Common Polymorphisms Identified In Patients with Type 2 Diabetes Mellitus Revealed From Next-Generation Sequencing Analysis

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Abstract

Objective: Type 2 diabetes mellitus (T2DM) is a multifactorial genetic condition caused by the combination of genes and environmental factors. Several variations linked to T2DM have been discovered in recent genetic investigations, particularly genome-wide association studies (GWAS). This study aimed to investigate genes involved in T2DM, focusing on the NGS analysis and studying the genetic basis of T2DM to improve diagnosis, prevention, and treatment.

Materials and Methods: We selected 5 families based on the diagnosis of diabetes at the age of 30 years or earlier in at least 3 consecutive generations for NGS analyses.

Results: For each of the 5 participants tested thus far, a mean of 11 to 21 variants of clinical significance were detected. These variants were located in different genes, which indicate the association of these genes with susceptibility to diabetes. *WFS1* and INS gene mutations were present in all five diabetic patients analyzed. Specifically, mutations in *WFS1, KCNJ11, ABCC8, HNF1B, INS, GCKR, HNF1A* and *PCSK1N* account for 25%, 13%, 8%, 7%, 7%, 6%, 6% and 6% of patients, respectively.

Conclusion: *WFS1* is the most often altered gene in our participants with putative alterations, according to our findings (25%). *WFS1* mutations were discovered in all of the probands.

Keywords: diabetes mellitus, Type 2, Next generation sequencing

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Introduction

Diabetes mellitus type 2 (T2DM) is a multifactorial illness. This condition is brought on by a complex interaction between genetic predisposition and environmental variables. The most significant environmental causes of the disease are undoubtedly obesity, high-calorieintake, and physical inactivity. However, genetic factors also determine a person's susceptibility to type 2 diabetes (1).

T2DM presently affects more than 400 million people worldwide, and 552 million cases are anticipated by 2030 (2).

Insulin resistance and beta-cell dysfunction are traits of T2DM. The illness may significantly lower the overall quality of life (3). Diabetes patients have a higher risk of stroke, cardiovascular disease, kidney failure, and a possible 5–10 year reduction in life expectancy (4).

Studies based on families and twins provide further evidence of the major genetic component in the aetiology of T2DM. The probability of contracting the condition is approximately 40% when one parent has T2DM, and it is 70% when both parents have the condition (5-7). Early detection of people at high T2DM risk with pharmaceutical therapies and/ or effective lifestyle changes can postpone or prevent the onset of T2DM. There is still a great deal of interest in identifying T2DM risk factors because early detection has been shown to save healthcare expenses (6).

Over the past ten years, the search for genetic associations has advanced significantly with the development of genotyping technologies, computational software, and statistical tools that are both affordable and high-throughput.

New susceptibility loci for T2DM were found in the first genome-wide association study (GWAS) conducted in 2007. Over 100 T2DM susceptibility loci have so far been identified. The technology of next-generation sequencing (NGS) has a wide range of applications in the analysis of the genetic causes of T2DM, including:

1. Identifying common and rare genetic variants linked to the disease.
2. Functional studies for describing the function of genes in disease pathogenesis.
3. Assessing the disease's environmental influence using microbiome profiling techniques.

NGS approaches have increased our understanding of genetic and epigenetic T2DM risk factors, but it is still unclear whether and to what extent this knowledge will be applied in clinical settings (7).

This study aimed to investigate genes involved in T2DM focusing on the NGS analysis and studying the genetic basis of T2DM to improve diagnosis, prevention, and treatment.

Materials and Methods

The Diabetes Research Center in Yazd, Iran, keeps a database of diabetic families. Using quota non-probability sampling, we chose 5 family based on the following criteria: 1) At least 3 generations have the T2DM, and 2) at least 4 members at a pedigree have had diabetes diagnosed when they were 30 years old or younger.

A subjective (i.e., non-random) way of selecting units from a population is known as non-probability sampling.

Forcing the inclusion of individuals of various subpopulations makes quota sampling preferable to other non-probability sampling techniques (like judgement sampling).

All family members are of Iranian origin. before starting this investigation, written informed consent for genetic research was obtained per Shahid Sadoughi University of Medical Sciences-approved standards. Five people from five different family trees were analyzed. There were three generations in every pedigree. An extensive clinical history was taken to make a clinical diagnosis.

