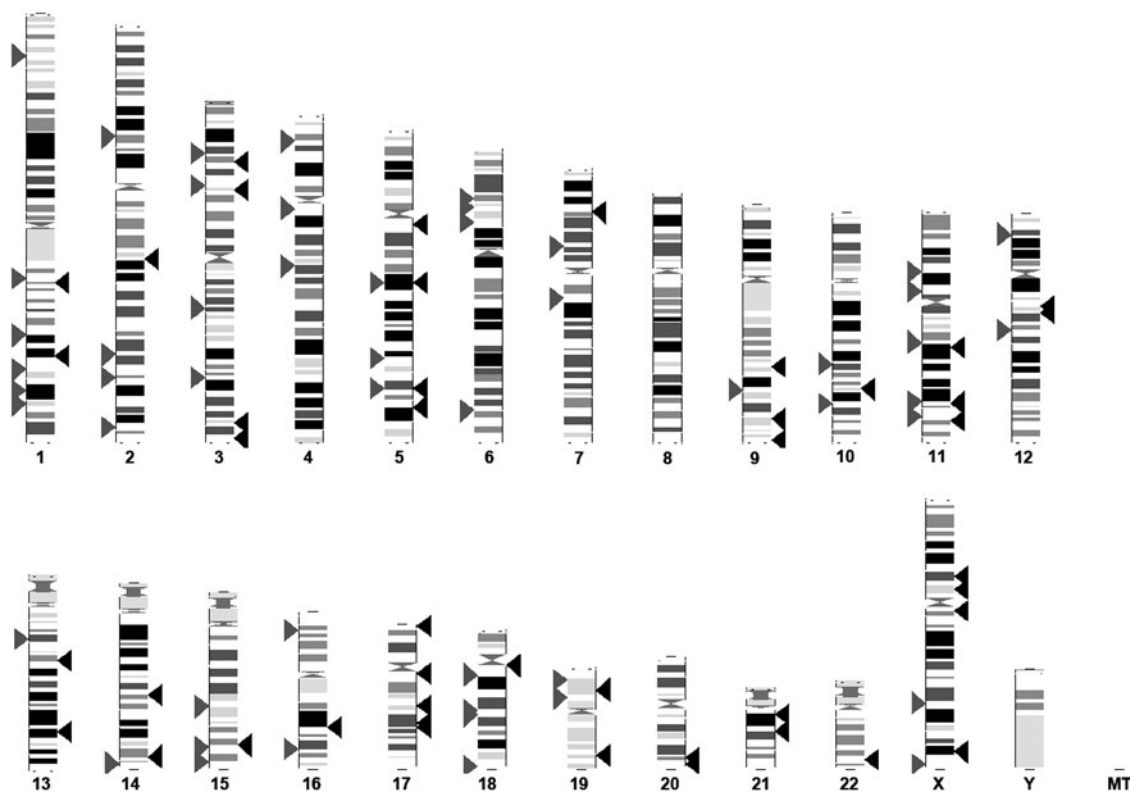


FIG. 2. Genomic locations of miRNA and target genes associated with asthma risk. *Black arrows on the right* represent the genomic locations of miRNA genes and *gray arrows on the left* represent the genomic locations of the miRNA target genes.

TABLE 2. GENOMIC DISTANCES BETWEEN THE CLOSEST GENE PAIRS, ASSOCIATED WITH ASTHMA

<i>Gene pairs</i>	<i>Distance between gene pairs (bp)</i>	<i>Chromosome number</i>
MIR17, MIR18A	63	13
MIR18A, MIR19A	70	13
MIR17, MIR19A	203	13
MIRLET7A1, MIRLET7F1	311	9
MIR221, MIR222	749	X
MIRLET7A3, MIRLET7B	864	22
MIR143, MIR145	1623	5
MIRLET7F1, MIRLET7D	2401	9
MIRLET7A1, MIRLET7D	2798	9
MIR323A, MIR485	29,602	14
MIR125B1, MIRLET7A2	46,678	11
MIR9-2, MEF2C	50,178	5
MIRLET7C, MIR125B2	50,326	21
POU2AF1, MIR34C	57,809	11
IL5, IL13	99,425	5
AKT1, MIR203A	651,835	14
PPP2R5D, VEGFA	757,842	6

genes closer than 0.7 Mb were identified and are presented in Table 3.

Discussion

The connection between miRNAs and asthma is emerging as an important study field. So far, several miRNAs have been identified that positively or negatively affect the progression of allergic inflammation and asthma and many miRNA targets have been found that play a crucial role in the development and expression of asthma (Lu and Rothberg, 2013). Differential regulation of MTIs can affect asthma in different ways. While downregulation of certain protein coding genes by miRNA can be associated with decreased airway remodeling (Yin et al., 2017) or regulation of proinflammatory cytokine expression (Huo et al., 2016), upregulation of others can have implications for expression of numerous other inflammatory mediators (Roff et al., 2014).

