Original Article

Suggested Drugs for Human Strabismic Extraocular Muscle

Nasim Afhami [1], MS; Mazaher Maghsoudloo [1,2,*],PhD; Ahmad Shojaei [3], MD; Farhad Adhami Moghadam [4], MD; Seyed-Hashem Daryabari [5], MD

1. The Center of Research and Training for Occupational Technical Safety and Health, Tehran, Iran.
2. Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
3. Basir Eye Health Research Center, Iran University of Medical Sciences, Tehran, Iran.
4. Department of ophthalmology, Fauclty of Medicine, Tehran Medical Sciences Islamic Azad University, Tehran, Iran.
5. Chemical Injures Research Center, Systems Biology and Poisonings Institutes, Baghiyatallah University of Medical Sciences. Tehran, Iran.

***Corresponding Author:** Mazaher Maghsoudloo

E-mail: m.maghsoudloo@ut.ac.ir

Abstract

Background: Misalignment of the eyes is called strabismus that is one of the most common disorders in ophthalmology. This disorder must be rapidly diagnosed because late diagnosis increases the probability for surgery. Genetic and environmental risk factors are involved in the prevalence of strabismus. This study aimed to investigate differentially expressed genes in patients with the extraocular muscles (EOMs) and heathy individuals, and also elucidating suggestive drugs for the treatment of the disease.

Material and Methods: The data were collected from Gene Expression Omnibus, comprising series of GSE38780. To detect hub genes with dysregulated expression, microarray data were used. Statistical methods extract differentially expressed genes and network analyses were used to detect potential biomarkers of EOMs. Then drugs were suggested based on potential biomarkers.

Results: 2009 DEGs were identified by help of adjusted P value and log fold change. DEGs were mapped on PPI data obtained from STRING database and PPI network was extracted after considering interactions. Centrality of nodes in network was calculated and 10 nodes with highest centrality as marker genes were identified. Ten potential biomarker including CYCS, NDUFV1, COX5A, NDUFB9, SDHA, NDUFS2, UQCR10, UQCR11, MDH2 and UQCRC1 were identified and Six candidate drugs based on them were suggested including NV-128, ME-344, Metformin Hydrochloride, Famoxadone, Albumin Human and Cisplatin.

Conclusion: This work was conducted to identify potential biomarker for strabismus and seeking the candidate drugs for it. The marker genes are the most important genes based on statistical and network analysis. By use of potential biomarkers, six drugs were suggested.

Keywords: Strabismic Human Extraocular Muscle; Drug; Biomarker; Differential Expressed Genes; PPI Network.

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Introduction

Strabismus is an uniocular or binocular anomaly in the eyes [1]. In the extraocular muscles (EOMs) of strabismus, muscles appearance lies outside the common phenotypic. Strabismus is one of the most common observed phenotypes. The prevalence of strabismus has been estimated to be 2.5-4 % in all over the world and it is known as one of the most common problems in ophthalmology [2].

In order to investigate the EOM, a health care must consider the function of the movement of eye muscles in opposite directions. Strabismus comprises a group of disorders such as constant or intermittent ocular deviation that is also associated with uniocular and binocular failure. Strabismus disorders can be associated with failure in eye's development. It may decrease vision and/or leads to blindness [3].

Early detection of people involved with risk of Strabismus disorder can help to theirs treatment and also successes for the treatment of amblyopia. Early detection also reduces frequency of surgery. Two factor are introduced as causes for the disease including genetic and environmental risk factor [4, 5]. The lack of access the whole muscle's tissue is a major challenge in pathology of eye's muscles disorders that is a deadlock for both healthy people and patients with strabismus [6].

Strabismus has two syndromic and non-syndromic forms. The most cases have non-syndromic form. The genes contributing to syndromic forms are known but these are elusive in non-syndromic forms [7]. For first time, Hippocrates observed that strabismus inherited from parents to offspring. Several attempts have been made to identify the difference between strabismic and healthy EOMs, such as histological investigations [8] and ultrastructural analyses [9]. Abnormalities were subtle and non-discriminative unless in specific molecules. Several studies have been identified differences in molecular composition. It was recently reported that gene expression of strabismic muscles is different in myosins, extracellular matrix and myogenic regulatory factors [10].