We collected two milliliters of peripheral blood. The DENAzist Blood DNA Isolation Kit was used to isolate genomic deoxyribonucleic acid (gDNA) from the participants' blood samples utilizing a filter-based technology from blood leucocytes of the proband. The DENAzist Blood DNA Isolation Kit was intended to extract of genomic DNA from cultured mammalian cells or whole blood (with citrate, heparin, or EDTA added). With the use of this kit, the greatest quantity (yield) of superior-quality genomic DNA can be obtained in the shortest amount of time. Numerous subsequent procedures, such as PCR, genotyping, DNA digestion, and sequencing, could use the extracted DNA. DNA aliquots were re-precipitated and subjected to an RNAse treatment to remove leftover proteins and RNA. A total of 1.0 µg genomic DNA per sample was used as input material for the DNA sample preparation.

NGS and bioinformatics analysis

Following the manufacturer's instructions, sequencing libraries were created using the Agilent SureSelect Human All ExonV7 kit (Agilent Technologies, CA, USA), and x index codes were added to attribute sequences to the sample. Finally, hydrodynamic shearing was used to create 180-280bp pieces. The remaining overhangs were transformed into blunt ends by exonuclease/polymerase activity, and enzymes were eliminated. After adenylating the 3' ends of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on both ends were chosen specifically during PCR. Captured libraries were enriched in a PCR reaction to incorporate index tags in order to prepare for hybridization.

The products were purified using the Beckman Coulter AMPure XP system and quantified using the Agilent high-sensitivity DNA assay on the Agilent Bioanalyzer 2100 system. The Illumina NovaSeq 6000 sequencers received the qualifying libraries. Then, using a Unix-based operating system, data quality control, analysis, and interpretation were performed on an HP server of the G9 generation

Using the bcl2fastq programme (version 2.18), we transformed raw data (.bcl) from Hiseq2000 to fastq files. Next, BWA (version 0.7.12), GATK (version 3.5), SAM tools, and ANOVA software were utilized for aligning sequences, local realignment, variants calling, and annotating, respectively. On the basis of their position on the genome, kind of variation, function, frequency, and inheritance patterns, we next filtered 101,815 discovered annotated variations. The correlation between diabetes-related Reference SNP (RSs) and RSs reported in global GWAS was calculated after diabetes-related RSs were extracted.

Ethical considerations

The ethics committee of Shahid Sadoughi University of Medical approved the study proposal (Code: IR.SSU.MEDICINE. REC.1399.294).

Written consent was obtained from the probonds and all family information of the patients will be kept confidential in the database of the Diabetes Center.

Results
Ethical statement, proband, and clinical assessment

A total of five probands (1 male and 4 female) participated in this study based on the diagnosis of diabetes. Four of these families, have more than 10 known members and one was a family with 6 members. The pedigree information of the families is shown in figure 1.

DNA extraction, and NGS

For each of the 5 participants, a mean of 11 to 21 variants of clinical significance were detected.

These variants were located in different genes which indicate the association of these genes with susceptibility to diabetes. *WFS1* and *INS* genes mutations were present in all five diabetic participants. Identified variants in genes *WFS1, KCNJ11, ABCC8, HNF1B, INS,*

GCKR, HNF1A and *PCSK1N* account for 25%, 13%, 8%, 7%, 7%, 6%, 6% and 6% of participants, respectively.

