Gene Biomarkers in Congenital Hyperinsulinism

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Abstract: Congenital hyperinsulinism (CHI) is a rare type of disease that causes a severe drop in blood sugar in infants. This disease prevents reaching enough sugar to the child's brain and causes lifelong and permanent damage. This study aims to investigate gene biomarkers in congenital hyperinsulinism. In this study, after reviewing the texts and searching for the bioinformatics databases of NCBI, Genecards, Swiss-prot, Diseasome, etc., the genes involved in the disease based on at least one of the methods in-vivo, in-vitro, and in-silico has been extracted as candidate genes. The expression data obtained from each group was standardized compared to the control group to compare the results in case and control groups. Then, the connection network of expression data of candidate genes in patients and healthy people were drawn separately with the help of MATLAB software (Version 9.1), and the correctness of these networks and determined biomarkers were checked using the rectome and diseasome database. All statistical calculations were done using R and Matlab software. In the present study, the essential genes of CHI disease were identified using 5 central criteria, including maximum neighborhood component, degree, closeness, radiality, and betweeness. Based on the results of the central criteria method, INS-PRKACA-PRKACB-PRKACG-AKT1 genes had the most repetitions. According to the identification of the most effective genes related to CHI disease in the present study, it is suggested that further studies need to be designed at the in vitro and clinical levels on the identified effective genes as diagnostic biomarkers of CHI disease.

Keywords: Congenital Hyperinsulinism, Gene Biomarker, Predictive biomarkers, GDA score, Bioinformatics databases.

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1. INTRODUCTION

Hypoglycemia in children has a plasma glucose level less than 2.8 mmol/l. This condition can be a potential threat to a person's health, calling for careful evaluation and serious intervention to enable correct diagnosis of its cause and prevent brain damage and other complications.[1,2] Congenital hyperinsulinism (CHI) is a rare disease that causes newborns and children to have low blood sugar due to abnormal insulin secretion. In a healthy person, insulin is secreted when blood sugar levels reach high levels, but in infants and children with CHI, even if they have low blood sugar, insulin is produced and secreted abnormally[3]. Endogenous insulin-induced hypoglycemia is defined in patients with pancreatic tumors, which Whipple's triad can recognize, including hypoglycemic symptoms, low plasma glucose concentration, and relief of general symptoms following resolution of hypoglycemia.[4,5] Whipple's triad is used today in modern guidelines for the diagnosis and management of hypoglycemia and its complications to differentiate patients with true hypoglycemia and to determine those in need of being investigated for the cause of hypoglycemia.[6] The prevalence of this disease is between 1 in every 30,000 to 50,000 live births.[7,8] In Iran, until 2014, about 3 cases of this disease have been reported in infancy.[9,10] The incidence rate of this disease is about 1 in every 40,000 cases in Northern Europe. Disruption in the Sulfonylurea receptor-1 (SUR1) gene has been diagnosed in less than 60% of patients with CHI. Therefore, the incidence rate of this disease is high in areas of the world where consanguineous marriage is high, such as Saudi Arabia and areas where Ashkenazi Jews live; this rate in Saudi Arabia has been reported to be about 1 in every 2500 births.[11] Most cases of the disease are sporadic.[12] However, a study reported that, more than 67% of them already had this disease in their family.[8] Computational bioinformatics includes computer programs in genomic and biological studies aiming to understand better the genetic basis of disease, unique adaptations, desirable properties, or differences between populations[13]. Bioinformatics is an interdisciplinary knowledge that includes methods and software for understanding biological information. Bioinformatics, as a multidisciplinary field of knowledge, combines computer science, statistics, mathematics, and engineering to analyze and interpret biological information.[14-16] Biomarkers related to the disease provide information about 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 (prognostic biomarkers).[17] Predictive biomarkers provide information about possible responses to a specific type of treatment, while prognostic biomarkers provide information about disease progression, whether the patient is treated or not.[18,19] The present study aims to investigate gene biomarkers in congenital hyperinsulinism.