Studies have reported the regulatory network of myogenic factors and other factors involved in prevalence of eye's muscles disease such as strabismus. They also stated that abnormality in myogenesis-related gene that increases the disorder of concomitant strabismus [10].

A review article was conducted on non-syndromic strabismus such as the gene expression, linkage, twin and family studies and showed that identified non-syndromic strabismus genes helping for early treatments [7]. It was also reported subcellular and molecular changes in strabismic muscles and inconsistency in plasticity mediated-signaling pathways [11].

Other study was conducted on gene expression profiling of orbital muscles and extracted eight downregulated gene expressions [12]. Other study also elucidated a understand about structure, pathophysiological and metabolic properties of EOMs [13].

Another study has identified gene related to POAG disease [14]. Different analyses were done for predicating DEGs functions. 20 top genes were identified by help of centrality analysis. This literature used PPI network for module mining. In our literature, DEGs were also extracted and then PPI network centrality properties were used for extracting potential biomarkers. Article study has suggested drugs for AMD disease [15]. The PPI network was constructed and modules were discovered by its help. Enrichment analyses were then done and drug-gene network was reconstructed. In our article, the candidate drugs were suggested

by used of query score and interaction score between genes and drugs. Suggested drugs are represented in table 3.

In the present study, we investigated differentially expressed genes (DEGs) between two groups. PPI network was extracted by pairwise correlation analysis. DEGs were mapped on PPI network using String portal [16]. The hub genes in network were detected based on centrality analysis. We evaluated these potential biomarker genes by help of biomarker genes identified in previous works. Candidate drugs for strabismus disease were suggested by use of DGIdb portal [17, 18].

Material and Methods

Dataset

Input dateset is affymetrix microarray data from eight subjects who suffered strabismic EOMs (n=4) that obtained from patients undergoing corrective surgery and healthy human organ donors (n=4). The dataset was deposited from Gene Expression Omnibus for Homo sapiens organism in series GSE38780 [19]. The dataset was also collected from previous studies [19] submitted to National Center for Biotechnology Information (NCBI) site (https://www.ncbi.nlm.nih.gov/) [20].

The expression of genes in normal EOMs differed from those in strabismic EOMs. The affymetrix expression microarray used in this work had 54675 gene probs.

Preprocessing

Affymetrix Probes must be converted to gene symbol. At the first, affymetrix data were pre-processed. Preprocessing stage has four stages:

Stage 1: Background correcting

Stage 2: Normalizing

Stage 3: Removing outliers

Stage 4: Calculating the expression

Hierarchical clustering was used to remove outliers form dataset. Hierarchical clustering is an unsupervised learning method that divides similar samples into same cluster. It is used to build a dendrogram or tree structures from data that show similarity between them. By help of hierarchical clustering, we can see how data points are far apart [21, 22]. Affy package was used for Background correcting and stats package was applied for Hierarchical clustering and detecting outliers.

Identifying DEGs

Affymetrix Probes were matched to genes after preprocessing. The mean was calculated for probs matched with same gene. Ebayes is empirical bayes statistics for differential gene expression and used in order to find different expression. It uses a liner model fitting with data. It computes log-odds of differential expression and moderated t-test and F-test. Ebayes used empirical Bayes for moderating the standard errors towards a global value. Ebayes give us P value, log for change, t-value and other metrics for different between samples. We use P value and log for change for DEG findings [23].

Log fold change is a parameter that gives us distinction and distance between samples. The largest distances between samples correspond to leading log fold changes [24]. Fold change (FC) was computed as a ratio of changes between final value and initial value over initial value. Log2 was also used to make leveled FC [25].

We select genes that have two condition P value < 0.05 and $-0.80 < \log FC < 0.80$ as DEGs. We empirically select these thresholds. The values of two parameter P value and logFC of DEGs were represented in volcano plot in figure 3.

Extracting Network

The gene expression pattern is similar in healthy people, while it is dysregulated in patient subjects. Gene relations can represented with network. Network feature for nodes was measured that implicating on their importance. To consider connection between DEGs, they were mapped on PPI network using String database [16] portal (https://string-db.org/). Node centrality represents importance and effect of the corresponding DEG. Centrality analysis was performed and MCC properties were calculated for each nodes in the network. CytoHubba [26] toolkit (https://cytoscape.org/) [27] was used to explore genes with highest centrality [28, 30].