A Pie Chart of mutated genes in our diabetic patient is shown in Figure 2. The pieces of the graph are proportional to the fraction of the
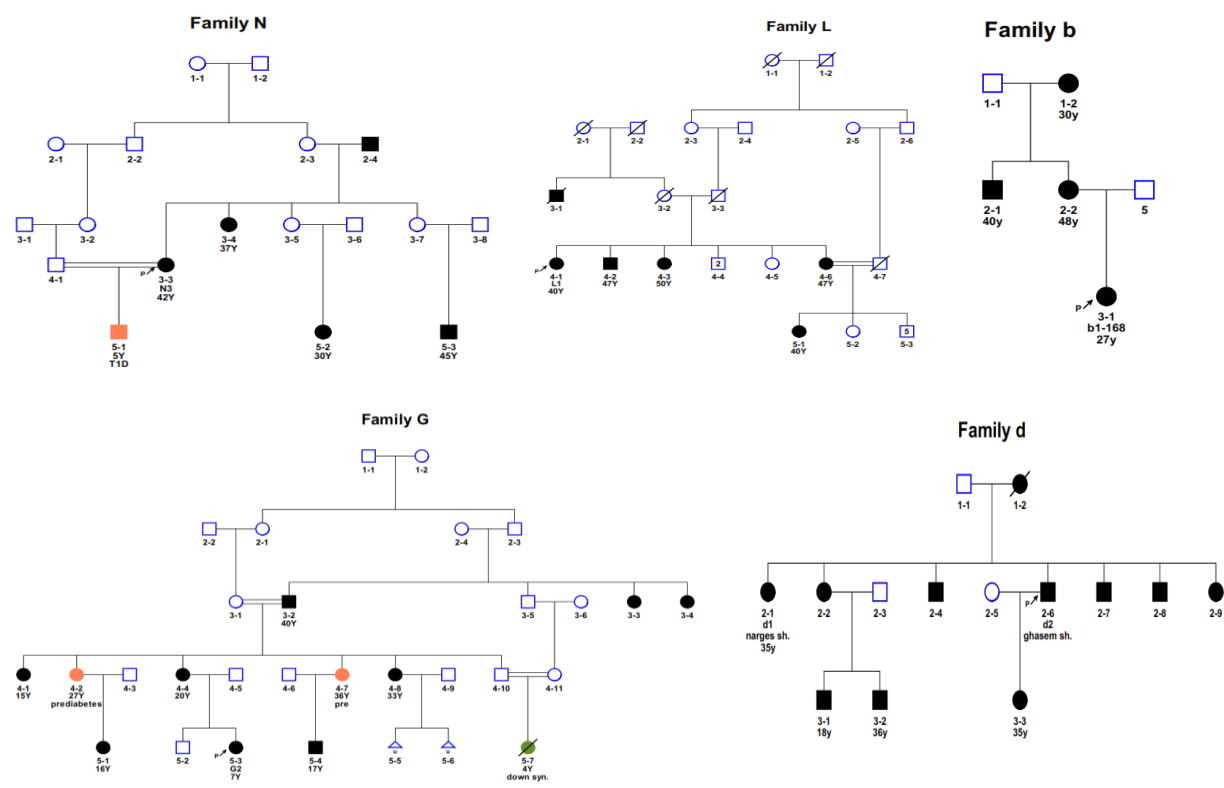
Figure 1. Studied three-generational pedigree. Normal individuals are shown as clear circles (females) and squares (males), and the affected individual is shown as a filled symbol. The patient above the arrow indicates a proband.
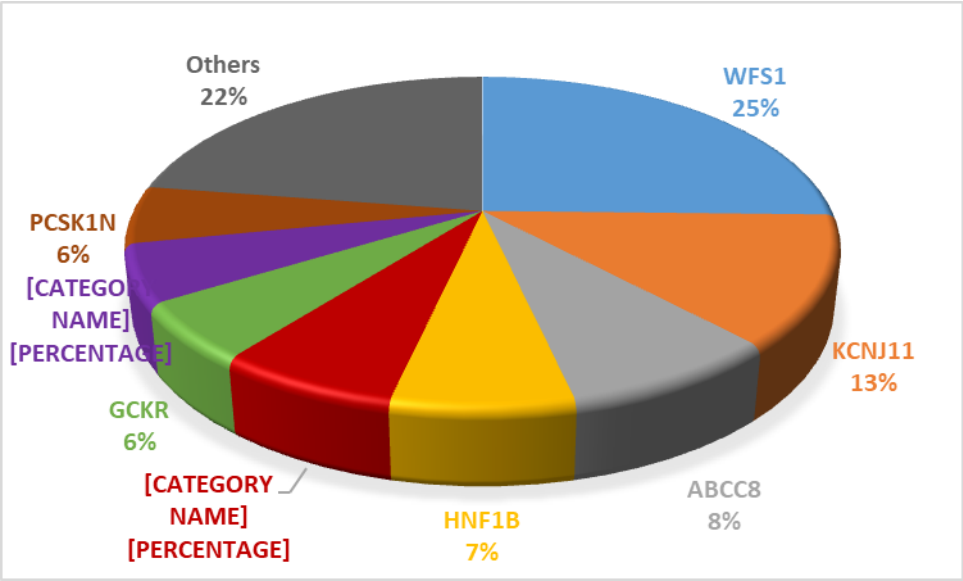

