1. INTRODUCTION

1.1 Cardiovascular disease

Cardiovascular disease includes all heart conditions where there is presence of structural problems, blood clots or diseased vessels. Also known as Coronary Heart Disease (CHD), Cardiovascular disease manifests itself in human beings in three major forms, namely- myocardial infarction, sudden cardiac death and angina pectoris (stable and unstable forms). Cardiovascular disease is the number one cause of death globally, according to WHO, as of May 2017. This indicates that more people die annually from Cardiovascular disease than any other disease (World Health Organization, 2017).

In 2015, an estimated 17.7 million people died from Cardiovascular disease representing 31% of total global deaths. Of these deaths, 7.4 million were due to Coronary Heart Disease (CHD) specifically (Mendis S, 2011).

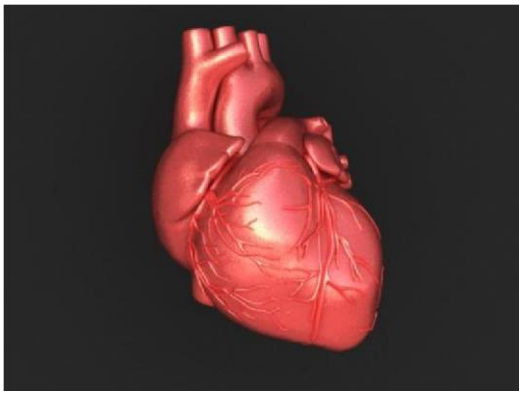


Figure 1.1: Human Heart

1.1.1 Myocardial Infarction

Myocardial Infarction is one of the first manifestations of Cardiovascular disease. Commonly referred to as heart attack, Myocardial Infarction occurs when blood supply to the heart muscle is abruptly cut off due to blockage in one or more arteries. This leads to either temporary or permanent damage of the heart tissue.

In India, more than 10 million cases of Myocardial Infarction are registered every year. The global mortality rate of Myocardial Infarction is 34% to 42% (Aso S, 2011).

1.1.2 Biomarkers as diagnostic tools for Myocardial Infarction

Biomarkers are biological molecules whose presence or absence can identify a particular process or disease. When blood levels of specific biomarkers are increased or decreased, myocardial injury can be detected. Such biomarkers for Myocardial Infarction can be identified by routine monitoring of cardiac biomolecules in high-risk patients and comparing the observed data with data from a healthy individual. Cardiac specific biomolecules and their expression have been identified such as proteins and genes (coding molecules) or RNA (non-coding molecules). This study focusses on discovering the role of these molecules as potential biomarkers for Myocardial Infarction.

1.2 Non-coding molecules

The central dogma of molecular biology describes the relationship between DNA, RNA and protein. A small fraction of the DNA is transcribed and translated to give proteins. The larger fraction is termed as non-coding DNA, although some non-coding DNA transcribe to produce functional non-coding RNA molecules.

RNA that is transcribed from DNA but not further translated into proteins is called noncoding RNA. The abundant and functionally important types of non-coding RNA include transfer RNA (tRNA), ribosomal RNA (rRNA) and small RNA such as microRNA(miRNA), small interfering RNA (siRNA), piwi interacting RNA (piRNA), small nuclear RNA (snRNA), and long non-coding RNA (lncRNA). Typically, short non-coding RNAs are less than 30 nucleotides in length whereas long non-coding RNAs are greater than 200 nucleotides in length. The various types of non-coding RNA have been schematically represented in Figure 1.2

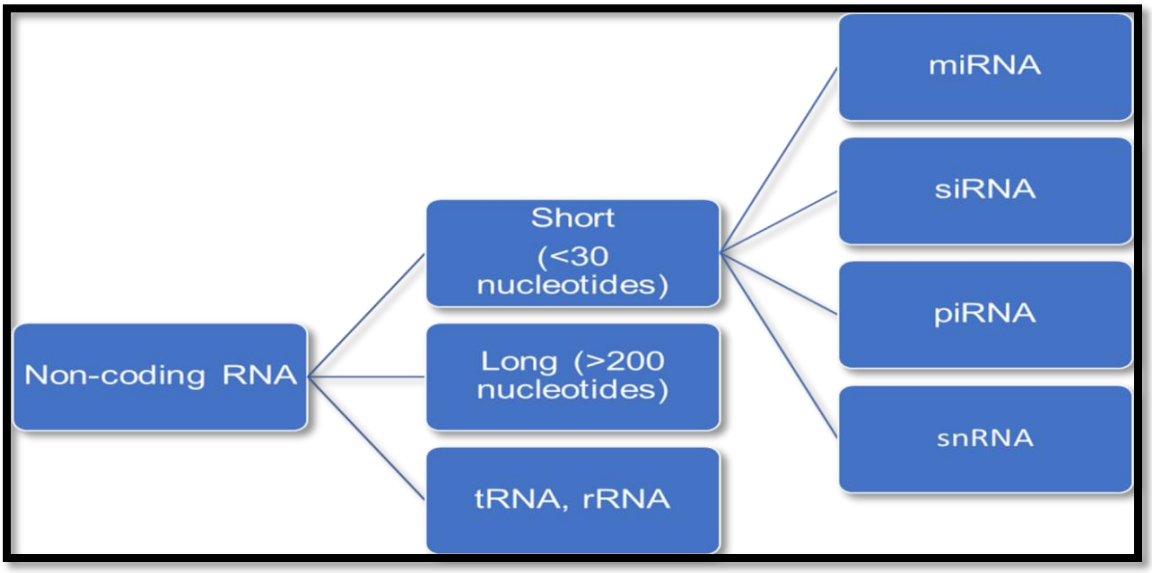


Figure 1.2: Schematic representation of classification of non-coding RNA

1.2.1 Importance of non-coding RNA in Myocardial Infarction

Regulation of gene expression is the main function of most non-coding RNA. Through survey of literature it was found that miRNAs and other non-coding RNA molecules are widely studied for their modulation of several pathological aspects of Myocardial Infarction. Mass scale data of non-coding RNAs related to Myocardial Infarction have been generated (Gomes CPC, 2017). Cardiac-specific miRNAs (miR-208, miR-499, and miR-1) and stress-related miRNA (miR-21) are found to be highly expressed in Myocardial Infarction.

Cumulative analysis of such non-coding RNA along with other coding molecules will result in a set of key biomolecules associated with Myocardial Infarction. Further analysis will provide potential biomarkers for the disease.

1.3 PubMed

The National Center for Biotechnology Information (NCBI) advances science and health by providing access to biomedical and genomic information. PubMed is a free resource that is developed and maintained by the National Center for Biotechnology Information (NCBI), at the U.S. National Library of Medicine (NLM), located at the National Institutes of Health (NIH). The database is maintained as part of the Entrez system of information retrieval. PubMed accesses more than 28 million citations for biomedical literature from MEDLINE, life science journals, and online books (PubMed, 1996).

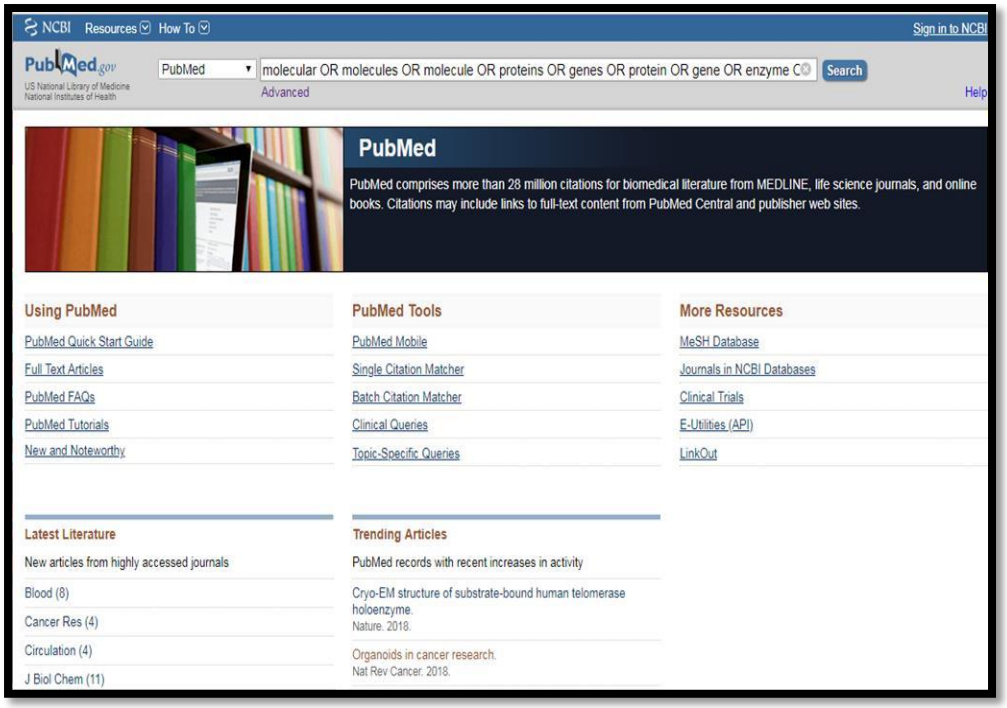


Figure 1.3: Snapshot of PubMed web page

1.3.1 History of PubMed

PubMed was first released in January 1996 as an experimental database under the Entrez retrieval system with full access to MEDLINE. The use of PubMed has grown exponentially since its introduction. PubMed searches numbered approximately 2 million for the month of June 1997, while current usage typically exceeds 3.5 million searches per day.

1.3.2 Utilization of PubMed

PubMed offers a wide range of information which often makes it challenging for its users to quickly identify data relevant to their individual needs. Standard literature search is enhanced by suggesting specific queries to help find the bibliographic information about a specific article, or publication pertinent to a specific topic (e.g. a disease). Search words are typed into the search box connected with Boolean connectors ‘AND’, ‘OR’ or ‘NOT’. Display settings can be changed according to ‘format’,’ no. per page’ and ‘sort by’ required. Additional filters can be added along with basic filters. Selected information can be saved by using ‘send to’ option on the top righthand side. Email alerts can be received with new articles that have been published on a particular topic or from a particular journal.

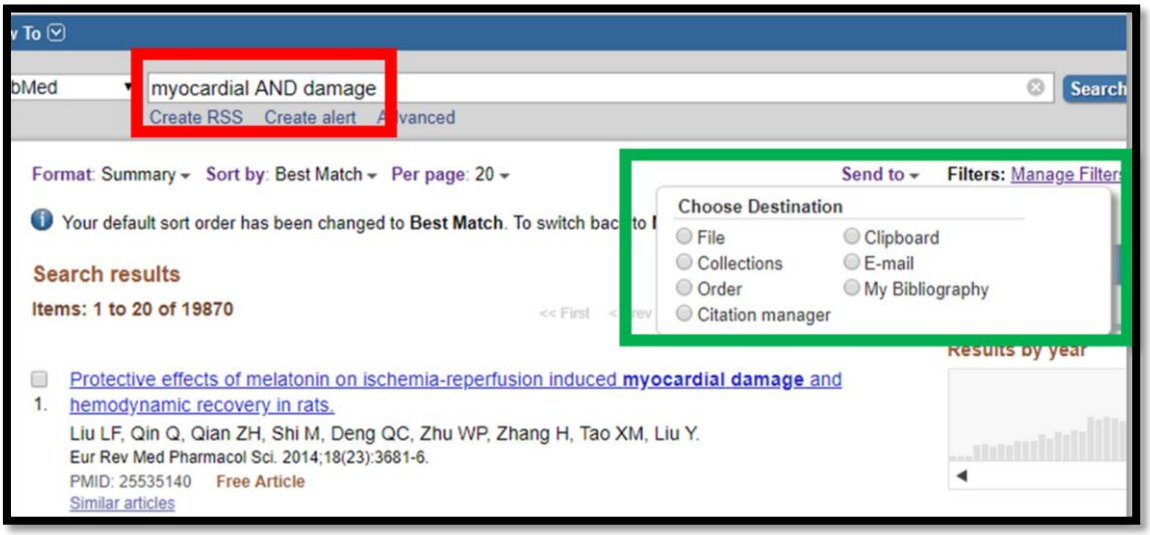


Figure 1.4: Snapshot of PubMed page showing the use of 'Boolean operator' in red and the use of 'send to' option in green