2. MATERIALS AND METHODS

The present analytical study datas were extracted from NCBI, SWISS prot, Genrcards, and Disaesome bioinformatics databases from samples of 417 CHI patients and 400 healthy individuals. The genes involved in the disease were extracted based on at least one *in vivo or in vitro and silico* method and were considered Candidate Genes. To investigate the network connection of genes involved in CHI disease and to calculate essential factors, genes involved in the disease were determined using the text mining method. Then, this disease's target gene was ranked using the Gene-Disease-Association-score (GDA score). Expression data were collected after determining the candidate genes from related studies. The expression data obtained from each group was standardized compared to the control group to compare the results of the two experimental and control groups. Then, the communication network of expression data of candidate genes was drawn in sick and healthy individuals separately using MATLAB (Version 9.1), and the structural parameters of communication networks of expression data were calculated and compared. Significant parameters were introduced as potential biomarkers, and using rectome and diseasome databases, the validity of these determined networks and biomarkers was checked for a second time.

2.1. Statistical analysis

All statistical calculations in this research were done using R and Matlab. Advanced descriptive and analytical statistical methods were used to analyze the data. Moreover, machine learning methods based on advanced bioinformatics algorithms were used to calculate features and network data analysis to extract biomarkers related to the structural characters of the network.

3. RESULTS

This study used the GDA score to define and rank the essential genes for disease diagnosis and treatment. The essential genes were identified using 5 criteria: degree, closeness, radiality, betweenness, and the maximum neighborhood component. Here, 5 genes, i.e., PRKACA - INS - AKT1 - PRKACG - PRKACB, had the most repetitions based on all the results of the above 5 central criteria methods. In this study, the communication network between candidate genes and the structural focus criteria of the network were calculated to determine essential genes and proteins (Figure 1).
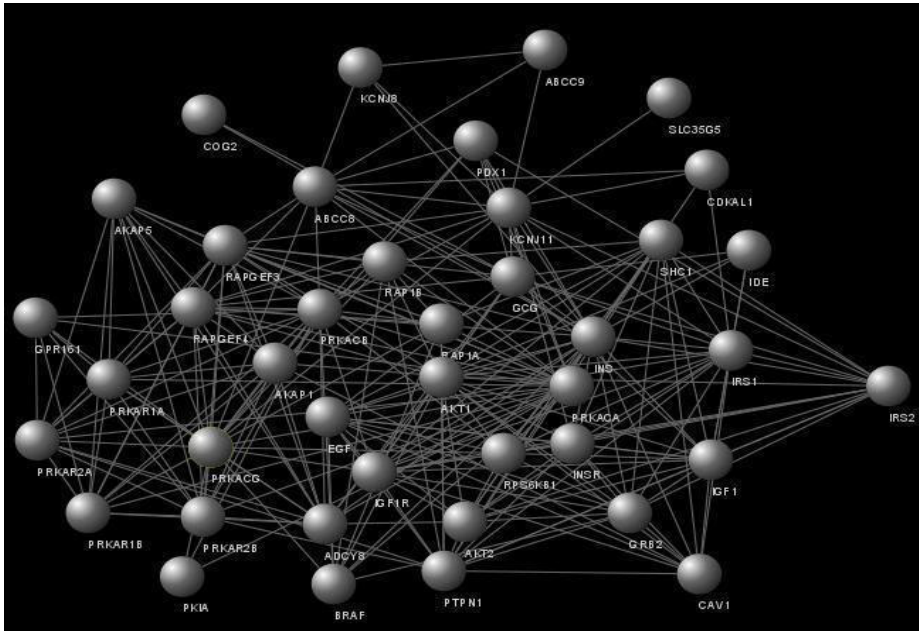
Fig 1. The communication network between proteins in CHI disease is as follows. In this drawn communication network, each node is a protein, and the link between them is a physical or functional connection calculated based on at least one type of in vivo, in vitro, or silico gene studies based on the GDA criteria.

The results indicated that regarding the maximum neighborhood component (MNC) and radiality indices, the most effective biomarker of the CHI disease network is related to INS, and the least effective is GCG. Regarding the degree index, the most effective biomarker of the CHI disease network is associated with INS, and the least effective is related to PTPN1. As for the closeness criterion, the most effective biomarker of the CHI disease network is related to INS, and the least effective is IRS1. Regarding the betweeness criterion, a gene with the highest score may have the most significant effect on the transmission of information in the biological network compared to other vertices of the network, and their removal from the network disrupts the entire network communication. Based on this criterion, the most effective biomarker of the CHI disease network is related to INS, and the least effective is related to PTPN1 (Table 1).

Table 1. The most effective genes related to CHI are arranged according to the chromosomal location, the highest level of expression, and diseases related to that gene. Based on the results in this table, the organs responsible for the incidence of CHI include the pancreas, cardio-skeletal muscles, brain, breast and testicle, adipocytes, and adrenal glands.