Figure 2. A Pie Chart of mutated genes in our diabetic patients.

whole in each category. In other words, each slice of the pie is relative to the number of mutated genes as a whole. The entire "pie" represents 100 percent of a whole, while the pie "slices" represents portions of the whole.

We found that *WFS1* is the most frequently mutated gene in our participants with putative mutations (25%). Mutations in *WFS1* were found in all of our probands. The *WFS1* gene maps to chromosome 4p16.3 and consists of eight exons. The binomial distribution of polymorphisms results is shown in Figure 3.

Discussion

T2DM causes macrovascular and microvascular complications that lead to deep physical and psychological distress to patients and their care. T2DM encompasses more than 90% of patients with diabetes and put a massive burden on healthcare systems. The prevalence and incidence of T2DM ascend worldwide, however, the knowledge about the risk factors and prevention programs continues to rise (8).

Screening programs, early detection, and the accessibility of effective and secure treatments decrease mortality and morbidity by preventing or delaying complications. As has been established in patients with maturity-onset diabetes of the young, a better understanding of individual diabetes phenotypes and genotypes may lead to more personalized and tailored care of patients with T2DM (9).

Traditional GWAS can identify associated loci, but because the method is restricted to pre-selected common variants discovered by the HapMap project at the turn of the millennium (10), it cannot be used to map causal variants, many of which are anticipated to be rare in the population.

On the other hand, NGS provides a strong substitute for chip-based methods. For genotyping, NGS results are matched to a reference genome and a variety of statistical methods are utilised to identify variance spots (11). Thus, regardless of their frequency, the majority of genetic variations found in a person's genome can be directly identified by NGS, enabling the testing of all variant associations. We focused on NGS datasets for the identification of causal variants for T2DM, and the loci that have been identified by these methods.

As previously mentioned, GWASs have discovered a large number of novel genetic
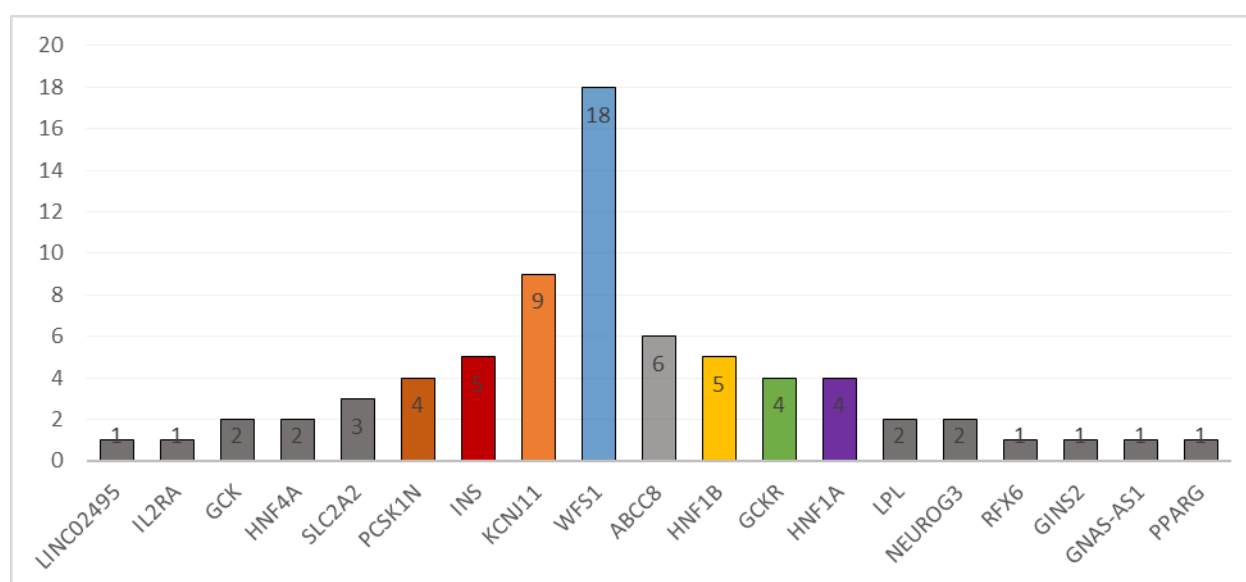