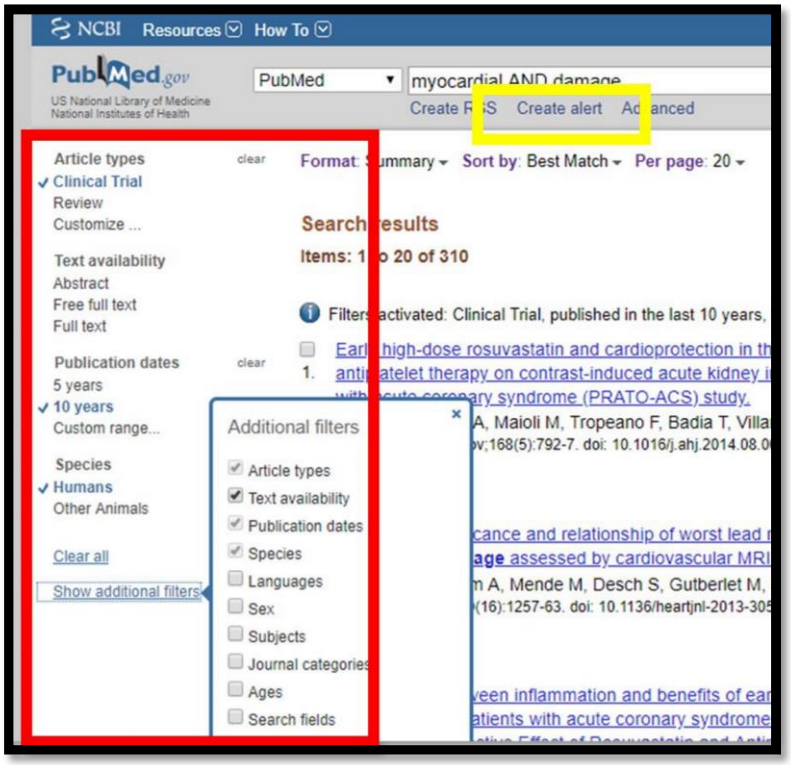


Figure 1.5: Snapshot of PubMed web page showing 'create alert' option in yellow and 'filter application' in red

1.4 Biocuration

The exponential growth in the amount of biological data requires large scale data management, analysis and accessibility. Biocuration is the activity of organizing, representing and making biological information accessible to both humans and computers. Extracting, tagging with controlled vocabularies, and representing data from the literature, are some of the most important and time-consuming tasks in biocuration.

Curated information from literature serves as the gold-standard data set for computational analysis, quality assessment of high-throughput data and benchmarking of data-mining. It has become an essential part of biological discovery and biomedical research.

1.4.1 Role of biocurators in biological and biomedical research

Biocurators extract knowledge from literature, typically by reading full text articles and transferring the essence into a database. They must connect information from various sources in a coherent and comprehensible way. Biocurators also focus on correction of inconsistencies and errors in data representation and help compile data from individual research publications to provide useful and meaningful pathbreaking results.

2. REVIEW OF LITERATURE

2.1 Historical papers on MI

Previous papers suggested that conditions such as obesity, Diabetes mellitus and syphilis increased the incidence of Myocardial infarction in both the sexes (Smith FJ, Keyes JW, et al., 1950). There were studies that also suggested that blood volume is an effective biomarker in Myocardial infarction (Agress CM, 1950). Agress stated that in general, patients with a clear-cut picture of peripheral circulatory failure had a reduced blood volume, in contrast to the expanded blood volumes of patients with congestive heart failure. In several instances there was a reduction of the blood volume during the shock and later expansion during the failure phase. Hamer in 2002 published that Evan's Blue Dye, is an azo dye that binds quantitatively with serum albumin, has the potential to be a useful vital stain of myofiber permeability in other models of skeletal muscle injury and membrane-associated fragility, therefore Evan's Blue Dye is used to identify the onset of muscle damage (Hamer P, 2002).

Lactate dehydrogenase is released when cells die, therefore, it is used as a biomarker for Myocardial infarction (Wilson HT, 1958). In 1954 and 1955 Hill and Levi and other investigators reported the serum content of the enzyme lactic acid dehydrogenase (LDH) to be elevated in neoplastic diseases. They studied 51 patients with cancer. This enzyme was also shown to be increased in the serum of patients with Myocardial infarction (ULMER DD, 1956). Schott studied high T waves as an early transient sign in Myocardial infarction. The unusually high T waves recorded seven hours after the attack of occlusion represent an early and transient stage in the ECG findings, preceding that of displacement of the R-T (S-T) intervals (Schott A, 1949).

In 1950, Charles stated that anticoagulants can be used to treat Myocardial infarction, the use of the anticoagulants in the treatment of certain types of heart disease is based upon the frequent occurrence of thromboembolic complications and upon the importance of these complications in increasing the morbidity and mortality rate (Marple CD, 1950).

2.2 ncRNA in other diseases

Several of the intrinsic properties of ncRNAs suggest that the non-coding transcriptome can be a useful source of disease and stage-specific biomarkers. For example, there are many more ncRNAs than mRNAs, hence the chance of finding a specific marker is higher. Furthermore,

ncRNAs are the final gene product, and thus biologically relevant levels are measured. The highly specific tissue and/or disease expression of ncRNA can provide the high discriminative power required for a successful biomarker. ncRNAs, and RNAs in general, have uniform biochemical properties that make it easier to manufacture clinical assays. Finally, ncRNAs can be detected in body fluids, enabling the development of minimally invasive "liquid biopsy" assays (Majem B, 2015).

The very first PCa-associated ncRNA to be discovered was *PCA3* (a.k.a. *DD3, PCAT3*), a lncRNA identified in 1999 via differential display analysis (Bussemakers MJ, van Bokhoven A, et al., 1999). *PCA3* is specifically expressed in prostate epithelial cells, and—compared with benign tissue-*PCA3* is highly overexpressed in PCa and high-grade prostatic intra-epithelial neoplasia (Popa I, Fradet Y, et al., 2007). *PCA3* is an antisense intronic lncRNA located in the tumour-suppressive protein-coding gene *PRUNE2*. Recently it was proposed that *PCA3* controls *PRUNE2* mRNA levels via the formation of a *PRUNE2/PCA3* double-stranded RNA that undergoes adenosine deaminase, RNA specific (ADAR)-mediated adenosine-to-inosine RNA editing. PCa-associated transcript 1 (non-protein coding) (*PCAT1*) is a prostate-specific lncRNA that is upregulated in high-grade PCa (Gleason score ≥7), metastatic disease, and castration-resistant PCa (CRPC). With respect to prognostic value, *PCAT1* has a favourable expression pattern compared with *PCA3.*

There have been many reports on the aberrant over-expression or downregulation of miRNAs in various cancer tissues and subsequent alternations in the expression of their target genes, which are involved in proliferation and malignant transformation. In addition, miRNA expression is tissue specific (Liang Y, 2007). and alteration in specific miRNAs have been associated with cancer development. Consequently, an altered miRNA expression profile could be a biomarker of malignant tumors, as well as an attractive therapeutic target in cancer. Wang analyzed the expression of miRNAs in the plasma of patients with pancreatic ductal adenocarcinoma and have identified miR-21, miR-210, miR155, and miR-196a, which have been reported to be upregulated in pancreatic cancer tissue and cell lines, as candidate biomarkers. Similarly, miR-200a/b, miR-18a, miR-221, and miR196a/b has been found to be upregulated in the serum/plasma in parallel with cancer tissues (Kishikawa T, 2015)

Different groups have studied the miRNA composition of whole saliva, including the cell content, cell debris and bacteria present in oral cavity. Patel *et al* described miR-223, miR-191, miR-16, miR-203, and miR-24 as the five most abundantly expressed miRNAs; they have also been reported in other studies. Park *et al.*, by comparing both cell free saliva and whole

saliva from 12 healthy donors and a cohort of 50 cancer patients, found that miR-125a and miR-200a were differentially expressed in patients with oral cancer. In 2011, pursuing the same disease, Wiklund *et al.* described a panel of miRNA and DNA methylation patterns. The panel, consisting of aberrant miR-375 and miR-200a expression and miR-200c-141 methylation, was initially found in oral squamous cell carcinoma (OSCC) tissues and then validated in oral rinse and saliva from OSCC patients and healthy controls, suggesting a potential clinical application for OSCC diagnosis. Later on, in 2012, Liu CJ *et al.* described miR-31 as a clinical biomarker of OSCC in oral lesions, plasma, and saliva. They found miR-31 significantly increased in saliva from patients with oral carcinoma at all clinical stages, including very small tumors. When comparing miR-31 expression in different body fluids, they found that miR-31 was more abundant in saliva than in plasma, suggesting that salivary miR-31 was a more sensitive biomarker for oral malignancy.

The development of gastrointestinal cancer is a complex process, although several serum tumor markers such as carcinoembryonic antigen (CEA) and CA19.9 are recommended for clinical applications, their low sensitivity and specificity remain a severe challenge for clinicians. Recent observations demonstrate that lncRNAs are aberrantly expressed in gastrointestinal cancer and have roles in tumorigenesis. Among the most prominent deregulated lncRNAs in gastrointestinal cancer are HOTAIR, MALAT1, and H19, which are upregulated in various cancers and are involved in migration, invasion, metastasis, dissemination and are associated with a more advanced tumor, node, metastasis (TNM) stage. For example, high HOTAIR expression in cancerous tissues closely correlates with poor prognosis in gastrointestinal cancer. Surprisingly, for colorectal cancer (CRC) patients, plasma levels of HOTAIR were significantly higher than those in healthy controls, and high levels of HOTAIR were associated with an unfavorable prognosis. This indicated that the HOTAIR plasma level could act as a new biomarker for CRC patients (Svoboda et al 2014). In gastrointestinal cancer, circulating H19 in plasma was also higher than in healthy controls, and after surgery, circulating H19 was reduced. In gastrointestinal cancer tissues, lncRNA AA174084 expression was found to be downregulated compared to expression in the paired normal tissues, although the AA174084 plasma level was positively associated with invasion and metastasis. Plasma levels of AA174084 dropped after surgical treatment, and A174084 levels in gastric juice were significantly higher in GC patients. These observations provide evidence that A174084 could be a potential biomarker for early diagnosis in gastrointestinal cancer.

2.3 Biomarkers in MI

Biomarker is a naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified. Various biomarkers were discovered for Myocardial infarction, most of them being coding molecules like proteins. Ideally, biomarkers affect therapy by identifying patients likely to benefit from a therapeutic intervention or in whom a more aggressive diagnostic strategy should be undertaken. In the setting of cardiovascular disease (CVD), biomarkers, particularly cardiac troponins (cTn) have become an integral part of diagnostic strategies. Other biomarkers alone or in combination have been shown to identify patients at high risk for subsequent adverse outcomes but have not been shown to predict a response to a particular therapeutic strategy.

Traditionally, creatinine kinase (CK), lactate dehydrogenase, and aspartate aminotransferase were used to evaluate for myocardial damage among patients presenting with chest pain. While sensitive for detecting cellular death, these biomarkers lacked specificity for myocardial injury. Later, the myocardial band (MB) isoform of CK (CK-MB) and serum myoglobin levels were utilized to improve specificity for cardiac damage and reduce the time of diagnosis. In the last two decades, the measurement of serum troponin level has become the predominant biomarker for the detection of myocardial necrosis. Brain natriuretic peptide (BNP) is a natriuretic hormone produced by myocytes and was initially identified in the brain. A prohormone is released in response to increased wall tension or myocardial stretch. Cleavage of the prohormone in circulation releases BNP and the more stable N-terminal fragment (NTproBNP). Plasma concentrations of both of these hormones are elevated in patients with left ventricular dysfunction (systolic or diastolic) and are frequently used to aid in the diagnosis of clinical heart failure (Maisel et al 2003). The measurement of NT-proBNP and BNP levels have become commonplace in the setting of heart failure. It has become evident, however, that NT-proBNP and BNP levels are also elevated in a variety of other situations associated with myocardial dysfunction, such as pulmonary embolism, sepsis, and Myocardial infarction (Charpentier et al 2004).