Identifying Biomarkers

The most important DEGS with highest centrality were identified and called as biomarker of strabismus EOMs. The selected biomarkers with MCC (maximal clique centrality) parameter are represented in Table 1. The MCC intuition is the tendency for clustering in a yeast interaction network and calculated as largest size clique of the part in which node is belonged it.

The potential biomarkers were evaluated based on biomarkers identified in the previous studies. For this propose, platform DisGeNET was used [31-33]. 716 potential biomarkers were introduced for this disorder. Five of our biomarkers were introduced in previous lectures. The network between biomarkers is depicted in figure 4.

Identifying pathway that the potential biomarkers exists

The pathways that potential biomarkers of strabismis EOMs is involved in them were identified. The pathways were identified by help of KEGG portal [34-36].

Suggestion of drugs for the potential biomarkers

We investigated the current drugs for ten potential biomarkers. In this article, six drugs were suggested for this disease. Drugs were suggested by using DGIdb portal (https://www.dgidb.org/) [17, 18]. Drugs for five potential biomarkers were reported in Table 3.

For searching interactions between gene and drug two score, Query and Interaction are calculated. The query score is based on the overlap of interactions [37, 38]. The Query score is given in Equation2. It depends on the publication count and source count also gene counts from the search set that totally interact with the drug, and relative drug specificity (Equation 1).

$$\text{Relative drug specificity} = \frac{\text{average known gene partners for all drugs}}{\text{known gene partners for drug d}}$$

Equation 1

$$\text{Query Score} = (\text{publication count} + \text{source count})$$

Equation 2

queried genes interacting with drug d relative drug specificity

The Interaction score is based on the evidence of an interaction, as shown in Equation 4. Interaction score depends on the publication count and source count, also relative gene specificity (Equation 3) and relative drug specificity (Equation3).

$$\text{Relative gene specificity} = \frac{\text{average known drug partners for all genes}}{\text{known drug partners for gene d}}$$

Equation 3

Results

Dataset

The dataset was deposited from GEO with

GSE38780 series. The input dataset had eight RNA samples. After preprocessing, eighth sample was distinguished as outlier and deleted from dataset and preprocessing was then replicated. Following remove outlier, seven samples were remained that three samples were belonged to EOM patients undergoing corrective surgery and four samples were belonged to healthy human organ donors. Platform used were Affymetrix.

Eighth sample was removed and seven samples were remained. Hierarchical clustering was conducted and new hierarchical clusters are represented in figure 1. figure 2 presentes the box plot for displaying the distribution of samples.

As show in figure 2, mean of seven samples become similar after normalization.

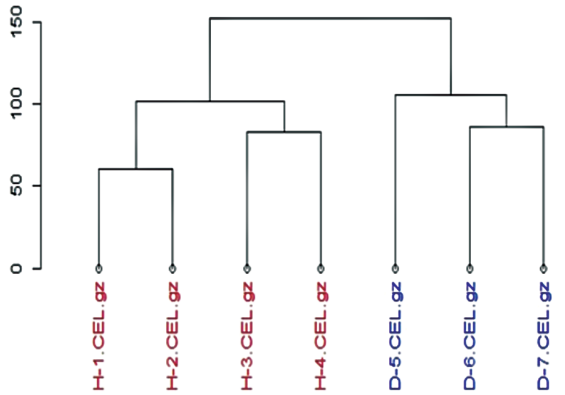

Figure 1: Hierarchical clustering of seven samples, representing distinguish between samples of two classes

Identification of DEGs

Ebayes for differential gene expression were used. Thresholds for P value and logFC was empirically selected. DEGs were identified by value of 0.05 as cut-point for P value and value of 0.80 for logFC. The dysregulated genes as DEGs were slected based on thresholds and 2009 DEGs were identified. LogFC and P value of DEGs are represented in figure 3.

Extracting PPI network

We construct network between DEGs with respect to connection between them. DEGs were mapped on PPI data and PPI network was constructed. This network was used for extracting the most important nodes as hub genes.

The identification of potential biomarkers

Ten genes were selected by using networks property. MCC network property can represent the most important and influencing genes. Considering MCC features, we selected genes with highest centrality. Ten centrality genes were presented in Table 1.