Figure 3. The binomial distribution of polymorphisms. The horizontal axis shows the names of the genes and the vertical axis shows the number of polymorphisms in each gene . As shown most polymorphisms are located in WFS1 and KCNJ11 genes respectively.

relationships relating to T2DM, despite the fact that these linkages represent common and mid-frequency genetic variants with modest effect sizes that only contribute to a small portion of the heritability of the condition. The sequencing approach enables more complete analyses of uncommon and low-frequency genetic variations, which may be advantageous in the investigation of complex traits (12).

Finding T2DM susceptibility loci using NGS data has been the subject of numerous published investigations (13-17).

In our diabetic participants with putative mutations, *WFS1* was the most frequently mutated gene (25%). Every one of our probands had *WFS1* mutations. The *WFS1* gene has eight exons and is located on chromosome 4p16.3 (18).

A monogenic form of diabetes known as Wolfram syndrome is brought on by mutations in the *WFS1* gene (19).

A single gene that causes juvenile diabetes and optic atrophy is responsible for Wolfram syndrome (MIM 222300), a kind of diabetes and neurodegeneration (20).

The most common type of DM is type 2, which has several subtypes. The insulin secretion pathway involves a number of genes and their interactions. The ATP-sensitive potassium (KATP) channel in pancreatic beta cells mediates insulin secretion (21). This channel is a heteromeric protein made up of sulfonylurea receptor 1 subunits surrounding the pore and four inward-rectifier potassium ion channel (Kir6.2) tetramers that form the KATP channel's pore. The potassium channel gene KCNJ11, a member of the potassium channel gene family, is responsible for encoding Kir6.2 (22). Numerous studies have documented how the KCNJ11 gene's single nucleotide polymorphisms and their interactions affect DM susceptibility (23-25).

The beta-cell proteins sulfonylurea receptor (SUR1) and inward-rectifying potassium channel component Kir6.2 make up the ATP-sensitive potassium (KATP) channel, which is a crucial regulator of insulin release (26).

Adenine nucleotide binding to subunit Kir6.2, which closes the channel, inhibits it. Nucleotide binding to or hydrolysis on SUR1, which opens the channel, activates it. The low open-channel probability, which regulates the excitability of pancreatic beta cells, is determined by the balance of two conflicting processes (27).

The faster disclosure of T2DM pathophysiology due to the finding of several loci through GWAS and sequencing technology presents an impressive potential to apply genetic knowledge to therapeutic treatment (28).

Identification of common polymorphisms in diabetes could be helpful for clinical management of patients, such as modifying treatment regimens to ensure that affected people receive the most benefit from their therapy while avoiding complications, as well as for disease risk prediction, such as identifying subjects at risk of developing disease early on.

Employing precision medicine to treat diabetes has been prompted by the expanding accessibility of genetic and electronic health data in large populations would be interesting (29).

Conclusions

WFS1 was the most frequently gene gene in our participants with putative alterations, according to our findings (25 %). *WFS1* mutations were discovered in all of our probands. The WFS1 gene has eight exons and is located on chromosome 4p16.3 (29).

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Conflict of Interest

The Authors declare that there is no conflict of interest.