Xin, in 2013, has reported elevated serum sPLA2-IIa was associated with an increased risk of mortality and readmission for heart failure. In 2014, Jin suggested that IL-6 -174 G/C polymorphism may contribute to Myocardial infarction susceptibility. Thus, detection of IL-6-174 G/C polymorphisms may be a promising biomarker for the early detection of Myocardial infarction. In 2015, Gong established that S100A4 may be considered as a biomarker for

predicting the probability of Myocardial infarction because S100A4 in plasma is found to be elevated in patients with Myocardial infarction.

In 2012, Li has reported plasma and serum miRNAs may be as novel biomarkers for diagnosis and prognosis of Myocardial infarction. miR-499 is an evolutionary conserved muscle-specific miR that is located in an intronic region of the *MYH7B* gene and plays a role in myosin gene regulation. Although miR-499 is highly expressed under normal conditions in the heart, its expression decreases in the ischemic heart, suggesting its release from the damaged tissue; miR-499 levels could be detected in plasma of patients with Myocardial infarction. Besides parallel expression of miR-499 and TnI in an animal model of Myocardial infarction, miR-499 correlates with Troponin T (TnT) and CK-MB in patients with Myocardial infarction. Other miRNAs encoded by the myosin genes are miR-208a and miR-208b, which are located in *MYH6* and *MYH7* genes, respectively. MiR-208a and miR-208b are abundantly and exclusively expressed in the heart, making them most suitable candidates to be used as biomarkers for the Myocardial infarction. Indeed, miR-208a and miR-208b levels are increased in the circulation after the Myocardial infarction. Two studies showed that miR-208b was the most abundantly elevated miR in the plasma, with 1600 (Corsten et al) and 3000 (Gidlof et al) times more expression in the patients with Myocardial infarction than in the controls. Furthermore, miR-208b levels correlated with TnT levels reflecting myocardial damage.

2.4 Microarray technique for detection of ncRNA

Thiolated C-probes were reduced in buffer A for 1 h at room temperature and then DTT was removed using a column equilibrated with buffer B. C-probes were then mixed with Silane PEG-Maleimide reagent at room temperature in buffer B. After a 1-hour incubation, DTT was added to quench the reaction. A printing solution was then prepared containing C-probe-PEG silane and betaine in buffer B. A non-treated coverslip was inserted on an ink-jet machine, and printing solution droplets were spotted and arrayed on it. The coverslip was transferred to a humid chamber immediately after spotting and incubated, then washed with buffer C to remove C-probes bound non-specifically to the glass surface. The spotted coverslips were dried in clean air and stored until use. A custom DNA microarray was used for quantification of miRNAs in total RNA prepared from human blood. Designs of C-probes were 5′-(GTGCTTCATCTC-$(ACA)_{15}$-AC)-3′ for miR-143, 5′(AGTCTGATAAGCTA-$(ACA)_{15}$-A)-3′ for miR-21, 5′-(TTTACGTGCTGCTA-$(ACA)_{15}$-A)-3′ for miR-16, 5′-(GGACAAGTGCAATA-$(ACA)_{15}$-A)-3′ for miR-92a and 5′-(TTTACACCCGGTGA-$(ACA)_{15}$-A)-3′ for cel-miR-39. The 5′ ends of

C-probes need to be phosphorylated for the Ligase Assisted Sandwich Hybridisation assay; therefore, C-probes, which were synthesized and immobilized on a DNA microarray via their 3′ ends, were incubated. The phosphorylated custom DNA microarray was rinsed, then dried and stored until use (Ueno T, 2014).

2.5 RNA sequencing technique for detection of ncRNA

Probes of 400 to 500 nucleotides were created based upon unique non-conserved sequences and constructed. In brief, multiple antisense probes targeting different parts of each of the lncRNA sequences were developed based upon predictions of the lncRNA secondary structures. Sequences that had high evolutional conservation were avoided, as they may be preferentially involved in tertiary RNA structures that could be difficult to hybridize to in a FFPE environment. In addition, sense stranded probes (opposite strand to the targeting antisense probe) were constructed for each lncRNA to evaluate for non-specific hybridization. The sense and antisense RNA probes labelled were generated by PCR amplification (Chisholm KM, 2012).

3. OBJECTIVES

i Screening and identification of potential biomarkers in Myocardial Infarction through bioinformatics approach

ii Determination of role of identified biomarkers in Myocardial Infarction through technique of biocuration

4. MATERIALS AND METHODS

4.1 Workflow

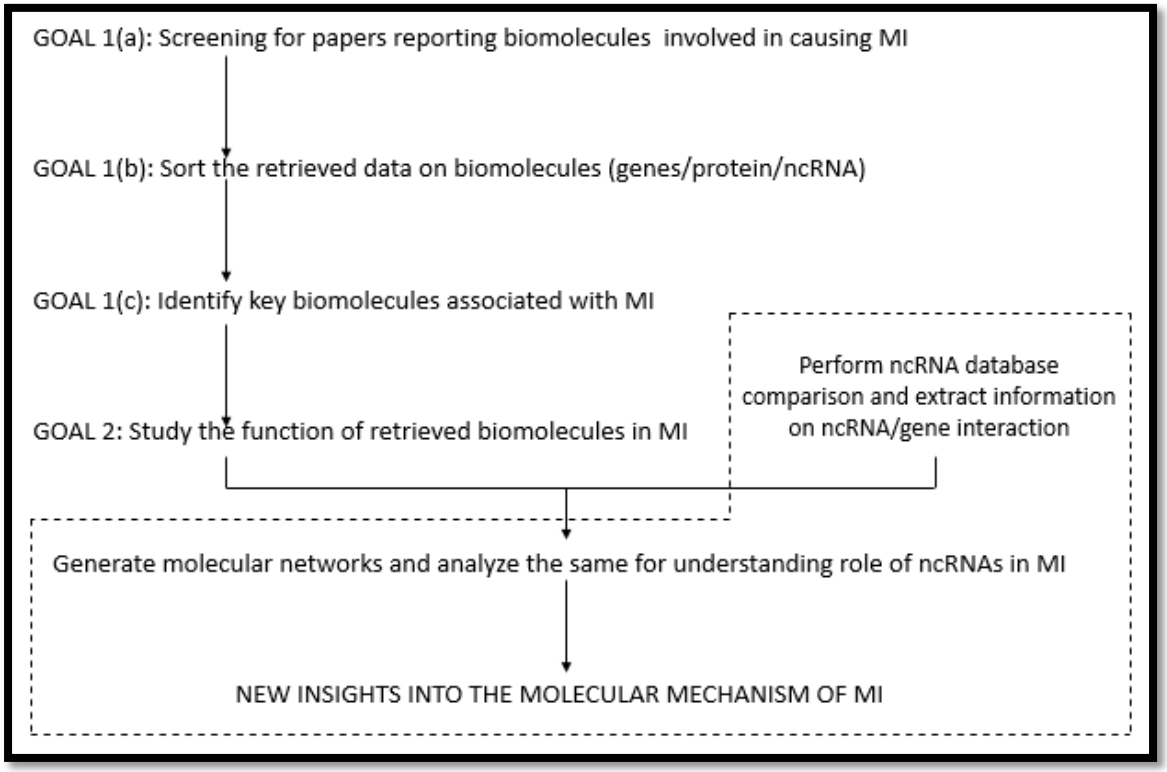


Figure 4.1: Workflow (future work enclosed within '---')

4.2. Protocol to execute Goal 1(a)

4.2.1 Screening for Research papers that reported biomolecules involved in causing MI

Screening was performed using standard and comprehensive search technique on PubMed. Two Query sets as follows were designed:

Query set #1

"Acute myocardial infarction" OR AMI OR "Myocardial infarction" OR "heart attack" OR "heart attacks" OR "cardiac arrest" OR "cardiac arrests"

Query set #2

molecular OR molecules OR molecule OR proteins OR genes OR protein OR gene OR enzyme OR enzymes OR enzymatic OR transcripts OR transcript OR transcriptom* OR proteom* OR biomarker OR biomarkers OR bio-marker OR bio-markers OR "non-coding RNA" OR "noncoding RNAs" OR ncRNA OR ncRNAs OR lncRNA OR lncRNAs OR vlncRNA OR vlncRNAs OR lincRNA OR lincRNAs OR miRNA OR miRNAs OR microRNA OR microRNAs OR piRNA OR piRNAs OR siRNA OR siRNAs OR molecular OR genes OR gene OR protein OR proteins

Query set designing is important for the search of relevant papers. Although 20% error limit is allowed, the strategically designed query sets ensure elimination of extraneous papers, hence allowing definite standardization of biomolecules obtained.

4.2.2. The standard query sets #1 and #2 were subjected to comprehensive search on PubMed.

i. Comprehensive search performed for Query #1

(a) Free full text

(b) Species: Humans and

(c) Search fields: Title

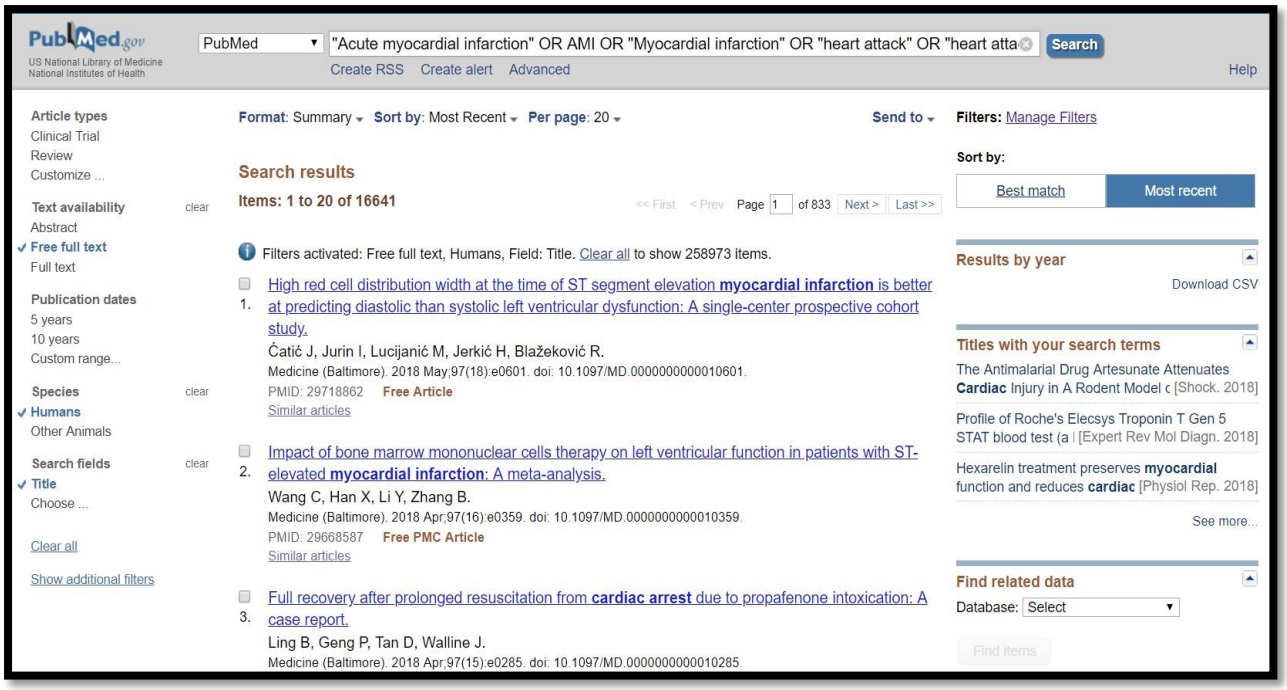


Figure 4.2: Screenshot of #1 Query set comprehensive search result

The search restriction was applied in the above order and the number of papers displayed was 16,569; as shown in Figure 4.2

ii. Comprehensive search performed for Query #2

(d) Free full text

(e) Species: Humans and

(f) Search fields: Title/Abstract

The search restriction was applied in the above order and the number of papers displayed was 9, 14,814; as shown in Figure 4.3