These genes are the most important genes in incident strabismic EOMs based on logFC and network analysis. The network and interaction

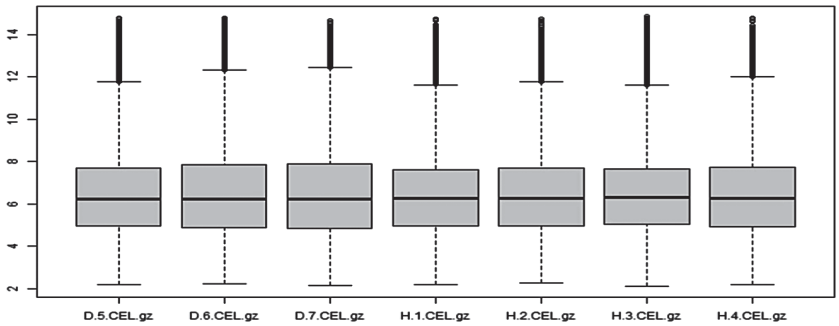

Figure 2: Box plot of samples, that displaying the distribution of samples after normalization

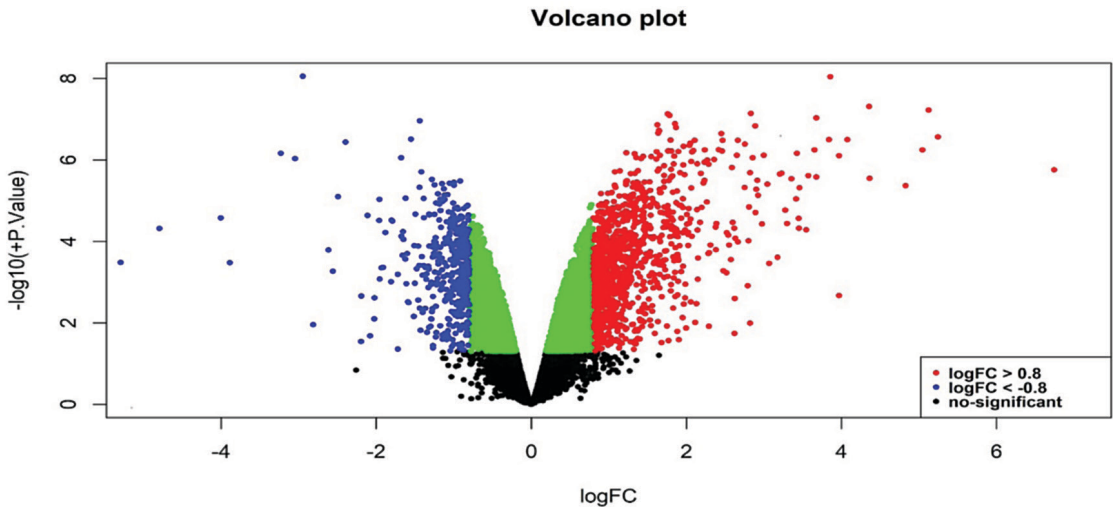

Figure 3: Volcano plot for DEGs. The green spot have P value < 0.05 and the red spot have P value < 0.05 & logFC > 0.8, Also blue spot have P value < 0.05 & logFC < - 0.8. Black spot are not significant

Table 1: Ten centrality genes for strabismic (EOMs)

Rank	Name	Score
1	CYCS	1641077
2	NDUFV1	1629328
3	COX5A	1624674
4	NDUFB9	1623656
5	SDHA	1580803
6	NDUFS2	1566030
7	UQCR10	1534580
8	UQCR11	1533144
9	MDH2	870607
10	UQCRC1	828334

between of selected genes are shown in figure 4.

Out of 10 selected genes, five genes were identified as potential biomarkers of strabismus in pre-searching in this field. The identified potential biomarkers are NDUFV1, NDUFB9, SDHA, NDUFS2 and MDH2.

Identifying pathway that the potential Biomarker exists

Pathways that the 10 selected genes exists in them were provided by using KEGG portal. Out of 40 pathways, 10 pathways with largest P value are represented in Table 2. P value and other statistical parameters are given in Table 2. The probability of thesse genes related with each disease is also shown.

