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A Bioinformatics Study of Gene Biomarkers in Congenital Hyperinsulinism

Abstract

Hyperinsulinism is a condition where insufficient sugar reaches the child's brain leading to lifelong and permanent damage. This state takes place when the beta cells in the pancreas produce large (excessive) values of insulin, leading to frequent drops in blood sugar in the infant. The study examined the gene biomarkers of this disease using bioinformatics methods. After determining candidate genes from in vivo, in vitro, and silico studies, their expression data was collected from bioinformatics databases. The expressive data obtained from each group was standardized compared to the control group. Then the communication network of expressive data of candidate genes in sick and healthy individuals was plotted separately in MATLAB software, and the structural parameters of communication networks of expression data were calculated and compared. Using Reactome and Disaesome databases, the correctness of these networks and the determined biomarkers were examined once more. All statistical calculations in the study were done using R and MATLAB software. Essential genes were identified using five criteria of centrality - degree, proximity, radius, between, and the greatest degree of the neighborhood. There, 5 genes INS, AKT1, PRKACG, PRKACB, and PRKACA possessed the most iterations according to all the results of the above 5 central criteria methods. The study indicated that AKT1, PRKACG, PRKACB, PRKACA, and INS genes could be considered diagnostic biomarkers of Congenital Hyperinsulinism (CHI).

Keywords:*Bioinformatics, Biomarker, Congenital Hyperinsulinism, Computational Biology*

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Introduction

CHI is a rare type of disease that leads to a severe drop in blood sugar in infants. The disease prevents enough sugar from reaching the child's brain and leads to lifelong and permanent damage. This happens when the beta cells in the pancreas produce large (excessive) values of insulin that result in frequent drops in blood sugar in the baby (a clinical condition unlike diabetes) (Hussain, 2005). The prevalence of the disease is between 1 in every 30,000 to 50,000 live births (Al-Nassar et al., 2006; De Visschere et al., 2007). In Iran, about 3 cases of the disease have been reported in infancy (this statistic is up to 2014) (Mosavati et al., 1994; BayatMokhtari et al., 2205). The incidence of the disease is about 1 in every 40,000 cases in Northern Europe. A disorder in the sulfonylurea receptor 1 (SUR1) gene has been diagnosed in less than 60% of patients with CHI. Hence, the incidence of the disease is more reported in areas of the world where consanguineous marriage is high, such as Saudi Arabia and Ashkenazi Jews. This rate has been reported in Saudi Arabia as about 1 in every 2500 births (Al-Nassar et al., 2006; Zumkeller, 1999). Indeed, most cases of the disease are sporadic, but Al-Nassar et al. reported some cases of the disease, and more than 67% of them had a history of this disease in their family (Al-Nassar et al., 2006).

Low or undetectable serum levels of insulin do not reject the diagnosis of HH (Brady et al., 2005; Ferrara et al., 2016; 8). In some cases, serum levels of C-peptide (≥ 0.5 ng/ml) and IGFBP-1 (≤ 110 ng/ml) could help confirm the diagnosis of HH (with a specificity of 100% and 96.6%) (Ferrara et al., 2016). The metabolic effect of high insulin secretion is shown through low serum levels of ketone bodies and fatty acids during hypoglycemic periods. There are no relationships between the measured insulin concentration and the severity of hypoglycemia (Palladino et al., 2008). In some difficult cases, HH diagnosis was not based on the serum insulin/c-peptide ratio, but on the clinical manifestations and biochemical profile of insulin action (Ferrara et al., 2016; Senniappan et al., 2012;Shah et al., 2017). Among the other diagnostic criteria for HH are negative urine ketones and an increase greater than or equal to 30 mg/dl of plasma glucose in response to the administration of 0.5 mg of glucagon intramuscularly (Arnoux et al., 2010; Al-Nassar et al., 2006). In some cases, some biochemical and clinical features could help diagnose certain forms to help CHI. The increase in serum ammonia concentration in patients with HH syndrome is called (HI/HA) hyperinsulinism/hyperammonemia (Stanley et al., 1998). Recently, some cases have been reported of new genetic mechanisms in patients with CHI and other syndromic features. The underlying molecular mechanisms of CHI are still not known in the vast majority of patients with HH who respond to diazoxide (Demirbilek and Hussain, 2017).