Gene name	full name	Chromosomal location	The highest level of expression	Diseases related to this gene
INS (173,172)	Insulin	11p15.5	Pancreas (RNAseq)	Permanent neonatal DM/MODY/DM1/ hyperproinsulinemia
PRKACA (175,174)	protein kinase cAMP-activated catalytic subunit alpha	19p13.12	Cardio-skeletal muscles (RNAseq)	primary pigmented nodular adrenocortical disease/fibrolamellar carcinoma/mixed fibrolamellar hepatocellular carcinoma
PRKACB (176)	protein kinase cAMP-activated catalytic subunit beta	1p31.1	Brain (RNAseq)	primary Pigmented nodular adrenocortical disease/cervical (non)keratinizing squamous cell carcinoma/ carney complex variant
PRKACG (178,177)	protein kinase cAMP-activated catalytic subunit gamma	9q21.11	Testicle (RNAseq)	Bleeding disorder/ primary Pigmented nodular adrenocortical disease/Stormorken syndrome/Friedreich ataxia/ thrombocytopenia absent radius syndrome
AKT1 (181,180,179)	AKT Serine/Threonine Kinase 1	14q32.33	Adipocyte, adrenal gland, breast (RNAseq)	Proteus syndrome/ breast, ovarian, and colorectal cancer/schizophrenia

4. DISCUSSION

CHI is the inappropriate secretion of insulin in the presence of low plasma glucose levels, which leads to severe and persistent hypoglycemia in infants and children.[20] In CHI, due to the inhibitory effect of insulin on lipolysis and ketogenesis, ketone body formation is suppressed, thus leading to an increased risk of brain damage.[21] Thus, rapid diagnosis and

immediate management of CHI are necessary to prevent brain damage and long-term neurological complications in children.[22] Today, progress in molecular genetic sciences, imaging techniques, medical treatments, and surgery outcomes has increased the recovery rate in patients with CHI.[23] The recently discussed biomarkers have helped to solve the difficulties and heterogeneities of CHI disease pathophysiology and can be a way to improve clinical tools for researchers and doctors. This study used bioinformatics to investigate essential genes for diagnosing and treating CHI. Based on this, the essential genes were identified using 5 centrality criteria, i.e., degree, closeness, radiality, betweenness, and maximum neighborhood component. Here, 5 genes, i.e., INS - PRKACA - PRKACB - PRKACG - AKT1, had the most repetitions based on all the results of the above 5 central criteria methods. The INS gene provides instructions for the production of the hormone insulin, which is necessary to control the glucose level in the blood.[24] Also, INS as a growth factor plays a role in the differentiation process of stem cells into brain and nerve cells[25,26] The present study showed that INS is the most effective biomarker related to CHI, in line with the results of other studies.[27] In a study conducted on patients with congenital hyperinsulinism, they found that a large percentage of infants with this disease have ventricular hypertrophy, which may be related to the severity of CHI at the time of diagnosis, making the previous care of these patients even more severe.[28] The results showed that PRKACA is the second most effective biomarker related to CHI. This gene encodes one of the catalytic subunits of protein kinase A, which exists as a tetrameric holoenzyme with two regulatory subunits and two catalytic subunits in its inactive form.[29] Also, the results showed that PRKACB is the third most effective biomarker related to CHI. The protein encoded by this gene is a serine/threonine protein kinase family member. PKA consists of two regulatory subunits and two catalytic subunits. The encoded protein is a catalytic subunit of cAMP-dependent protein kinase, mediating cAMP signaling.[30] In this study, PRKACG was reported as the third most effective biomarker associated with CHI. Also, the results showed that AKT1 is the fifth effective biomarker related to CHI in this study. Previous studies have suggested the role of AKT1 kinase in the cell-to-cell communication of neurons, nerve cells' survival, and memory formation. The AKT1 gene belongs to a group of genes known as oncogenes. If oncogenes are mutated, they have the potential to turn normal cells into cancerous ones.[31,32]

5. CONCLUSION

The results of the present study showed that the most effective genes related to CHI include INS-PRKACA-PRKACB-PRKACG-AKT1. Thus, in the future, additional studies at the laboratory and clinical levels can be designed on the determined effective genes as diagnostic biomarkers of CHI disease. Besides, by investigating the communication paths in the gene communication network of CHI disease, one can recognize and study the communication in the disease and the interaction of this disease with other diseases, which can help to understand more about the mechanism of the disease.