References

1. Park KS. The search for genetic risk factors of type 2 diabetes mellitus. Diabetes & Metabolism Journal. 2011; 35(1): 12-22.
2. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Research and Clinical Practice. 2011; 94(3): 311-21.
3. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. The Lancet. 2014; 383(9922): 1068-83.
4. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clinical Diabetes. 2008; 26(2): 77-82.
5. Rich SS. Mapping genes in diabetes: Genetic epidemiological perspective. Diabetes. 1990; 39(11): 1315-19.
6. Lindstrom J. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. The Lancet. 2006; 368: 1673-9.
7. Nasykhova YA, Barbitoff YA, Serebryakova EA, Katserov DS, Glotov AS. Recent advances and perspectives in next generation sequencing application to the genetic research of type 2 diabetes. World Journal of Diabetes. 2019; 10(7): 376-395.
8. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. The lancet. 2017; 389 (10085): 2239-51.
9. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ. Type 2 diabetes mellitus. Nature Reviews Disease Primers. 2015; 1 (1):1-22.
10. Consortium IH. A haplotype map of the human genome. Nature. 2005; 437 (7063): 1299-320.
11. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. Current Protocols in Bioinformatics. 2013; 43(1): 11-0.
12. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nature Genetics. 2011; 43 (10): 984-9.
13. Ellard S, Lango Allen H, De Franco E, Flanagan SE, Hysenaj G, Colclough K, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia. 2013;56:1958-63.
14. Szopa M, Ludwig-Sł omczyń ska A, Radkowski P, Skupień J, Zapał a B, Pł atek T, et al. Genetic testing for monogenic diabetes using targeted next-generation sequencing in patients with maturity-onset diabetes of the young. Polish Archives Of Internal Medicine. 2015;125(11). 845-51.
15. Fareed M, Chauhan W, Fatma R, Din I, Afzal M. Next-generation sequencing technologies in diabetes research. Diabetes Epidemiology and Management. 2022:100097.
16. Ang SF, Lim SC, Tan CS, Fong JC, Kon WY, Lian JX, et al. A preliminary study to evaluate the strategy of combining clinical criteria and next generation sequencing (NGS) for the identification of monogenic diabetes among multi-ethnic Asians. Diabetes Research and Clinical Practice. 2016; 119: 13-22.
17. Khan IA. Do second generation sequencing techniques identify documented genetic markers for neonatal diabetes mellitus?. Heliyon. 2021;7(9): e07903.
18. Cryns K, Sivakumaran TA, Van den Ouweland JM, Pennings RJ, Cremers CW, Flothmann K, et al. Mutational spectrum of the WFS1 gene in Wolfram syndrome, nonsyndromic hearing impairment, diabetes mellitus, and psychiatric disease. Human Mutation. 2003; 22(4): 275-87.
19. Rigoli L, Lombardo F, Di Bella C. Wolfram syndrome and WFS1 gene. Clinical Genetics. 2011; 79(2): 103-17.
20. Minton JA, Hattersley AT, Owen K, McCarthy MI, Walker M, Latif F, et al. Association studies of genetic variation in the WFS1 gene and type 2 diabetes in UK populations. Diabetes. 2002; 51(4): 1287-90.
21. Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, Daly A, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nature Genetics. 2007; 39(8): 951-3.
22. Franks PW, Rolandsson O, Debenham SL, Fawcett KA, Payne F, Dina C, et al. Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. Diabetologia. 2008; 51: 458-63.
23. Haghvirdizadeh P, Mohamed Z, Abdullah NA, Haghvirdizadeh P, Haerian MS, Haerian BS. KCNJ11: genetic polymorphisms and risk of diabetes mellitus. Journal of Diabetes Research. 2015; ID 908152..
24. Phani NM, Guddattu V, Bellampalli R, Seenappa V, Adhikari P, Nagri SK, et al. Population specific

impact of genetic variants in KCNJ11 gene to type 2 diabetes: A case-control and meta-analysis study. PLoS One. 2014; 9(9): e107021.

25. Qiu L, Na R, Xu R, Wang S, Sheng H, Wu W, et al. Quantitative assessment of the effect of KCNJ11 gene polymorphism on the risk of type 2 diabetes. PloS one. 2014; 9(4): e93961.

26. Babenko AP, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, et al. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. New England Journal of Medicine. 2006; 355(5): 456-66.

27. Haghverdizadeh P, Haerian MS, Haghverdizadeh P, Haerian BS. ABCC8 genetic variants and risk of diabetes mellitus. Gene. 2014;545(2):198-204.

28. Dorajoo R, Liu J, Boehm BO. Genetics of type 2 diabetes and clinical utility. Genes. 2015; 6(2): 372-84.

29. Floyd JS, Psaty BM. The application of genomics in diabetes: barriers to discovery and implementation. Diabetes Care. 2016; 39(11): 1858-69.