Mutations in the genes that encode K-ATP channel proteins are the most prevalent cause of severe CHI (Thomas et al., 1995, 1996). Moreover, these mutations (inactivating or reducing the function) are mainly the cause of diffuse CHI that does not

respond to treatment (Gutgold et al., 2017; Kapoor et al., 2013; Lord et al., 2013; Snider et al., 2013). Autosomal dominant genetic mutations are mostly the cause of mild CHI types (Arya et al., 2014; Pinney et al., 2008).

Systemic biomedicine is the use of computational sciences to model and simulate biomedical systems to understand and evaluate and predict these systems accurately, particularly in the field of disease modeling. Furthermore, bioinformatics is a knowledge that provides the base for this type of modeling using advanced computational methods. This emerging knowledge, as an interdisciplinary knowledge, makes efforts to use the techniques available in computer science, mathematics, genetics, chemistry, physics, and other related sciences to solve different biological problems accurately, quickly, and at a lower cost compared to other methods.

In their study of patients with CHI, Banerjee et al. found that a large percentage of infants with this disease had congenital hyperinsulinism, which may be related to the severity of hyperinsulinism at diagnosis, which may require early care of these patients. makes it more serious (Banerjee et al., 2012), Glaser et al. (2011) calculated the risk of focal CHI in a fetus with a paternal recessive mutation of the K-ATP channel. Dunne et al. (2004) stated that disorders in 9 genes have been involved in the occurrence of CHI and are divided into 2 categories of metabolopathy and channelopathy.

Pinney et al. (2008) stated that in 20% (23 of 118) of patients responding to diazoxide, single-allelic K-ATP missense mutations were identified: 19 in ABCC8 and 4 in KCNJ11. All these mutations were found by gene expression studies. Bruining in one study and Mathew et al. in another stated that the estimated incidence of CHI is from 1 case in every 50,000 births in the Netherlands to 1 case in every 2,500 births in Saudi Arabia {which means a lot of connection with the level of kinship (ethnicity and kinship)} (Bruining, 1990; Mathew et al., 1988). Glaser et al. (2011) claimed that the incidence of the disease was estimated to be 1 in 10,816 among Ashkenazi Jews based on the carrier frequency for 2 K-ATP recessive mutations (Glaser et al., 2011). Sayed in one study and Snider et al. in another considered diazoxide as the first-line drug to control hypoglycemia in hyperinsulinism, but only in types in which K-ATP and GCK mutations occurred (Sayed et al., 2012; Snider et al., 2013). Maiorana et al. reported prescribing an experimental ketogenic diet for a child with CHI because of a spontaneous active GCK mutation, with frequent episodes of hypoglycemia despite medical treatment. The EEG was normal, and a significant improvement in his mental development and quality of life was seen after the first 6 months, the patient did not have epileptic seizures (Aronson, 2005).

Biomarker means a measurable index of some biological or biological states and conditions. Overall, a medical biomarker refers to anything that can be used as an index of a specific disease or some physical state of an organism. Disease-related biomarkers provide information on the possible effects of treatment on the disease (predictive biomarkers), the presence of the disease (diagnostic biomarkers), and how a disease develops regardless of the type of disease (pre-warning biomarkers). Predictive biomarkers provide information about possible responses to a particular type of treatment, whereas prognostic biomarkers provide information about disease progression, whether the patient is treated or not (Maiorana et al., 2005). Given the points stated, the purpose of the study is the bioinformatics study of gene biomarkers in CHI.