6. AUTHOR'S CONTRIBUTION STATEMENT

Dr. Hossein Seidkhani, designed the model and the computational framework and analyzed the data. Dr. Reza Valizadeh, Critical revision of the manuscript for intellectual content, administrative, technical, and material support.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. Arnoux JB, de Lonlay P, Ribeiro MJ, Hussain K, Blankenstein O, Mohnike K, Nihoul-Fékété, C. Congenital hyperinsulinism. Early human development. 2010; 86(5):287-94. https://doi.org/10.1016/j.earlhumdev.2010.05.003
2. Thornton PS, Stanley CA, De Leon DD. Congenital hyperinsulinism: A historical perspective. Hormone research in pediatrics. 2022; 95(6):631-7. https://doi.org/10.1159/000526442
3. Banerjee I, Raskin J, Arnoux JB, De Leon DD, Weinzimer SA, Hammer M, Thornton PS. Congenital hyperinsulinism in infancy and childhood: challenges, unmet needs and the perspective of patients and families. Orphanet journal of rare diseases. 2022; 17(1):1-12. https://doi.org/10.1186/s13023-022-02214-y
4. Whipple AO, Frantz VK. Adenoma of islet cells with hyperinsulinism: A Review. Ann Surg. 1935; 101(6):1299–335. https://doi.org/10.1097/00000658-193506000-00001
5. Hewat TI, Johnson MB, Flanagan SE. Congenital hyperinsulinism: current laboratory-based approaches to the genetic diagnosis of a heterogeneous disease. Frontiers in Endocrinology. 2022; 13(1):873254. https://doi.org/10.3389/fendo.2022.873254
6. Belabed W, Mnif F, Missaoui AM, Elleuch M, Charfi N, Mejdoub N, Abid M. Endogenous hyperinsulinemic hypoglycemia: A retrospective analysis of 10 cases. Pituitary and Neuroendocrinology. 2023; 90(1): EP733. https://doi.org/10.1530/endoabs.90.EP733
7. De Visschere PJL, Seynaeve P, Vanrietvelde F, Senepart M. Brain injury due to persistent hyperinsulinemic hypoglycemia of infancy. European Journal of Radiology Extra. 2007; 62(2):35-8. https://doi.org/10.1016/j.ejrex.2007.01.005
8. Al-Nassar S, Sakati N, Al-Ashwal A, Bin-Abbas B. Persistent hyperinsulinaemic hypoglycemia of infancy in 43 children: long-term clinical and surgical follow-up. Asian J Surg. 2006; 29(3):207-11. https://doi.org/10.1016/S1015-9584(09)60089-0
9. Bayat Mokhtari M, Raeis Sadat MA, Masoumian M, Vakili R. Persistent hyperinsulinemic hypoglycemia of infancy (PHHI). Med Sci J Islamic Azad University of Mashhad. 2005; 1(3):62-5.
10. Mosavati SM, Ahmadi J, Kalantari M. Nearly total pancreatectomy in hypoglycemic patients resistant to medical treatment. Iran J Pediatr. 1994; 5(20):287-93.
11. Zumkeller W. Nesidioblastosis. Endocr Relat Cancer. 1999; 6(3):421-8.
12. Arnoux JB, de Lonlay P, Ribeiro MJ, Hussain K, Blankenstein O, Mohnike, K, Nihoul-Fékété, C. Congenital hyperinsulinism. Early human development. 2010; 86(5):287-94. https://doi.org/10.1016/j.earlhumdev.2010.05.003

13. Anyaso-Samuel S, Sachdeva A, Guha S, Datta S. Bioinformatics Pre-Processing of Microbiome Data with An Application to Metagenomic Forensics. Statistical Analysis of Microbiome Data. 2021; 1(1):45-78. https://doi.org/10.1007/978-3-030-73351-3_3

14. Liu YY, Harbison S. A Review of bioinformatic methods for forensic DNA analyses. Forensic Science International: Genetics. 2018; 33(1):117-28. https://doi.org/10.1016/j.fsigen.2017.12.005

15. Muntaha ST, Hasnain MJU, Khan WA, Rafiq F, Pervez MT. Role of bioinformatics in forensic science. FUUAST Journal of Biology. 2018; 8(1):133-8.