Suggestion of drug

We suggest six drugs for strabismic EOMs based on current drug for the introduced 10

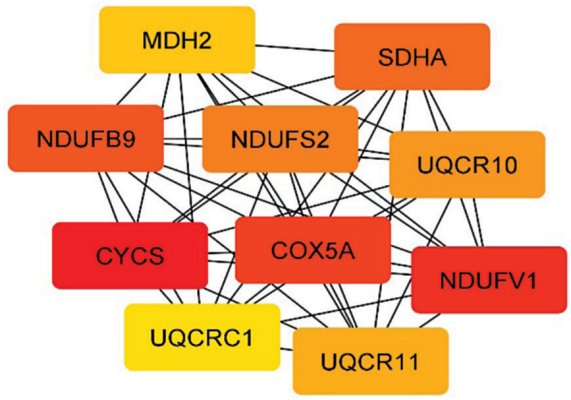

Figure 4: PPI Network between selected ten genes

Table 2: KEGG Human disease pathway that the ten selected genes

Term	Genes	P value	Adjusted P value	Odds Ratio	Combined Score
Non-alcoholic fatty liver	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	7.92E-19	3.09E-17	1223.26	50985.76
Parkinson	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	6.15E-17	1.20E-15	740.625	27645.83
Oxidative phosphorylation	NDUFB9;UQCRC1;NDUFS 2;UQCR11;UQCR10;SDHA; NDUFV1;COX5A	1.37E-16	1.39E-15	635.68	23217.21
Prion	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	1.42E-16	1.39E-15	672.47	24537.09
Huntington	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	4.03E-16	3.14E-15	596.75	21153.59
Amyotrophic lateral sclerosis	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	1.95E-15	1.23E-14	497.78	16859.58
Alzheimer	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	2.21E-15	1.23E-14	490.75	16560.38
Diabetic cardiomyopathy	NDUFB9;UQCRC1;NDUFS 2;UQCR11;UQCR10;SDHA; NDUFV1;COX5A	4.34E-15	2.11E-14	406.05	13428.84
Thermogenesis	NDUFB9;UQCRC1;NDUFS 2;UQCR11;UQCR10;SDHA; NDUFV1;COX5A	1.28E-14	5.55E-14	352.964	11290.78
Pathways of neurodegeneration	NDUFB9;UQCRC1;CYCS; NDUFS2;UQCR11;UQCR10; SDHA;NDUFV1;COX5A	2.18E-14	8.52E-14	377.07	11860.86

selected genes. New current drugs for five of selected genes are reported in table 3. In this table, query score and interaction score for each pair of gene and drug are shown.

Discussion

Strabismus is one of the most common disorders in ophthalmology. Early diagnosis and treatment of strabismus are accompanied with excellent results. Genetic is one of risk factor of the prevalence of strabismus. In this article, ten genes that with abnormal expression related to strabismus EOMs disease are selected. A set of ten genes with analysis of microarray data and with respect to differential gene expression between disease and control

Table 3: Suggested drug for the 10 selected genes

Gene	Drug	Query Score	Interaction Score
NDUFV1	NV-128	0.26	0.42
NDUFB9	NV-128	0.26	0.42
NDUFS2	NV-128	0.26	0.42
NDUFV1	ME-344	0.26	0.42
NDUFB9	ME-344	0.26	0.42
NDUFS2	ME-344	0.26	0.42
NDUFV1	METFORMIN HYDROCHLORIDE	0.24	0.39
NDUFB9	METFORMIN HYDROCHLORIDE	0.24	0.39
NDUFS2	METFORMIN HYDROCHLORIDE	0.24	0.39
UQCR10	FAMOXADONE	13.13	190.95
MDH2	ALBUMIN HUMAN	0.58	4.24
MDH2	CISPLATIN	0.06	0.44

groups was identified. Dysregulated special genes are identified. These genes are involved in the increased risk of disease and must be considered for the treatment. These genes were identified by help of statistical and network analysis methods.