MATERIALS AND METHODS

The genes involved in CHI disease were determined using the text mining method in the study to examine the network connection of genes involved in CHI and to calculate essential factors. Determining the relationship of these genes with CHI disease is done using at least one in vivo, in vitro, and silico method.

The population was all CHI patients. The initial data for network construction were extracted from NCBI and Swiss-Prot databases as well as the Disaesome database from samples of 417 CHI patients and 400 healthy individuals. The data is of the gene expression type, downloaded from the standard database with the help of the algorithm written in MATLAB.

The set of target genes in this disease was ranked using the Gene-Disease Association (GDA) score defined below.

$$GDA = C+M+I+L$$

$$C = \begin{cases} 0.6 & N\ sources\ i > 2 \quad if \\ 0.5 & N\ sources\ i = 2 \quad if \\ 0.3 & N\ sources\ i = 1 \quad if \\ 0 & \text{otherwise} \end{cases}$$

Where *N sources i* is the number of specialized confirming sources (scoring) along with the desired gene with the target disease.

(CTD, UNIPROT, PSYGENET, CGL, GENOMICS, CLINGEN, ORPHANET)

$$M = \begin{cases} 0.2 & N\ sources\ i > 0 \quad if \\ 0 & \text{otherwise} \end{cases}$$

J ϵ CTD, MGD, RGD mouse or rat databases included

$$I = \begin{cases} 0.1 & N\ sources\ i > 0 \quad if \\ 0 & \text{otherwise} \end{cases}$$

$$K \in HPO,\ CLINVAR,\ GWASCAT,\ GWASDB$$

$$L = \begin{cases} 0.1 & N\ pubs > 9 \quad if \\ N\ pubs * 0.01 & N\ pubs < 9 \quad if \end{cases}$$

Where *N pubs* was the number of papers (textual sources) that confirm the relationship between gene and disease in BEFREE and LHGDN databases.

The candidate genes in CHI disease were arranged as follows after calculating the GDA score (Table 1).

In the next step, the expression levels of the sorted candidate genes, Gene Entrez and Uniprot, were extracted. In the next step, the communication network between candidate genes was plotted using the *Gephi* platform, and while determining the communication structure network between candidate genes, the structural concentration criteria of the network were calculated to determine essential genes and proteins. In this network, the weight of the edges was determined according to the expression level of the corresponding genes and proteins.

The expression data obtained from each group was standardized compared to the control group after collecting the expression data to compare the results in the case and control groups. Then the communication network of the expression data of the candidate genes in the diseased and healthy individuals separately with the help of software MATLAB software was plotted and the structural parameters of communication networks of expressive data were calculated and compared. Significance parameters could be introduced as potential biomarkers, then again using the Reactome and Disaesomedatabases, we examined the validity of these networks and determined biomarkers. All the statistical calculations in this study were carried out using R and MATLAB.

RESULTS

Network Structural Parameters

Maximum Neighborhood Component (MNC). Like *a*, every vertex has several neighbors that are directly connected with it N(a). The *MNC* score for vertex a is defined as the size of the largest element connected to vertex a. According to this parameter, the highest score, respectively, is given to the following 10 biomarkers (Table 2).

And the communication network will be as follows (Fig. 1).

Degree. The number of edges connected to a vertex defines the degree of that vertex. According to this criterion, the most effective biomarkers of the CHI network will be as follows (Table 3, Fig. 2).

Closeness. This criterion in a connected network is defined as the total length of the shortest paths between a vertex and other vertices. One of the key criteria for determining the biomarker in biological networks is that it determines the proximity of a protein to other proteins. According to this criterion, the key biomarkers of CHI will be as follows (Table 4, Fig. 3).

Radiality. This measure introduces the vertex with the shortest distance from other vertices in its neighboring set of vertices. The highest score according to this criterion was calculated for the following proteins (Table 5, Fig. 4).