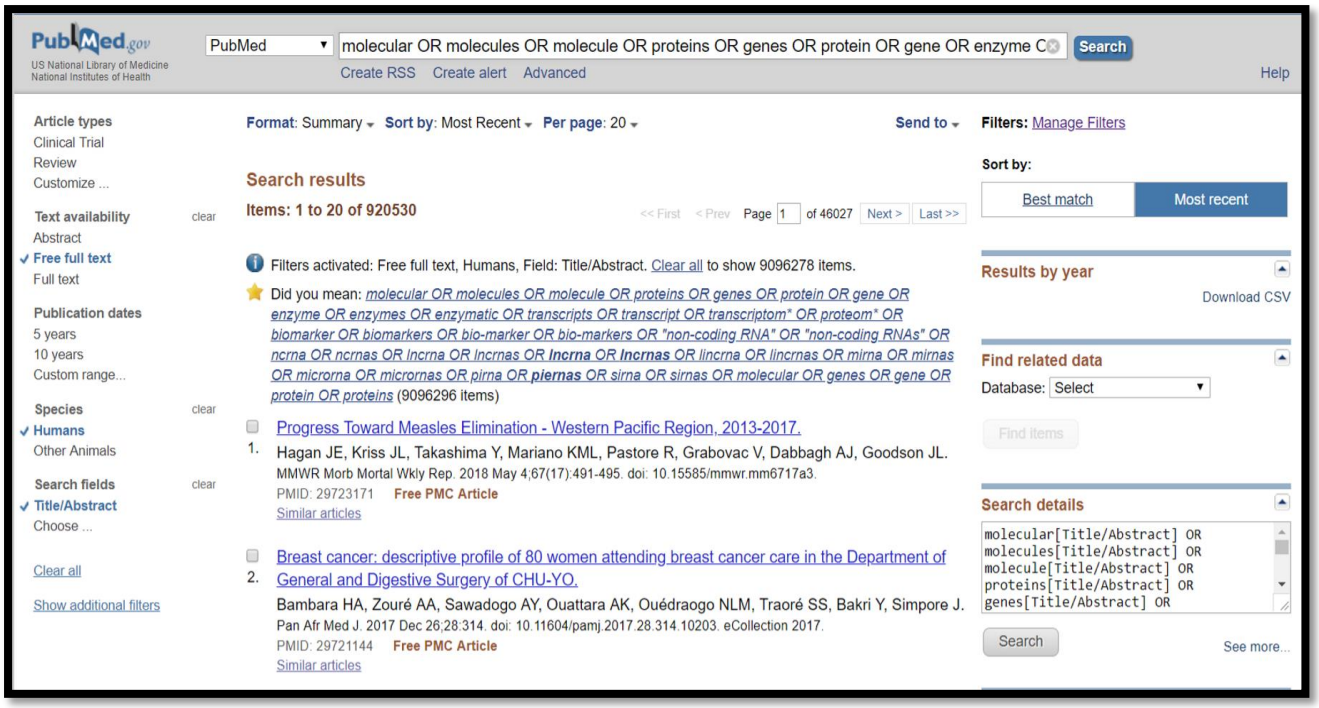


Figure 4.3: Screenshot of #2 Query set comprehensive search result

4.2.3. Advanced search page

Advanced search page is one of the features of PubMed where previously used query searches with its filters are available. In the Search Builder, search results for #1 AND #2 (#8 AND #9) were selected and subjected to advanced search.

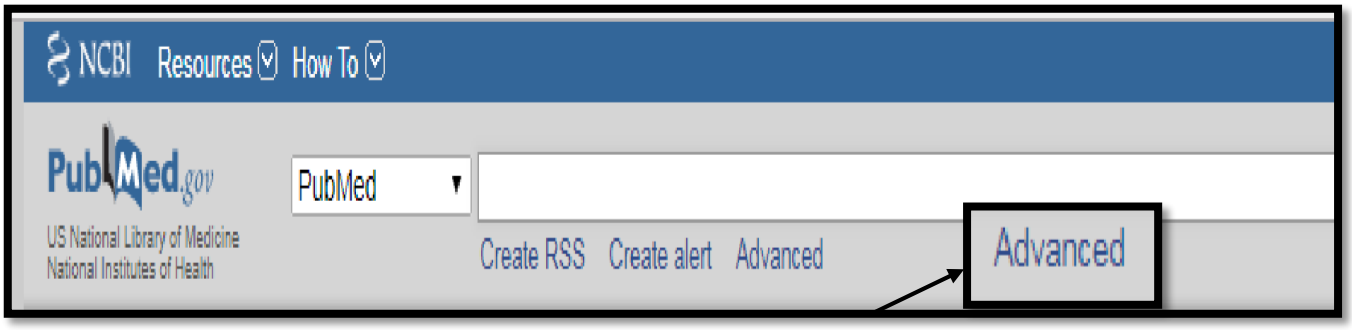


Figure 4.4: Screenshot displaying the 'Advanced' option on PubMed

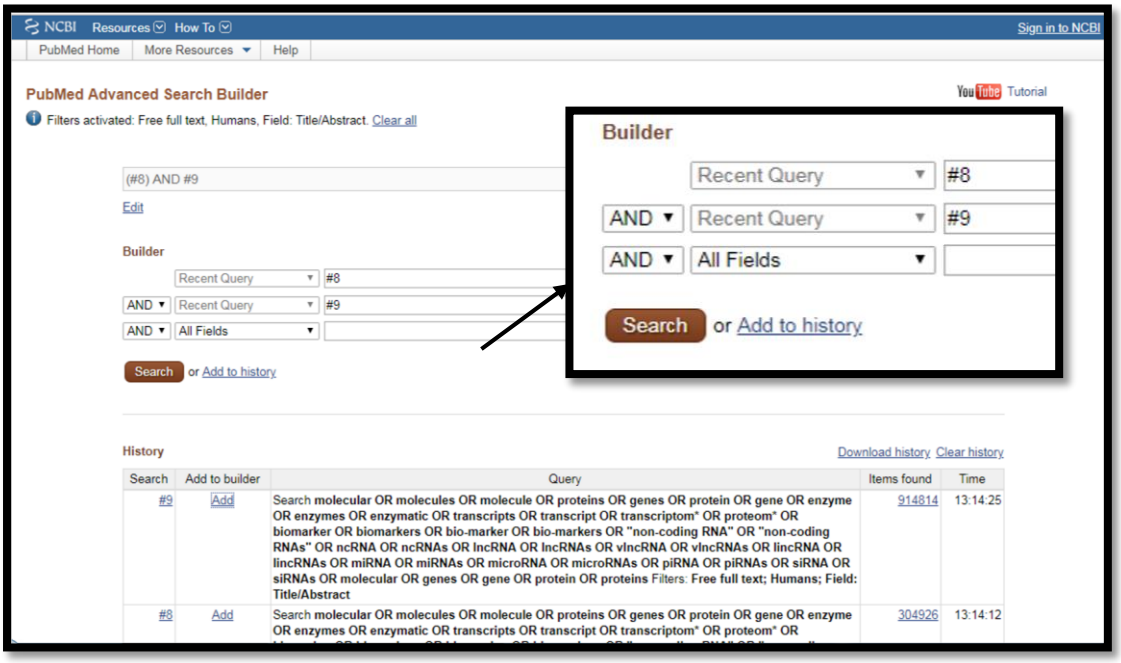


Figure 4.5: Screenshot of Advanced search page on PubMed

4.3. Protocol to execute Goal 1(b)

The papers obtained through combined comprehensive search is subjected to thorough biocuration and the retrieved coding & non-coding molecules is sorted, column wise on MS Excel sheet.

MS Excel is a spreadsheet developed by Microsoft. It features calculation, graphing tools, grids of cells arranged in numbered rows and columns. Microsoft Excel can be well exploited for its user-friendly features and is widely used in Bioinformatics to sort biomolecules data obtained from databases.

Figure 4.6 shows a snapshot of the Excel sheet for non-coding molecules.

Sno.	PMID	MOLECULE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION	ROLE/FUNCTION
1	26676325	RP11-1277A3.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
2	26676325	RP11-806L2.2	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
3	26676325	RP11-573D15.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
4	26676325	RP11-414K1.3.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
5	26676325	RP11-540A21.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
6	26676325	RP11-407N17.5.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
7	26676325	RP11-611L7.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
8	26676325	RP11-353N14.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
9	26676325	RP11-723G8.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
10	26676325	RP11-480A16.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
11	26676325	RP11-167N24.3.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
12	26676325	RP11-24D15.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
13	26676325	RP11-65L19.4	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
14	26676325	RP11-442O18.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
15	26676325	RP11-731J8.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways causing MI
16	25111390	miR-133a	miR-133 and its isoforms	miRNA	Upregulated	early biomarker for identifying perioperative myoc infarction
17	25111390	miR-133b	miR-133 and its isoforms	miRNA	Upregulated	early biomarker for identifying perioperative myoc infarction
18	21672190	miR-133a	miR-133 and its isoforms	miRNA		involved in post-transcriptional regulation of gene expression/affects both the stability and translatio mRNAs

Figure 4.6: Compilation of the non-coding biomolecules data retrieved from PubMed

4.4. Protocol to execute Goal 1(c)

Upon sorting retrieved coding and non-coding biomolecules data involved in MI, the frequency of a particular biomolecules being reported is calculated and presented in 2 columns on the Excel sheet.

(a) Molecule Name

(b) Frequency

The most frequently reported molecules have high possibility of showing significant association with MI.

4.5. Protocol to execute Goal 2

A thorough search (of most frequently reported biomolecules in MI) conducted using protein, mRNA and gene databases can help determine the involvement of coding and non-coding biomolecules in causing MI.

This can provide new insights into deducing the molecular mechanism involved in triggering MI and may even help identify potential biomarkers to either mitigate or delay the progression of MI.

5. RESULTS AND DISCUSSION

5.1. Results for Protocol to execute Goal 1(a)

On subjecting Query #1 to search by free **full Text** followed by **Species: human and** then **Search field: Title only** in PubMed. The numbers of papers displayed upon applying each filter consecutively were **67651**; **49255** and **16641** respectively.

On subjecting Query #2 to search by free **full Text** followed by **Species: human and** then **Search field: Title/Abstract** only in PubMed. The numbers of papers displayed upon applying each filter consecutively were **30, 09,260**; **13, 98,646** and **9, 20,530** respectively.

By performing a combined search of the results from #1 (**16,641 papers**) and #2 (**9, 20,530 papers**) using Advanced option on PubMed, a total of **2247 papers** were found that were relevant to our study.