Previous papers have identified biomarkers of strabismic EOMs [28-31]. The NDUFV1, NDUFB9, SDHA, NDUFS2 and MDH2 genes are previously introduced as biomarkers for this disorder, and we proposed five genes (including: CYCS, COX5A, UQCR10, UQCR11, and UQCRC1) as novel genes in EOMs that can may affect in EOMs. We identified the human disease pathways as shown in Table 2. Other biomarkers pathways of human disease were researched by help of KEGG portal and 10 pathways were extracted with respect to highest P value. KEGG human disease pathway for markers included Non-alcoholic fatty liver, Parkinson, Oxidative phosphorylation, Prion, Huntington, Amyotrophic lateral sclerosis, Alzheimer, Diabetic cardiomyopathy, Thermogenesis and pathways of neurodegeneration and etc [34-36]

NDUFV1 encodes a protein that its function is related to activity of 4 iron, 4 sulfur cluster binding; nucleotide binding; NADH dehydrogenase (ubiqinone); and also in mitochondrial electron transport. The NDUFV1 gene is also found in several pathways including respiratory electron transport, heat production via uncoupling proteins, and also ATP synthesis through chemiosmotic coupling [39]. It has been reported in several disease classes such as, congenital, hereditary, neonatal diseases and abnormalities, nutritional and metabolic diseases, nervous system [40-42]. The potential biomarker COX5A belongs to protein class enzyme. The encoded protein is a Subunit of the mitochondrial respiratory chain complex IV. This complex containing 13 subunits, including mitochondrial and nuclear encoded subunits [39]. It has been reported in multiple disease classes including, congenital, hereditary, neonatal diseases and abnormalities, nutritional and metabolic diseases [43, 44] and etc.

NDUFB9 is a subunit of the NADH dehydrogenase (ubiquinone) complex with, the largest complex of the electron transport chain [45], but it is also involved in the transfer of in of electrons from NADH to the respiratory chain. This protein belongs to complex I that is not directly involved in catalysis process. Protein`s activities are NADH dehydrogenase and oxidoreductase. It is also seen in different disease classes such as nutritional and metabolic diseases [46], cardiovascular diseases [47] and etc.

SDHA plays a pivotal role in making one of subunit of the SDH enzyme. This enzyme related to function of mitochondria, which supply consuming energy of cell from food. This enzyme exists in two energy pathways, including the oxidative phosphorylation and citric acid cycle [48, 49]. It has been reported in several disease classes, such as congenital, hereditary, neonatal diseases and abnormalities, nutritional and metabolic diseases and nervous system diseases [50, 51], and also digestive system diseases, neoplasms [52].

NDUFS2 belongs to protein class enzyme. The encoded protein is a central part of mitochondrial membrane respiratory chain NADH dehydrogenase (complex I). When a mutation in this gene occurs, mitochondrial complex I deficiency is seen. This gene exists in several pathways, such as ATP synthesis via chemiosmotic coupling, respiratory electron transport and heat production through uncoupling proteins and Complex I biogenesis. This gene plays a major role in NADH dehydrogenase (ubiquinone) and ubiquitin protein ligase binding [53]. NDUFS2 is found in different disease classes including, nutritional and metabolic diseases [54], congenital, hereditary, neonatal diseases and abnormalities, eye diseases, nervous system diseases [55] and etc.

MDH2 is related to protein class enzyme. The encoded protein exists in mitochondria and it`s function is related to metabolic cycle of cytosol and mitochondria. This gene exists in several pathways including, respiratory electron transport, glycolysis, heat production via uncoupling proteins and ATP synthesis by chemiosmotic coupling. It is existed in neoplasms, female urogenital diseases and pregnancy complications, endocrine system diseases [56] and etc.

The first novel gene is CYCS. It is a protein coding gene. The CYCS gene encodes a highly conserved small heme protein that function related to mitochondrial ATP production and plays a role in initial stage of apoptosis [57]. The other biological processes are respiratory chain and electron transport. The predicated location is intracellular site. If CYCS gene mutate, autosomal dominant nonsyndromic thrombocytopenia can occur. Various CYCS pseudogenes are found in the human genome. It is seen in fatty liver disease [58], Alzheimer [59] and nervous system diseases [60] and etc.