Betweenness. This criterion measures the placement of a vertex in the path of other vertices. According to this criterion, a vertex with the highest score may have the highest effect on the transmission of information in the biological network compared to other vertices in the network, and their removal from the network will disrupt the entire network communication. According to this criterion, the highest score between CHI network proteins was calculated as follows (Tables 6, 7, Fig. 5).

DISCUSSION

The findings indicated that the INS gene provides instructions for the production of the hormone insulin, necessary to control the level of glucose in the blood (glucose is a simple sugar and the main source of energy for most cells in the body). Insulin is produced as a precursor called proinsulin which is made up of a single chain of building blocks of protein (amino acids). The proinsulin chain is cut (cleaved) and becomes separate pieces, A and B chains, connected by disulfide bonds forming insulin. INS is a growth factor with a role in the differentiation process of stem cells into cells such as the brain and nerves.

PRKACA encodes one of the catalytic subunits of protein kinase A that exists as a tetrameric holoenzyme with two regulatory subunits and two catalytic subunits in its inactive form. Moreover, cAMP dissociates the inactive holoenzyme into a dimer of four cAMP-bound regulatory subunits and two free monomeric catalytic subunits. Four various regulatory subunits and three regulatory subunits have been identified in humans. Phosphorylation of cAMP-dependent proteins by protein kinase A is critical for many cellular processes including differentiation, proliferation, and apoptosis. The constitutive activation of this gene is caused by somatic mutations or genomic duplication of regions that include this gene, associated with hyperplasia and adenoma of the adrenal cortex and are associated with corticotropin-independent Cushing's syndrome.

PRKACB, which is the protein encoded by this gene, is a member of the serine/threonine protein kinase family. PKA has

two regulatory subunits and two catalytic subunits. The encoded protein is a catalytic subunit of cAMP-dependent protein kinase (cyclic AMP), which mediates cAMP signaling. Furthermore, cAMP signaling is important for several processes including cell proliferation and differentiation. Protein kinase A (cAMP-dependent protein kinase) has a role in regulating lipid and glucose metabolism as well.

PRKACG gene creates a protein with the same name. furthermore, cAMP-dependent protein kinase (PKA) has two catalytic subunits and a regulatory subunit dimer. This gene encodes the gamma form of its catalytic subunit. This gene is intronless and is thought to be a retrotransposon derived from the gene for the alpha form of the PKA catalytic subunit. Among the pathways associated with it, one can mention DAG and IP3 signaling and ADP signaling through P2Y purinoceptor 12. Protein kinase A (cAMP-dependent protein kinase) has a role in regulating lipid and glucose metabolism.

The AKT1 gene provides instructions for making a protein called AKT1 kinase. The protein exists in many types of cells in the body, where it plays an essential role in many signaling pathways. For instance, AKT1 kinase helps regulate cell growth and division (proliferation), which is a process that cells carry out to perform specific functions (differentiation) and cell survival. AKT1 kinase also helps control apoptosis and the self-destruction of cells (when they are damaged or no longer needed). Signaling involving AKT1 kinase seems to be essential for the normal development and function of the nervous system. Some studies have suggested the role of AKT1 kinase in cell-to-cell communication between nerve cells (neurons), the survival of nerve cells, and the formation of memories. AKT1 gene belongs to a group of genes known as oncogenes. If oncogenes undergo mutation, they can turn normal cells into cancerous ones. AKT mediates insulin-stimulated protein synthesis through phosphorylating TSC2.

CONCLUSION

The findings indicated that among the 5 genes PRKACA - INS - AKT1 - PRKACG - PRKACB had the most iterations according to all the findings of the five central criteria methods. One of the critical weaknesses of the study is the lack of examination of the communication pathways of CHI according to the communication method between essential genes in this study, which is suggested to be examined in a supplementary study while examining communication sub-networks in CHI and the communication between this and other diseases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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ETHICS STATEMNT

None.