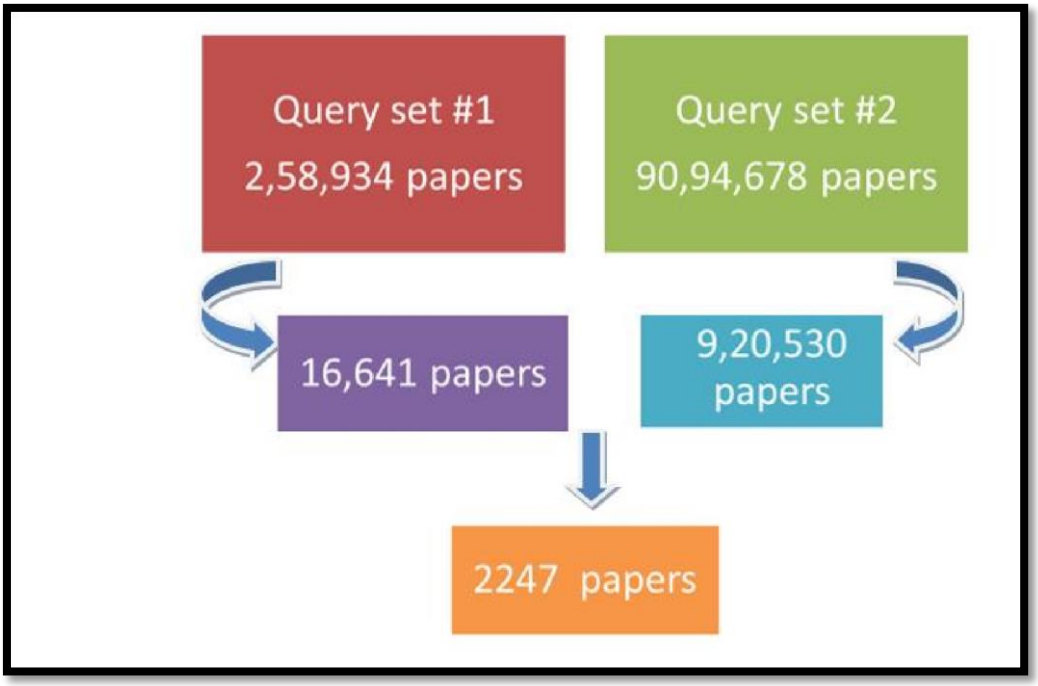


Figure 5.1: Brief explanation of Comprehensive search used on PubMed

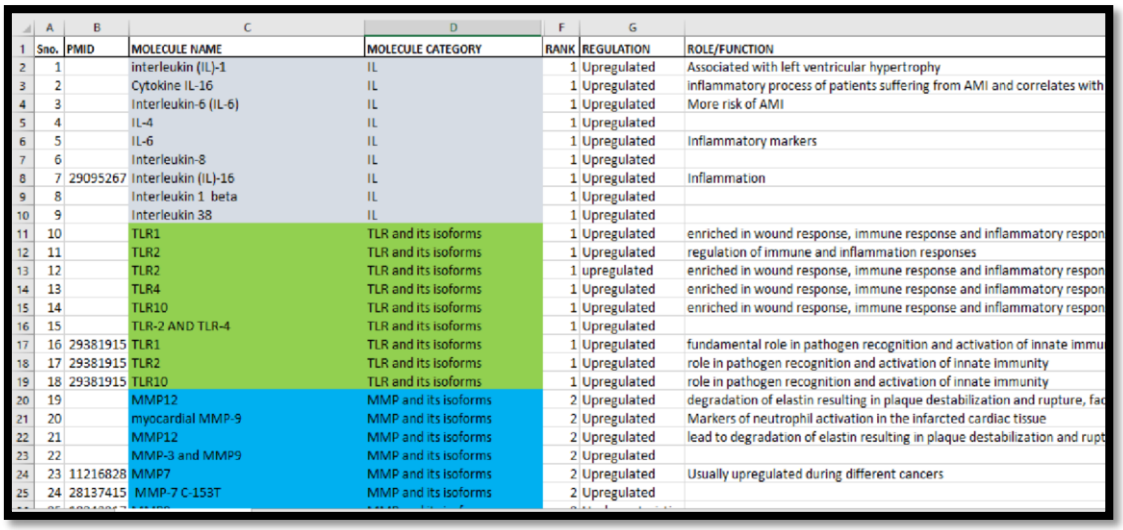
5.2. Results for Protocol to execute Goal 1(b)

Thorough biocuration of 2,247 papers was performed and both coding and non-coding biomolecules that showed differential expression among the diseased and normal population was sorted systematically into MS Excel sheets. Two separate Excel sheets were made for sorting coding and non-coding biomolecules respectively, each sheet having 6 columns.

1. PMID
2. Molecule name
3. Molecule category
4. Molecule type
5. Regulation
6. Role and function

	A	B	C	D	E	F	G	H	I
1	Sno.	PMID	MOLECULE NAME	MOLECULE CATEGORY	MOLCATEGORY_GROUPING	Rank	MOLECULE TYPE	REGULATION	ROLE/FUNCTION
2	1	26676325	RP11-1277A3.1.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
3	2	26676325	RP11-806L2.2	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
4	3	26676325	RP11-573D15.2.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
5	4	26676325	RP11-414K1.3.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
6	5	26676325	RP11-540A21.2.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
7	6	26676325	RP11-407N17.5.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
8	7	26676325	RP11-611L7.1.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
9	8	26676325	RP11-353N14.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
10	9	26676325	RP11-723G8.1.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
11	10	26676325	RP11-480A16.1.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
12	11	26676325	RP11-167N24.3.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
13	12	26676325	RP11-24D15.1.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
14	13	26676325	RP11-65L19.4	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
15	14	26676325	RP11-442O18.2.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
16	15	26676325	RP11-731J8.2.1	RP11 and its isoforms	RP11 and its isoforms	1	lncRNA	Uncharacteristic	Involved in >6 regulatory pathways
17	16	25111390	miR-133a	miR-133 and its isoforms	miR-133 and its isoforms	2	miRNA	Upregulated	early biomarker for identifying peric infarction
18	17	25111390	miR-133b	miR-133 and its isoforms	miR-133 and its isoforms	2	miRNA	Upregulated	early biomarker for identifying peric infarction
19	18	21672190	miR-133a	miR-133 and its isoforms	miR-133 and its isoforms	2	miRNA		involved in post-transcriptional expression/affects both the stab mRNAs

Figure 5.2: Snapshot of Excel sheet showing sorted non-coding biomolecules



	A	B	C	D	F	G	
1	Sno.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION	ROLE/FUNCTION
2	1		interleukin (IL)-1	IL	1	Upregulated	Associated with left ventricular hypertrophy
3	2		Cytokine IL-16	IL	1	Upregulated	inflammatory process of patients suffering from AMI and correlates with
4	3		Interleukin-6 (IL-6)	IL	1	Upregulated	More risk of AMI
5	4		IL-4	IL	1	Upregulated	
6	5		IL-6	IL	1	Upregulated	Inflammatory markers
7	6		Interleukin-8	IL	1	Upregulated	
8	7	29095267	Interleukin (IL)-16	IL	1	Upregulated	Inflammation
9	8		Interleukin 1 beta	IL	1	Upregulated	
10	9		Interleukin 38	IL	1	Upregulated	
11	10		TLR1	TLR and its isoforms	1	Upregulated	enriched in wound response, immune response and inflammatory respon
12	11		TLR2	TLR and its isoforms	1	Upregulated	regulation of immune and inflammation responses
13	12		TLR2	TLR and its isoforms	1	upregulated	enriched in wound response, immune response and inflammatory respon
14	13		TLR4	TLR and its isoforms	1	Upregulated	enriched in wound response, immune response and inflammatory respon
15	14		TLR10	TLR and its isoforms	1	Upregulated	enriched in wound response, immune response and inflammatory respon
16	15		TLR-2 AND TLR-4	TLR and its isoforms	1	Upregulated	
17	16	29381915	TLR1	TLR and its isoforms	1	Upregulated	fundamental role in pathogen recognition and activation of innate immu
18	17	29381915	TLR2	TLR and its isoforms	1	Upregulated	role in pathogen recognition and activation of innate immunity
19	18	29381915	TLR10	TLR and its isoforms	1	Upregulated	role in pathogen recognition and activation of innate immunity
20	19		MMP12	MMP and its isoforms	2	Upregulated	degradation of elastin resulting in plaque destabilization and rupture, fa
21	20		myocardial MMP-9	MMP and its isoforms	2	Upregulated	Markers of neutrophil activation in the infarcted cardiac tissue
22	21		MMP12	MMP and its isoforms	2	Upregulated	lead to degradation of elastin resulting in plaque destabilization and rupt
23	22		MMP-3 and MMP9	MMP and its isoforms	2	Upregulated	
24	23	11216828	MMP7	MMP and its isoforms	2	Upregulated	Usually upregulated during different cancers
25	24	28137415	MMP-7 C-153T	MMP and its isoforms	2	Upregulated	

Figure 5.3: Snapshot of Excel sheet showing sorted coding biomolecules

Upon biocuration of 2247 papers, 84 non-coding biomolecules were reported to have differential regulation amongst diseased and healthy population across 174 papers.

The following table shows the frequency of 17 molecules that were reported three times or more, followed by a graphical representation of the obtained data in figure 5.4. The complete list of 84 non-coding biomolecules can be found.

Molecule Name	Frequency
RP11	15
miR-133	9
miR-208	9
miR-21	8
miR-499	8
miR-122	6
miR-1	5
CTD	4
miR-150	4
miR-29	4
miR-92	4
ANRIL	3
Cdr-1	3
HOTAIR	3

Molecule Name	Frequency
LINC	3
miR-145	3
miR-19	3

Table 5.1. Tabulation of non-coding molecules that were reported three times or more.

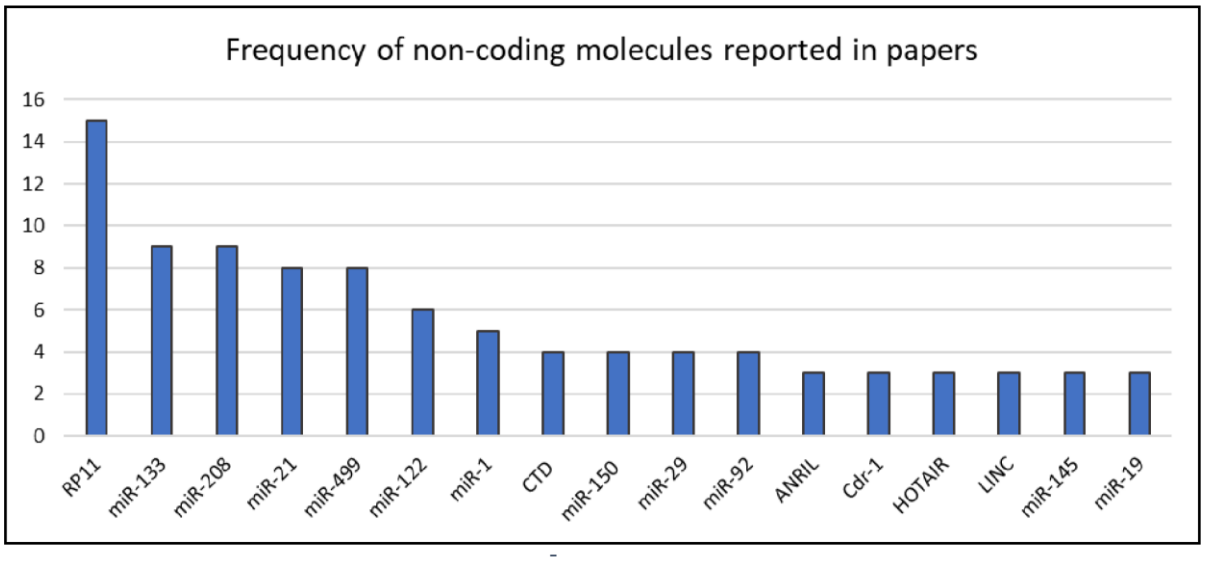


Figure 5.4. Graphical representation of the frequency of non-coding molecules reported three times or more in papers

Upon biocuration of 2247 papers, 117 coding biomolecules were reported to have differential regulation amongst diseased and healthy population across 194 papers. The following table shows the frequency of 19 molecules that were reported three times or more, followed by a graphical representation of the obtained data in Figure 5.5. The complete list of 117 coding biomolecules can be found.

Molecule name	Frequency
IL	**9**
TLR	**9**
MMP	**8**
CD	**6**
Troponin	**5**

Molecule name	Frequency
CRP	5
C4b-binding protein	4
CCL	4
Fibrinogen	4
ANG	3
Creatine kinase	3
GZM	3
Gelatinase	3
cMyC	3
GP	3
GSE	3
FOXO3	3
GBP5	2
GBP5	1

Table 5.2: Tabulation of coding molecules that were reported three times or more

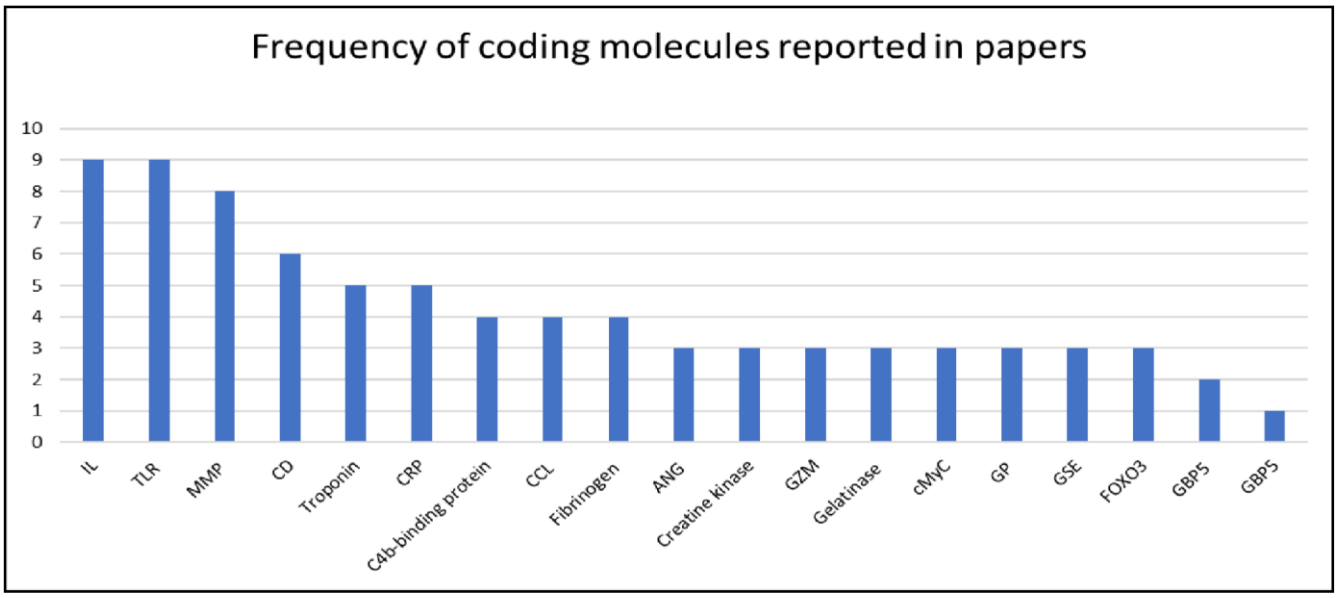


Figure 5.5: Graphical representation of the frequency of coding molecules reported three times or more in papers

5.3. Results for Protocol to execute Goal 1(c)

Based on the retrieval and sorting of coding and non-coding biomolecules the following list was compiled.