UQCR10 is another novel gene that belongs to complex III subunit X. This gene is in protein class enzyme. The encoded protein exists in the central unit of the respiratory chain of the inner mitochondrial membrane [61]. This gene also observed in several pathways, such as ATP synthesis via chemiosmotic coupling, respiratory electron transport, mitochondrial complex III assembly and heat production by uncoupling proteins. It has been observed in digestive system diseases and musculoskeletal diseases [62]. Also, this gene encodes a protein which is a subunit of complex III subunit XI [63] and has an important role in mitochondrial respiratory chain. This protein works as binding factor of iron-sulfur protein in this complex. It is found in neoplasms disease classes [64].

UQCRC11 is one of novel genes that is involved in mitochondrial electron transport, oxidative phosphorylation and in ubiquinol to cytochrome c. It is also found in several pathways including heat production via uncoupling proteins, ATP synthesis by chemiosmotic coupling, mitochondrial complex III assembly and respiratory electron transport. It is seen in multiple disease classes such as mental disorders [65], nervous system diseases [66] and etc.

We aimed to use ten selected genes of strabismus EOMs as targeted therapeutically. For suggesting appropriate drugs. New genes based on pathways, molecular functions and gene families are related to drugs.

The current drugs were searched for 10 selected genes and half of them have drug. These genes are including NDUFV1, NDUFB9, NDUFS2, UQCR10 and MDH2. The drugs are NV-128, ME-344, Metformin Hydrochloride, Famoxadone, Albumin Human and Cisplatin. The suggestive drugs for strabismus EOMs are reported in Table 3.

For NDUFV1 gene, three suggested drugs are including NV-128, ME-344 and Metformin Hydrochloride. There is an inhibitory relation between drugs with NDUFV1 gene. In inhibitory relation, drug is bond with its target and the expression level is decreased. Inhibitors have enzymatic inhibitory activities and decreases enzyme activity by binding with enzyme.

Three NV-128, ME-344 and Metformin Hydrochloride drugs have direct interaction with NDUFV1 gene and mechanism of interactions are mitochondrial complex and NADH dehydrogenase inhibitor.

Two genes of NDUFB9 and NDUFS2 have also have inhibitory interactions with drugs. They have direct interactions and mechanism of interactions is mitochondrial complex and NADH dehydrogenase inhibitor. For NDUFV1, NDUFB9 and NDUFS2 genes, query score and interaction score of drugs NV-128, ME-344 are higher than scores of metformin hydrochloride and source of drugs is the chEMBL bioactivity database.

For UQCR10 gene, Famoxadone drug is present. Directionalities are not identified and source of those drugs is drug target common. For gene MDH2, Two drugs albumin human and cisplatin have been introduced. Interaction type & directionality of two drugs are not identified and source of searched drugs is NCI cancer gene index.

Interaction score is evidence based on static score. Interaction score are calculated as multiple of evidence score and relative drug specificity and relative gene specificity. Relative drug specificity is ratio of average known gene partners for all drugs for knowing gene partners for given drug. Relative gene specificity is also a ratio of average known drug partners for all genes to the known drug partners for given gene.

Interaction score between gene NDUFV1 and drug NV-128 is 0.42. Interaction score between NDUFB9 and NDUFS2 with this drug is 0.42. Drug ME-344 has same interaction score with each of three genes NDUFV1, NDUFB9 and NDUFS2. Metformin Hydrochloride drug has interaction score of 0.39 with each of these three genes.

Famoxadone drug with UQCR10 gene has interaction score 190.95 and MDH2 gene has interaction score of 4.24 with Albumin Human drug and score 0.44 with drug cisplatin. Any drug has not been identified for CYCS, COX5A, SDHA, UQCR11 and UQCRC1 genes.

Conclusively, ten selected genes associated with strabismus EOMs are identified and based on these genes, six drugs including

NV-128, ME-344, metformin hydrochloride, famoxadone, albumin human and cisplatin are introduced as therapeutic of EOMs. One of the limitations of our study is selected genes are also involving in other diseases, and it is better to validate novel genes for further analysis.

Conclusions

In this study, five genes are introduced as novel genes in EOMs based on statistical and network analysis. And also, some drugs for this disorder were then suggested. As a result, co-expression network analyses help to identify novel therapeutic genes and drugs in EOMs.

Authors ORCIDs

Nasim Afhami:
https://orcid.org/0000-0001-6360-0394
Mazaher Maghsoudloo:
https://orcid.org/0000-0002-3218-1059

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.