While the reliability of MTIs associated with asthma or other diseases can be divided into two groups, strong and less strong, depending on the methodology used in the study (Piletič and Kunej, 2017), it is important to note that studies use various definitions of the connection between genes and asthma. Studies describe genes as being relevant to, associated with, likely to be involved in contributing or having a role in asthma, the pathogenesis of asthma, or the asthmatic disease process (Bartel et al., 2017; Davis et al., 2017; Dileepan et al., 2014; Greene and Gaughan, 2013). A new way of describing the connection of genes to asthma should

be introduced, which would be based on level of validation or the evidence level, consequently showing more clearly if those genes are relevant to different underlying disease processes related to asthma.

The developed miRNA-target network presents just a small part of a more extensive system of interconnected miRNAs and targets, all associated with asthma, which has to be revealed in the future studies. Additional miRNAs and targets need to be found, so the regulome can become more robust and further increase its value for researchers. Recently, a study has presented a larger miRNA regulome, reviewing genetic variability sorted according to gene function and genetic variation type (Hrovatin and Kunej, 2018).

Moreover, a recent study has used a multiomics approach to expose several asthma candidate biomarkers apparent in two or more omics levels (Pecak et al., 2018). Combining the presented miRNA network with a larger miRNA regulome in addition to using new multiomics approaches could enable higher efficiency and significantly faster advances in potential biomarker discovery in the future.

Several miRNAs affect many different targets and many targets are affected by different miRNAs. Networks and pathways associated with miRNAs and their targets have been constructed previously, mostly associated with a single GWAS study (Bulik-Sullivan et al., 2013). Our study presents the most comprehensive miRNA regulome associated with asthma, because our data set synthesizes information from several publications and databases displaying validated interconnections of miRNAs and their targets. While several other miRNAs have been linked to asthma development and pathogenesis, only those with experimentally linked targets associated with asthma, are shown here.

Including new data to the current data set and additional experimental validation could enable to create a larger miRNA regulome, exposing the most important miRNAs and targets with the highest effect on asthma development. This miRNA regulome could then be used for additional research, for example, for prediction of novel MTIs and setting novel hypotheses. Furthermore, data analyzed in this study have a potential to assist in the identification of a disease module subnetwork, consisting of MTIs.

Developed miRNA regulome displays interconnectedness of various molecules with diverse metabolic roles. Our results show that IL-13, SMAD2, miR-21, and miR-155 are central molecules within the generated asthma regulome, involving many interacting partners. An analysis using the CPDB tool did not reveal a common pathway for all four central molecules. However, those central molecules in the regulome are important for asthma pathogenesis, since they are involved in critical mechanisms leading to the disease, specifically Th2 inflammation (IL-13, miR-21) and airway remodeling (SMAD2, miR-155). Furthermore, a recent study has shown there is evidence of mutual regulation between miR-21 and the TGF β /Smad signaling pathway, a pathway involving SMAD2, in human airway remodeling (Yu et al., 2018).

Much research has been done in the search for asthma susceptibility loci, with the 17q21 locus being repeatedly associated with risk for childhood development of asthma in the past (Kavalar et al., 2011; Ober and Yao, 2011; Žavbi et al., 2016). Another region linked with asthma is 21q21 (Ober et al., 1998). Interestingly, 21q21 is the genomic

TABLE 3. ASTHMA CRITICAL REGIONS CONSISTING OF AT LEAST THREE GENES LOCATED <1 Mb

<i>Genes</i>	<i>Genomic distance, first to last (bp)</i>	<i>Locus</i>
MIR17, MIR18A, MIR19A	203	13q31.3
MIRLET7A1, MIRLET7F1, MIRLET7D	2798	9q22.32

location for miR-155, suggesting further importance of this molecule, central in our regulome. Another central molecule was shown to be miR-21, which plays a key role in the immune system, and its dysregulation is suggested to promote disease pathogenesis (Sheedy, 2015). Most miR-21 MTIs shown here come from findings made in murine models. Their role in human asthma is, however, still unclear, showing the need for more studies with human asthma patients.

The present data synthesis and developed miRNA regulome, in combination with identification of further components of this network in future studies, will enable the testing of more targeted hypotheses and help future researchers select miRNA-target pairs, such as the central molecules, for wet laboratory experiments in different asthma phenotypes. Furthermore, the central molecules also present promising candidate loci for laboratory trials and clinical studies. Such research could provide a novel network-based biomarker cluster for diagnosis or treatment of asthma.

Additional studies of miRNA associated with asthma can lead to the discovery of new loci connected with risk for asthma. Locations where several genes are clustered nearby are potentially critical regions for asthma. The genomic locations of the genes associated with asthma obtained by this study have been investigated, showing several genes less than 0.7 Mb apart from each other. These regions present new critical regions for asthma and therefore also interesting candidates for further study.

Conclusions

The results of the present study show several central molecules within a subnetwork involving many interacting partners. Developed miRNA regulome displays interconnectedness of various molecules with diverse metabolic roles. The central molecules, IL-13, SMAD2, miR-21, and miR-155, present promising candidate loci for laboratory trials and clinical studies. Such research could provide a novel network-based biomarker cluster for diagnosis or treatment of asthma.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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Abbreviations Used

CPDB = ConsensusPathDB

CREB1 = CAMP responsive element binding protein

CRTC1-3 = CREB regulated transcription coactivator 1–3

HGNC = HUGO Gene Nomenclature Committee

HLA-G = human leukocyte antigen G

IL-13 = interleukin-13

miRNA = microRNA