Molecule Name	Frequency
RP11	15
miR-133	9
miR-208	9
miR-21	8
miR-499	8
miR-122	6
miR-1	5
CTD	4
miR-150	4
miR-29	4
miR-92	4
ANRIL	3
Cdr-1	3
HOTAIR	3
LINC	3

Molecule Name	Frequency
miR-145	3
miR-19	3
IL	9
TLR	9
MMP	8
CD	6
Troponin	5
CRP	5
C4b-binding	4
CCL	4
Fibrinogen	4
ANG	3
Creatine kinase	3
GZM	3
Gelatinase	3
cMyC	3
GP	3
GSE	3
FOXO3	3
GBP5	2
GBP5	1

Table 5.3. List of identified non-coding (pink) and coding biomolecules (blue) reported three times or more and concluded as significant in MI

5.4. Results for Protocol to execute Goal 2

5.4.1. Retrieval of coding and non-coding biomolecules through biocuration

Studies done in the past four decades prove that ncRNAs have the ability to affect the stability and regulation of protein coding messenger RNAs. It can not only lead to differential expression of coding and non-coding biomolecules, but it can also trigger the expression of genes whose transcriptional products have no particular function in the regular metabolic pathway expression. It can obstruct a wild metabolic pathway or re-direct it to produce proteins with different property by affecting the mRNAs translation. This indicates that there is a

correlation between the expression of coding and non-coding biomolecules which makes it important to study these two biomolecules together.

5.4.2. Comment on non-coding biomolecules retrieved through biocuration

According to the data collected, **RP11** which is a class of long non-coding RNA which were reported through myocardial infarction-related differential lncRNA-mRNA co-expression network (MILMN) (Wang P, 2016).

Long non-coding RNAs are a large and diverse class of transcribed RNA molecules with a length of more than 200 nucleotides that do not encode proteins. LncRNAs are thought to encompass nearly 30,000 different transcripts in humans, hence lncRNA transcripts account for the major part of the non-coding transcriptome (Durham, et al., 2012).

There is increasing evidence that **miRNA133** family of microRNAs is enriched in muscle tissues and myogenic cells, and its aberrant expression could induce the occurrence and development of cardiac disorders. This implied that diagnosis of miRNA 133 could be a potential indicator for CVD (Liu Y, 2017).

Another repeatedly found miRNA associated with CVD was **miRNA 208**.The primary transcript of microRNA 208 is cleaved by ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA products. The mature miRNA is incorporated into an RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA and most commonly results in translational inhibition or destabilization of the target mRNA (MIR208A, Gene, 1996).

miRNA 499 was reported 9 times in different papers and were upregulated in the blood plasma as per four different researches carried out across the globe. (PMIDs: 25111390, 27423422, 25111390, 27423422). MicroRNA (miRNA) 499 is an evolutionary conserved muscle-specific miRNA that is encoded by an intron of the myh7 gene and is likely to play a role in myosin gene regulation. It has been shown to be involved in inhibiting apoptosis and myocardial infarction induced by ischemia and anoxia (Sharkawy EM, 2017). It is possible that up regulation of miRNA 499 could indicate damage to the heart muscle tissue which can cause formation of clots and plaque formation in the blood vessels which can indeed lead to cardiovascular diseases.

ANRIL is a long non-coding RNA (lncRNA) that is encoded in the chromosome 9p21 region. This locus has been determined to be the hotspot for disease-associated polymorphisms, and it has been consistently associated with cardiovascular diseases. ANRIL is transcribed by RNA polymerase II and spliced into multiple linear isoforms. Although most of the splicing variants are polyadenylated some circular non-polyadenylated variants have also been described by several studies. Some of the splicing variants have been reported to be tissue-specific suggesting their physiological relevance and underlining the complexity of its regulatory function (Congrains A, 2013).

Table 5.4. Results of biocuration of non-coding molecules

S.no.	PMID	MOLECUlE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION
1	26676325	RP11-1277A3.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic
2	26676325	RP11-806L2.2	RP11 and its isoforms	lncRNA	Uncharacteristic
3	26676325	RP11-573D15.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic
4	26676325	RP11-414K1.3.1	RP11 and its isoforms	lncRNA	Uncharacteristic
5	26676325	RP11-540A21.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic
6	26676325	RP11-407N17.5.1	RP11 and its isoforms	lncRNA	Uncharacteristic
7	26676325	RP11-611L7.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic
8	26676325	RP11-353N14.1	RP11 and its isoforms	lncRNA	Uncharacteristic
9	26676325	RP11-723G8.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic
10	26676325	RP11-480A16.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic
11	26676325	RP11-167N24.3.1	RP11 and its isoforms	lncRNA	Uncharacteristic
12	26676325	RP11-24D15.1.1	RP11 and its isoforms	lncRNA	Uncharacteristic
13	26676325	RP11-65L19.4	RP11 and its isoforms	lncRNA	Uncharacteristic
14	26676325	RP11-442O18.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic
15	26676325	RP11-731J8.2.1	RP11 and its isoforms	lncRNA	Uncharacteristic
16	25111390	miR-133a	miR-133 and its isoforms	miRNA	Upregulated
17	25111390	miR-133b	miR-133 and its isoforms	miRNA	Upregulated
18	21672190	miR-133a	miR-133 and its isoforms	miRNA	
19	21881276	miR-133	miR-133 and its isoforms	miRNA	Upregulated
20	21881276	miR-133	miR-133 and its isoforms	miRNA	Upregulated
21	23066896	miR-133,	miR-133 and its isoforms	miRNA	Downregulated
22		miR-133	miR-133 and its isoforms	miRNA	Upregulated
23	24885383	miR-133	miR-133 and its isoforms	miRNA	Upregulated

S.no.	PMID	MOLECUlE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION
24	24053180	microRNA-133a	miR-133 and its isoforms	miRNA	Upregulated
25		miR208	miR-208 and its isoforms	miRNA	Upregulated
26	26528525	miRNA-208a	miR-208 and its isoforms	miRNA	
27	21672190	miR-208b	miR-208 and its isoforms	miRNA	
28	22252325	miRNA-208b	miR-208 and its isoforms	miRNA	Upregulated
29	29381915	miR-208a	miR-208 and its isoforms	ncRNA	
30		miR-208b	miR-208 and its isoforms	miRNA	Upregulated
31	26044724	miR-208b	miR-208 and its isoforms	miRNA	Upregulated
32	27423422	miR-208b	miR-208 and its isoforms	miRNA	Downregulated
33	20395621	miRNA 208 (miR208)	miR-208 and its isoforms	miRNA	Upregulated
34	23066896	miR-21	miR-21 and its isoforms	miRNA	Downregulated
35		hsa-miR-21-5p	miR-21 and its isoforms	miRNA	Downregulated
36		hsa-miR-21-5p	miR-21 and its isoforms	miRNA	Downregulated
37	26875904	microRNA-21	miR-21 and its isoforms	miRNA	Upregulated
38		miR21	miR-21 and its isoforms	miRNA	Upregulated
39	29049183	hsa-miR-21-5p	miR-21 and its isoforms	miRNA	
40	26258537	miR-21-5p	miR-21 and its isoforms	miRNA	
41	26875904	miR-21	miR-21 and its isoforms	miRNA	Upregulated
42	25111390	microRNA-499	miR-499 and its isoforms	miRNA	Upregulated
43		miR-499	miR-499 and its isoforms	miRNA	Upregulated
44	27423422	miR-499-5p	miR-499 and its isoforms	miRNA	Downregulated
45	25111390	miR- 499	miR-499 and its isoforms	miRNA	Upregulated
46	27423422	miR-499-5p	miR-499 and its isoforms	miRNA	Upregulated
47	21672190	miR-499-5p	miR-499 and its isoforms	miRNA	Upregulated
48	29381915	miR-499	miR-499 and its isoforms	ncRNA	
49	22252325	miR-499	miR-499 and its isoforms	miRNA	Upregulated
50	26884877	miR-122-5p	miR-122 and its isoforms	miRNA	Upregulated

S.no.	PMID	MOLECUlE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION
51	27593229	miR-122-5p/133b ratio	miR-122 and its isoforms	miRNA	Upregulated
52	27423422	miR-122	miR-122 and its isoforms	miRNA	Upregulated
53	23066896	miR-122	miR-122 and its isoforms	miRNA	Upregulated
54	27423422	miR-122	miR-122 and its isoforms	miRNA	Upregulated
55	23066896	miR-122,	miR-122 and its isoforms	miRNA	Downregulated
56		miR-1	miR-1 and its isoforms	miRNA	Upregulated
57	21672190	miR-1	miR-1 and its isoforms	miRNA	
58	29381915	miR-1	miR-1 and its isoforms	ncRNA	
59	23066896	miR-1,	miR-1 and its isoforms	miRNA	Downregulated
60	20163779	MicroRNA miR-1	miR-1 and its isoforms	miRNA	Upregulated
61	26676325	CTD-2350C19.1	CTD and its isoforms	lncRNA	Uncharacteristic
62	26676325	CTD-2329C7.2	CTD and its isoforms	lncRNA	Uncharacteristic
63	26676325	CTD-2574D22.5.1	CTD and its isoforms	lncRNA	Uncharacteristic
64	26676325	CTD-2144E22.6.1	CTD and its isoforms	lncRNA	Uncharacteristic
65	24900964	miR-150-3p	miR-150 and its isoforms	miRNA	Upregulated
66	22048267	miR-150	miR-150 and its isoforms	miRNA	Upregulated
67	26077801	miR-150	miR-150 and its isoforms	miRNA	Upregulated
68	23547171	MicroRNA-150	miR-150 and its isoforms	miRNA	Upregulated
69	26258537	miR-29a-3p	miR-29 and its isoforms	miRNA	
70	26258537	miR-29b-3p	miR-29 and its isoforms	miRNA	
71	26258537	miR-29c-3p	miR-29 and its isoforms	miRNA	
72	27423422	miR-29c	miR-29 and its isoforms	miRNA	Upregulated
73		*miR-92a*	*miR-92a and its isoforms*	miRNA	Upregulated
74	27411964	MiR-92a	*miR-92a and its isoforms*	miRNA	Upregulated
75	22153007	miR-92a	*miR-92a and its isoforms*	miRNA	Upregulated
76		*miR-92a*	*miR-92a and its isoforms*	miRNA	Upregulated
77	25035150	ANRIL	ANRIL	lncRNA	Downregulated
78	28107200	ANRIL(9p21.3 locus)	ANRIL	antisense ncRNA	
79		ANRIL	ANRIL	lncRNA	
80	26928231	CDR1AS	Cdr-1 and its isoforms	lncRNA	Upregulated
81	26928231	Cdr1	Cdr-1 and its isoforms	antisense ncRNA	