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Tables

Table 1. CHI gene network connection score table

Gene	GDA score
KCNJ11	0.7

ABCC8	0.5
GCK	0.5
FOXA2	0.34
HNF4A	0.2
GLUD1	0.2
HADH	0.2
HNF1A	0.15
INSR	0.14
SLC16A1	0.13
UCP2	0.13
PDX1	0.12
MEN1	0.12
MAFA	0.1
CEL	0.1
MPI	0.1
APPL1	0.1
CDKN1A	0.1
CDKN1B	0.1
KLF19	0.1
CDKN2B	0.1
CDKN2C	0.1
BLK	0.1
PAX4	0.1
NEUROD1	0.1

Table 2. MNC score for CHI communication network

Row	Biomarker
1	INS
2	PRKACA
3	PRKACB
4	PRKACG
5	AKT1
6	RAPGEF3
7	RAPGEF4
8	IGF1R
9	IRS1
10	GCG

Table 3. Degree score for CHI protein communication network

Row	Biomarker
1	INS
2	PRKACA
3	PRKACB
4	PRKACG
5	AKT1
6	RAPGEF3
7	RAPGEF4
8	IGF1R
9	IRS1
10	PTPN1

Table 4. Proximity score for CHI protein communication network

Row	Biomarker
1	INS
2	PRKACA
3	PRKACB
4	PRKACG
5	AKT1
6	RAPGEF3
7	RAPGEF4
8	IGF1R
9	KCNJ11
10	IRS1

Table 5. Radius score for CHI protein communication network

Row	Biomarker
1	INS
2	PRKACA
3	PRKACB
4	PRKACG
5	RAPGEF3
6	RAPGEF4
7	AKT1
8	KCNJ11
9	IGF1R
10	GCG

Table 6. Betweenness score for CHI protein communication network

Row	Biomarker
1	INS
2	PRKACA
3	PRKACB
4	PRKACG
5	AKT1
6	GCG
7	RAPGEF3
8	RAPGEF4
9	KCNJ11
10	PTPN1

Table 7. Genes-2

Mechanism	Diseases related to this gene	The highest level of expression	Chromosomal location	Full name	Genes
1	*Permanent neonatal DM/MODY/DM1/ hyperproinsulinemia*	*Pancreas(RNAseq)*	*11p15.5*	*Insulin*	***INS (173,172)***
2	*primary pigmented nodular adrenocortical disease/fibrolamellar carcinoma/mixed fibrolamellar hepatocellular carcinoma*	*Cardio-skeletal muscles(RNAseq)*	*19p13.12*	*protein kinase cAMP-activated catalytic subunit alpha*	***PRKACA (175,174)***
3	*primary Pigmented nodular adrenocortical disease/cervical (non)keratinizing squamous cell carcinoma/ carney complex variant*	*Brain (RNAseq)*	*1p31.1*	*protein kinase cAMP-activated catalytic subunit beta*	***PRKACB (176)***

4	*Bleeding disorder/ primary Pigmented nodular adrenocortical disease/ stormorken syndrome/ Friedreich ataxia/ thrombocytopenia absent radius syndrome*	*Testicles (RNAseq)*	*9q21.11*	*protein kinase cAMP-activated catalytic subunit gamma*	***PRKACG (178,177)***
5	*Proteus syndrome/ breast, ovarian, and colorectal cancer/schizophrenia*	*Adipocyte, adrenal gland, breast(RNAseq)*	*14q32.33*	*AKT Serine/Threonine Kinase 1*	***AKT1 (181,180,179)***