16. Fakiha BS. Bioinformatics as a forensic tool in coronavirus outbreak. Journal of Indian Academy of Forensic Medicine. 2020; 42(3):219-23. https://doi.org/10.5958/0974-0848.2020.00057.3

17. Havasian MR, Panahi J, Khosravi A. Correlation between the lipid and cytokine profiles in patients with coronary heart disease (CHD)(Review article). Life Science Journal. 2012; 9(4):5772-77.

18. Havasian MR, Panahi J, Mahdieh N. Cystic fibrosis and distribution and mutation analysis of CFTR gene in Iranian patients. Koomesh. 2014; 15(4):431-40.

19. Wright JJ, Williams JM, Letourneau-Freiberg LR, Kandasamy B, Reyes D, Kanegusuku AG, Moore DJ. Insulin Deficiency From Insulin Gene Mutation Leads to Smaller Pancreas. Diabetes Care. 2023; 46(4):773-6. https://doi.org/10.2337/dc22-2082

20. Jiajue RZ, Xiao C, Liu YW, Li R, Zhang HB, Yu M. Diagnosis, treatment and genetic analysis of two cases of congenital hyperinsulinemic hypoglycemia caused by GCK gene mutation. Yi Chuan. 2022; 44(11):1056-62. https://doi.org/10.16288/j.yczz.22-226

21. Giri D, Hawton K, Senniappan S. Congenital hyperinsulinism: recent updates on molecular mechanisms, diagnosis and management. Journal of Pediatric Endocrinology and Metabolism. 2022; 35(3):279-96. https://doi.org/10.1515/jpem-2021-0369

22. Worth C, Yau D, Salomon Estebanez M, O'Shea E, Cosgrove K, Dunne M, Banerjee I. Complexities in managing hypoglycemia due to congenital hyperinsulinism. Clinical endocrinology. 2020; 92(5):387-95. https://doi.org/10.1111/cen.14152

23. Demirbilek H, Hussain K. Congenital Hyperinsulinism: Diagnosis and Treatment Update. J Clin Res Pediatr Endocrinol. 2017; 9(Suppl2):69-87. https://doi.org/10.4274/jcrpe.2017.S007

24. Milstein JL, Ferris HA. The brain is an insulin-sensitive metabolic organ. Molecular Metabolism. 2021; 52(1):101234. https://doi.org/10.1016/j.molmet.2021.101234

25. Lewitt MS, Boyd GW. The role of insulin-like growth factors and insulin-like growth factor–binding proteins in the nervous system. Biochemistry insights. 2019; 12(1): 1178626419842176. https://doi.org/10.1177/11786264198421

26. Hewat TI, Johnson MB, Flanagan SE. Congenital hyperinsulinism: current laboratory-based approaches to the genetic diagnosis of a heterogeneous disease. Frontiers in Endocrinology. 2022; 13, 873254. https://doi.org/10.3389/fendo.2022.873254

27. Banerjee I, Avatapalle B, Petkar A, Skae M, Padilla R, Ehtisham S. The association of cardiac ventricular hypertrophy with congenital hyperinsulinism. Eur J Endocrinol. 2012; 167(5):619-24. https://doi.org/10.1530/EJE-12-0632

28. Karamafrooz A, Brennan J, Thomas DD, Parker LL. Integrated Phosphoproteomics for Identifying Substrates of Human Protein Kinase A (PRKACA) and Its Oncogenic Mutant DNAJB1–PRKACA. Journal of proteome research. 2021; 20(10):4815-30. https://doi.org/10.1021/acs.jproteome.1c00500

29. Taylor SS, Wallbott M, Machal EM, Søberg K, Ahmed F, Bruystens J, Skålhegg BS. PKA Cβ: a forgotten catalytic subunit of cAMP-dependent protein kinase opens new windows for PKA signaling and disease pathologies. Biochemical Journal. 2021; 478(11):2101-19. https://doi.org/10.1042/BCJ20200867

30. Chen Y, Huang L, Dong Y, Tao C, Zhang R, Shao H, Shen H. Effect of AKT1 (p. E17K) hotspot mutation on malignant tumorigenesis and prognosis. Frontiers in Cell and Developmental Biology. 2020; 8(1):573599. https://doi.org/10.3389/fcell.2020.573599

31. Oktelik FB, Yilmaz V, Turkoglu R, Akbayir E, Tuzun E, Deniz G, Cinar S. Expression of Akt1 and p-Akt1 in peripheral T cell subsets of multiple sclerosis patients. Acta Neurologica Belgica. 2021; 121(1):1777-82. https://doi.org/10.1007/s13760-020-01518-9.