S.no.	PMID	MOLECUlE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION
82		CDR1	Cdr-1 and its isoforms	circRNA	Upregulated
83	29258067	HOTAIR	HOTAIR	lncRNA	Downregulated
84		HOX RNA (HOTAIR)	HOTAIR	lncRNA	Downregulated
85		HOTAIR - lncRNA	HOTAIR	lncRNA	Downregulated
86	26676325	LINC00174	LINC and its isoforms	lncRNA	Uncharacteristic
87	26676325	LINC00313	LINC and its isoforms	lncRNA	Uncharacteristic
88	26676325	LINC01482	LINC and its isoforms	lncRNA	Uncharacteristic
89	28051023	miRNA-145	miR-145 and its isoforms	ncRNA	
90	27423422	miR-145	miR-145 and its isoforms	miRNA	Upregulated
91		circulating miR-145	miR-145 and its isoforms	miRNA	Downregulated
92	26939053	miR-19b-3p	miR-19 and its isoforms	miRNA	Upregulated
93	25383678	miR-19a	miR-19 and its isoforms	miRNA	Upregulated
94	29381915	miR-19a	miR-19 and its isoforms	ncRNA	
95	26928231	aHIF	aHIF	lncRNA	
96	25035150	aHIF	aHIF	lncRNA	Upregulated
97	26676325	lncRNAs-H19	H19 and its isoforms		
98	26676325	H19	H19 and its isoforms	lncRNA	Tumorsupressor
99	29049183	hsa-miR-30c-5p	hsa-miR-30 and its isoforms	miRNA	
100		hsa-miR-30c-5p	hsa-miR-30 and its isoforms	miRNA	Downregulated
101	26928231	MIAT	MIAT	lncRNA	
102		MIAT	MIAT	lncRNA	Downregulated
103	24046434	miRNA125a-5p	miR-125 and its isoforms	miRNA	downregulated
104	23066896	miR-125a/b	miR-125 and its isoforms	miRNA	Downregulated
105	24900964	miR-126-3p	miR-126 and its isoforms	miRNA	Downregulated
106	23066896	miR-126,	miR-126 and its isoforms	miRNA	Downregulated
107	24900964	miR-191-5p	miR-191 and its isoforms	miRNA	Downregulated
108	26044724	miR-191	miR-191 and its isoforms	miRNA	Downregulated
109	26044724	miR-26a	miR-26 and its isoforms	miRNA	Downregulated
110	24900964	miR-26a-5p	miR-26 and its isoforms	miRNA	Downregulated
111	27423422	miR-34a	miR-34 and its isoforms	miRNA	Upregulated
112		miR34	miR-34 and its isoforms	miRNA	Upregulated
113	24900964	miR-486-3p	miR-486 and its isoforms	miRNA	Upregulated

S.no.	PMID	MOLECUlE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION
114	26077801	miR-486	miR-486 and its isoforms	miRNA	Upregulated
115	24885383	miR-663b	miR-663 and its isoforms	miRNA	Upregulated
116	27423422	miR-663b	miR-663 and its isoforms	miRNA	Upregulated
117	28051249	miR-99a	miR-99 and its isoforms	ncRNA	
118		miR-99a	miR-99 and its isoforms	miRNA	Downregulated
119	26676325	RP5-1142A6.3.1	RP5 and its isoforms	lncRNA	Uncharacteristic
120	26676325	RP5-828H9.3.1	RP5 and its isoforms	lncRNA	Uncharacteristic
121	26676325	AC004410.1	AC004410.1	lncRNA	Uncharacteristic
122	26676325	AC093611.1.	AC093611.1.	lncRNA	Uncharacteristic
123	26676325	AC145123.2.1	AC145123.2.1	lncRNA	Uncharacteristic
124	26676325	AL928742.12.1	AL928742.12.1	lncRNA	Uncharacteristic
125	26676325	AP000475.2.1	AP000475.2.1	lncRNA	Uncharacteristic
126	26676325	CTB-113P19.1.1	CTB-113P19.1.1	lncRNA	Uncharacteristic
127	26676325	CTC-378H22.2.1	CTC-378H22.2.1	lncRNA	Uncharacteristic
128	26928231	DIO3OS	DIO3OS	lncRNA	
129	26928231	FENDRR	FENDRR	lncRNA	
130	26676325	GS1-204I12.2.1	GS1-204I12.2.1	lncRNA	Uncharacteristic
131	26928231	HCG22	HCG22	lncRNA	
132	25035150	KCNQ1OT1	KCNQ1OT1	lncRNA	Upregulated
133	28062497	let-7b	let-7b	ncRNA	
134	25035150	MALAT1	MALAT1	lncRNA	Upregulated
135	26928231	MHRT	MHRT	lncRNA	
136	27423422	miR -330-3p	miR-330 and its isoforms	miRNA	Downregulated
137	27515482	MiR-103a	MiR-103 and its isoforms	miRNA	Upregulated
138	24885383	miR-1291	miR-1291 and its isoforms	miRNA	Upregulated
139	26939053	miR-134-5p	miR-134 and its isoforms	miRNA	Upregulated
140	23066896	miR-140	miR-140 and its isoforms	miRNA	Upregulated
141	22048267	miR-146a	miR-146 and its isoforms	miRNA	Upregulated
142	22048267	miR-155	miR-155 and its isoforms	miRNA	Upregulated
143		Circulating miR181a	miR-181 and its isoforms	miRNA	Downregulated
144	26939053	miR-186-5p	miR-186 and its isoforms	miRNA	Upregulated
145	23066896	miR-199a	miR-199 and its isoforms	miRNA	Upregulated
146	27423422	miR-200a	miR-200 and its isoforms	miRNA	Upregulated
147	27423422	miR-205	miR-205 and its isoforms	miRNA	Upregulated

S.no.	PMID	MOLECUlE NAME	MOLECULE CATEGORY	MOLECULE TYPE	REGULATION
148	27423422	miR-210	miR-210 and its isoforms	miRNA	Upregulated
149	25931214	miR-214	miR-214 and its isoforms	miRNA	Downregulated
150	27423422	miR-221	miR-221 and its isoforms	miRNA	Downregulated
151		miR-23a	miR-23 and its isoforms	miRNA	
152	23066896	miR-320a/b/c/d	miR-320 and its isoforms	miRNA	Upregulated
153	21881276	miR-328	miR-328 and its isoforms	miRNA	Upregulated
154	28062497	miR-378	miR-378 and its isoforms	ncRNA	
155	23066896	miR-483	miR-483 and its isoforms	miRNA	Upregulated
156	25110754	miR-497	miR-497 and its isoforms	miRNA	Upregulated
157	25184815	miR-519e-5p	miR-519 and its isoforms	miRNA	Downregulated
158	23066896	miR-574-3p/-5p	miR-574 and its isoforms	miRNA	Upregulated
159	27423422	miR-9-5p	miR-9 and its isoforms	miRNA	Downregulated
160	23066896	miR-98	miR-98 and its isoforms	miRNA	Downregulated
161		miRNA-124	miRNA-124 and its isoforms	miRNA	Upregulated
162		miRNA-184	miRNA-184 and its isoforms	miRNA	Upregulated
163	26676325	RP1-170O19.14.1	RP1 and its isoforms	lncRNA	Uncharacteristic
164	26676325	RP13-452N2.1.1	RP13 and its isoforms	lncRNA	Uncharacteristic
165	26928231	SENCER	SENCER	lncRNA	
166	26928231	*SRA*	*SRA*	lncRNA	
167	26676325	Z83851.1.1	Z83851 and its isoforms	lncRNA	Uncharacteristic
168	26928231	ZFAS1	ZFAS1 and its isoforms	antisense ncRNA	Downregulated
169	26676325	C21orf91-OT1	C21orf91-OT1	lncRNA	Uncharacteristic
170	26676325	C4orf38	C4orf38	lncRNA	Uncharacteristic
171	26928231	CARL	CARL	lncRNA	
172	26928231	NRON	NRON	lncRNA	
173	26928231	NESPAS	NESPAS	lncRNA	
174	26928231	SAF	SAF	lncRNA	

5.4.3. Comments on coding biomolecules retrieved through biocuration

i. Interleukin

Interleukin (IL), are a subset of a larger group of cellular messenger molecules called cytokines, which are modulators of cellular behavior. Interleukins regulates cell growth, differentiation

and are particularly important in stimulating immune responses such as inflammation. Interleukins are not stored within cells but are secreted rapidly, and briefly, in response to a stimulus such as infectious agent. Release of IL allows binding of IL to target cell surface and this interaction triggers a cascade of signals within the target cell that ultimately alter the cell's behavior (The Editors of Encyclopaedia Britannica et al., 1998).

ii. Toll-like receptors

Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRRs) that play a crucial role in recognition of foreign antigens and induction of immune response. Cells of the immune system (macrophages, dendritic cells, mast cells, eosinophils, neutrophils, B-lymphocytes), cardio-myocytes, epithelial cells and adipocytes all recognize pathogens via TLRs. TLRs also play a role in regulation of immune response via direct or indirect influence on the function of T regulatory cells, which results in the induction and subsequent suppression of the immune response or a reversal of suppression (contra-suppression) (Majewska M, 2006).

Table 5.5. Results of biocuration of coding molecules

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
1		interleukin (IL)-1	IL	1	Upregulated
2		Cytokine IL-16	IL	1	Upregulated
3		Interleukin-6 (IL-6)	IL	1	Upregulated
4		IL-4	IL	1	Upregulated
5		IL-6	IL	1	Upregulated
6		Interleukin-8	IL	1	Upregulated
7	29095267	Interleukin (IL)-16	IL	1	Upregulated
8		Interleukin 1 beta	IL	1	Upregulated
9		Interleukin 38	IL	1	Upregulated
10		TLR1	TLR and its isoforms	1	Upregulated
11		TLR2	TLR and its isoforms	1	Upregulated
12		TLR2	TLR and its isoforms	1	upregulated
13		TLR4	TLR and its isoforms	1	Upregulated
14		TLR10	TLR and its isoforms	1	Upregulated
15		TLR-2 AND TLR-4	TLR and its isoforms	1	Upregulated

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
16	29381915	TLR1	TLR and its isoforms	1	Upregulated
17	29381915	TLR2	TLR and its isoforms	1	Upregulated
18	29381915	TLR10	TLR and its isoforms	1	Upregulated
19		MMP12	MMP and its isoforms	2	Upregulated
20		myocardial MMP-9	MMP and its isoforms	2	Upregulated
21		MMP12	MMP and its isoforms	2	Upregulated
22		MMP-3 and MMP9	MMP and its isoforms	2	Upregulated
23	11216828	MMP7	MMP and its isoforms	2	Upregulated
24	28137415	MMP-7 C-153T	MMP and its isoforms	2	Upregulated
25	18342317	MMP9	MMP and its isoforms	2	Uncharacteristic
26	16629857	MMP-3	MMP and its isoforms	2	Downregulated
27		CD4+T cells	CD	3	Upregulated
28	28213360	CD31	CD	3	Upregulated
29	28213360	CD42b	CD	3	Downregulated
30	22426168	$CD4^{+}Foxp3^{+}$	CD	3	Downregulated
31		CD3+ T cells	CD	3	Downregulated
32		CD8+ T cells	CD	3	Downregulated
33		C-reactive protein (CRP)	CRP	4	Upregulated
34		hsCRP	CRP	4	Upregulated
35		C-reactive protein (CRP)	CRP	4	Upregulated
36		C-reactive protein CRP	CRP	4	Upregulated
37	28051279	hs C-reactive protein CRP	CRP	4	Upregulated
38		C4b-binding protein	C4b-binding protein	5	Downregulated
39		C4b-binding protein	C4b-binding protein	5	Upregulated
40	18682851	C4b-binding protein	C4b-binding protein	5	Upregulated
41	21971072	C4b-binding protein	C4b-binding protein	5	Upregulated
42		CCL5	CCL and its isoforms	5	Downregulated
43		CCL4	CCL and its isoforms	5	Downregulated
44	29381915	CCL5	CCL and its isoforms	5	Downregulated
45	29381915	CCL4	CCL and its isoforms	5	Downregulated
46		Fibrinogen	Fibrinogen	5	Upregulated