Figures

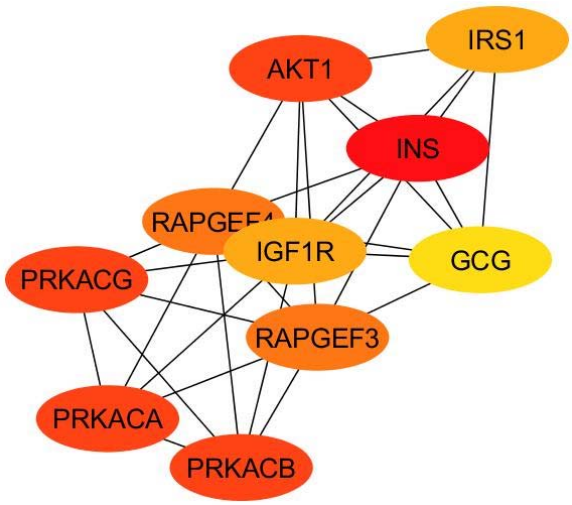

FIG. 1.Protein communication network of CHI according to MNC score

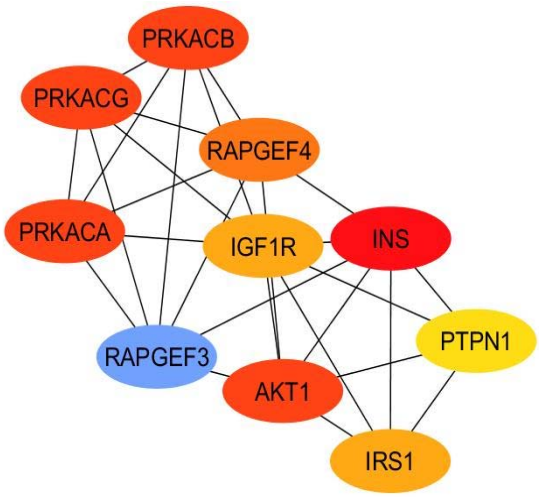

FIG. 2.CHI protein communication network according to degree score

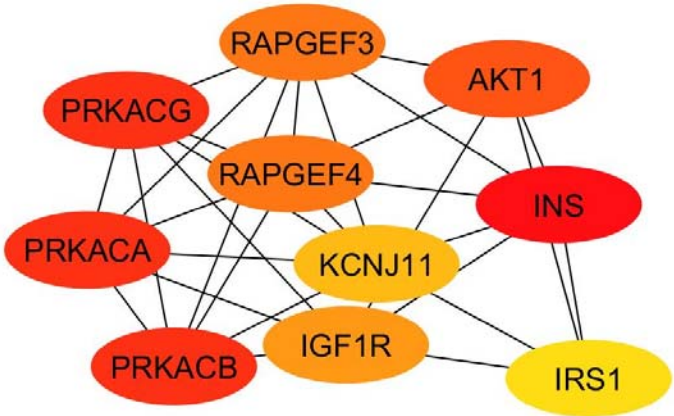

FIG. 3.CHI protein communication network based on proximity score

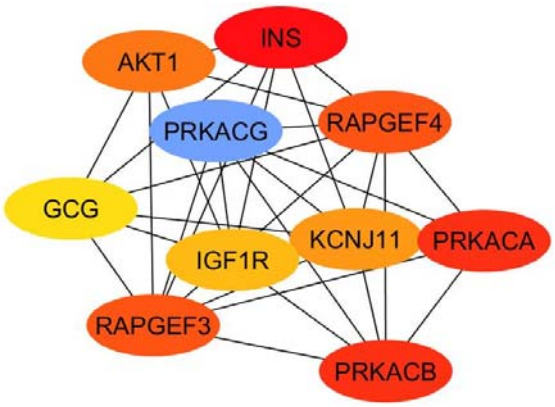

FIG. 4.Protein communication network of CHI according to radius score

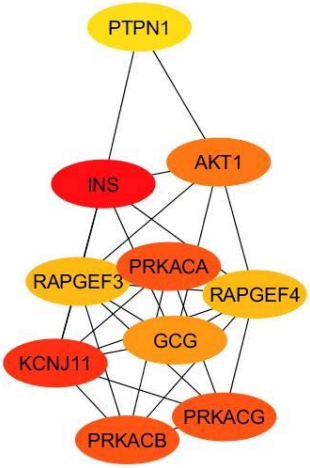

FIG. 5.Protein communication network of CHI according to betweenness score