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
47		Fibrinopeptide A (FPA)	Fibrinogen	5	Upregulated
48		Fibrinogen	Fibrinogen	5	Upregulated
49		Fibrinogen	Fibrinogen	5	Upregulated
50	26864512	Troponin T - TCG	Troponin	5	Upregulated
51	24927776	high sensitivity Troponin 1	Troponin	5	Upregulated
52	26884877	cTnI	Troponin	5	Upregulated
53		Cardiac troponin T fragments	Troponin	5	Upregulated
54		Cardiac troponin I (cTnI)	Troponin	5	Upregulated
55	26676325	ANGPTL2	ANG and its isoforms	6	Uncharacteristic
56		AngII	ANG and its isoforms	6	Upregulated
57	27329205	AngII	ANG and its isoforms	6	Upregulated
58		cMyC	cMyC	6	Upregulated
59		cMyC	cMyC	6	Upregulated
60	26258537	cMyC	cMyC	6	Upregulated
61	2404640	Creatine kinase	Creatine kinase	6	Downregulated
62		Creatine kinase	Creatine kinase	6	Upregulated
63		Creatine kinase	Creatine kinase	6	Upregulated
64		FOXO3	FOXO3	6	Upregulated
65		FOXO3	FOXO3	6	Upregulated
66	29049183	FOXO3	FOXO3	6	Upregulated
67		GBP5	GBP5	6	Upregulated
68		GBP5	GBP5	6	Downregulated
69	29381915	GBP5	GBP5	6	Downregulated
70		Gelatinase B	Gelatinase and its isoforms	6	Upregulated
71		Gelatinase A	Gelatinase and its isoforms	6	Upregulated
72		Gelatinase A	Gelatinase and its isoforms	6	Upregulated
73		GP VI 13254CC	GP and its isoforms	6	Upregulated
74		glutathione peroxidase (GPx)	GP and its isoforms	6	Upregulated
75		glutathione reductase (GR)	GP and its isoforms	6	Upregulated
76	29049183	GSE66360	GSE	6	Uncharacteristic
77	29049183	GSE34198	GSE	6	Uncharacteristic

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
78	29049183	GSE48060	GSE	6	Uncharacteristic
79		GZMB	GZM	6	Upregulated
80	29381915	GZMA	GZM	6	Downregulated
81		GZMA	GZM	6	Downregulated
82	26676325	CLDN10	CLDN and its isofroms	7	Uncharacteristic
83	26676325	CLDN19	CLDN and its isofroms	7	Uncharacteristic
84	26864512	Copeptin - CVS	Copeptin	7	Upregulated
85	26864512	Copeptin - AO	Copeptin	7	Upregulated
86	26340515	CPU	CPU	7	Upregulated
87	26340515	proCPU	CPU	7	Downregulated
88		CXCL5	CXCL5	7	Downregulated
89	29381915	CXCL5	CXCL5	7	Downregulated
90	26258537	ETS1	ET and its isoforms	7	Upregulated
91	24046434	ET-1	ET and its isoforms	7	Upregulated
92	29381915	FPR1	FPR1	7	Upregulated
93		FPR1	FPR1	7	Upregulated
94	4042327	LDL	LDL	7	Upregulated
95	16251892	LDL	LDL	7	Upregulated
96	17847002	LRP8 variant	LRP and its isoforms	7	Upregulated
97	24906453	LRP6	LRP and its isoforms	7	Upregulated
98		Mac-1	Mac-1	7	Upregulated
99		Mac-1	Mac-1	7	Upregulated
100	29049183	MYBL2	MYBL2	7	Upregulated
101		MYBL2	MYBL2	7	Upregulated
102	12939547	Osteopontin	Osteopontin and its isoforms	7	Upregulated
103	28253327	Osteoprotegerin	Osteopontin and its isoforms	7	Upregulated
104	16902161	phospholipase A2	Phospholipase A2	7	Upregulated
105		phospholipase A2	Phospholipase A2	7	Upregulated
106		Tissue plasminogen activator	Plasminogen	7	Upregulated
107		Plasminogen activator	Plasminogen	7	Upregulated
108	26676325	SLC5A4	SLC and its isoforms	7	Upregulated
109	26676325	SlC22a9	SLC and its isoforms	7	Upregulated
110		ST2	ST2	7	Upregulated
111		soluble ST2	ST2	7	Uncharacteristic

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
112	29049183	COL5A2	COL5A2	8	Upregulated
113	11693755	eNOS	eNOS	8	Upregulated
114		Lp-PLA2	Lp-PLA2	8	Upregulated
115		TIMP-2	TIMP-2	8	Uncharacteristic
116		rs3807989	rs3807989	8	Upregulated
117	26278136	HDC	HDC	8	Upregulated
118	22155456	*ABCG1*	*ABCG1*	8	Downregulated
119	21195082	Abro1	Abro1	8	Uncharacteristic
120	26258537	JUN	JUN	8	Upregulated
121		VAMP8	VAMP8	8	Uncharacteristic
122		HNRPUL1	HNRPUL1	8	Uncharacteristic
123	16175505	*KIAA0992*	*KIAA0992*	8	Uncharacteristic
124		STAT1	STAT1	8	Upregulated
125		SPZ1	SPZ1	8	Upregulated
126		B cells	B cells	8	Downregulated
127		caveolin-3	caveolin-3	8	Upregulated
128		SERCA2a	SERCA2a	8	Upregulated
129		18F-Fluciclatide	18F-Fluciclatide	8	Upregulated
130	27329205	KLK1	KLK1	8	Upregulated
131	27306684	sMICA	sMICA	8	Downregulated
132		Nox2	Nox2	8	Upregulated
133		Cys282Tyr mutation	Cys282Tyr mutation	8	Upregulated
134		TFPI	TFPI	8	Upregulated
135		GNB3 825T allele	GNB3 825T allele	8	Upregulated
136		sVCAM-1	sVCAM-1	8	Upregulated
137		CYP11B2	gene encoding aldosterone synthase (CYP11B2)	8	Upregulated
138		S100A4	S100A4	8	Upregulated
139		SCN5A	SCN5A	8	Uncharacteristic
140		C1019T polymorphism	C1019T polymorphism	8	Uncharacteristic
141		uPAR	uPAR	8	Upregulated
142		APC	APC	8	Downregulation
143	29095267	HOX	HOX	8	Uncharacteristic
144	29049183	SOCS3	SOCS3	8	Uncharacteristic
145	29049183	VAPA	VAPA	8	Uncharacteristic

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
146		Paraoxonase gene	Paraoxonase gene	8	Upregulated
147		Arg506Gln	Arg506Gln	8	Upregulated
148		GAG	GAG	8	Upregulated
149		Platelet factor IV	Platelet factor IV	8	Upregulated
150		Beta-enolase	Beta-enolase	8	Upregulated
151	25958931	p53	p53	8	Downregulated
152	25180781	PCSK9	PCSK9	8	Upregulated
153		IgM	IgM	8	Downregulated
154		IgG	IgG	8	Downregulated
155		Enterovirus antibodies	Enterovirus antibodies	8	Upregulated
156	16175505	*ROS1 (G)*	*ROS1 (G)*	8	Uncharacteristic
157	16175505	*TAS2R50* (G)	*TAS2R50* (G)	8	Uncharacteristic
158	16175505	OR13G1 (G)	OR13G1 (G)	8	Uncharacteristic
159	20424303	Q192R & L55M	Q192R & L55M	8	Upregulated
160		(BNP)	brain natriuretic peptide (BNP)	8	Uncharacteristic
161		SH2B3	SH2B3	8	Upregulated
162		CDKN2B-AS1 gene	CDKN2B-AS1 gene	8	Upregulated
163		R353Q	R353Q	8	Upregulated
164		stromelysin gene	stromelysin gene	8	Upregulated
165	26676325	Cilp2	Cilp2	8	Uncharacteristic
166		SOD	superoxide dismutase (SOD)	8	Upregulated
167	26258537	CEBPA	CEBPA	8	Uncharacteristic
168	26258537	AHR	AHR	8	Upregulated
169	26258537	RELA	RELA	8	Uncharacteristic
170		xanthine oxidase (XO)	xanthine oxidase (XO)	8	Upregulated
171		catalase	catalase	8	Uncharacteristic
172		Low plasma Serine	Low plasma Serine	8	Downregulated
173		Low plasma Glycine	Low plasma Glycine	8	Downregulated
174		Serum amyloid A protein	Serum amyloid A protein	8	Upregulated
175	27770663	Plasma Z	Plasma Z	8	Downregulated
176		ICAM-1	Plasma soluble ICAM-1 (sICAM-1)	8	Upregulated
177	26073931	Plasma ADAMTS-13	Plasma ADAMTS-13	8	Downregulated
178	26676325	Ptgis	Ptgis	8	Uncharacteristic
179	26676325	CORO6	CORO6	8	Uncharacteristic

S.no.	PMID	MOLECULE NAME	MOLECULE CATEGORY	RANK	REGULATION
180	26676325	mfap4	mfap4	8	Uncharacteristic
181	26676325	Hus1b	Hus1b	8	Uncharacteristic
182	26676325	PCGF1	PCGF1	8	Uncharacteristic
183	26676325	TROAP	TROAP	8	Uncharacteristic
184	26676325	zar1	zar1	8	Uncharacteristic
185	26676325	lmf2	lmf2	8	Uncharacteristic
186	26676325	ppyr1	ppyr1	8	Uncharacteristic
187	26676325	ephX1	ephX1	8	Uncharacteristic
188	26676325	Fkbp1b	Fkbp1b	8	Uncharacteristic
189	26676325	RNF207	RNF207	8	Uncharacteristic
190	26676325	HSPB8	HSPB8	8	Uncharacteristic
191		OPG	OPG	8	Upregulated
192		IFN-γ	IFN-γ	8	Upregulated
193		Glu298Asp variant	Glu298Asp variant	8	Upregulated
194		Total homocysteine	Total homocysteine (tHCY)	8	Upregulated

6. CONCLUSIONS

Myocardial infarction, commonly known as heart attack, occurs when the blood flowing to a part of the heart ceases or decreases, causing damage to the heart muscle. Myocardial infarction is widely studied in the field of medicine and in the field of biotechnology. It bears a global mortality rate of 30%. With a mortality rate that high, more diagnostic and prognostic tools are required. A lot of research has been done to establish various diagnostic tools which include assays to test for different proteins, mRNAs and ncRNAs. The current study also involves the compilation of coding biomolecules (genes, proteins and mRNAs) and noncoding molecules (ncRNAs) by utilizing different biocuration techniques. ncRNAs are studied because 95%-98% of the human genome is non-coding, thus ncRNAs are present in more number than mRNAs in patients with Myocardial infarction, hence the chance of finding a specific marker is higher. Coding molecules are compiled because the goal of this research was to analyze small scale individual studies to increase the efficiency of the results. To achieve this goal, a bioinformatics approach was taken to screen, identify and determine the key molecules (genes, proteins, ncRNAs) involved in Myocardial infarction. Further biocuration techniques were applied and screened to narrow down to 2247 papers, which gave us information about all the proteins, genes, mRNAs and ncRNAs involved in Myocardial infarction.

Our study has enlisted a total of 116 coding molecules and 83 ncRNAs which have been experimentally proved to have a role in Myocardial infarction.

1. Interleukin-6, a cytokine, and Toll like receptors (TLRs), proteins, have shown to be reported in several papers which indicates that they have a major role in Myocardial infarction.
    - Interleukin-6 and TLRs are shown to be upregulated in 100% of the papers screened.

2. miR-133 and miR-208 have shown to be reported in several papers which indicates that they have a major role in Myocardial infarction.
    - miR- 133 is shown to be upregulated in 78% of the papers screened.
    - miR- 208 is shown to be upregulated in 55% of the papers screened.

7. FUTURE SCOPE

1. Generation of molecular networks using pathway databases and analysis of the same will provide new insights into molecular mechanisms of MI and accelerate biomarker identification.

2. A comparison of various ncRNA databases and extraction of information on ncRNA/ gene interaction will prove to be a valuable resource for identification of biomarkers for various cardiovascular diseases.

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