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REVIEW

Recent advances and perspectives in next generation sequencing application to the genetic research of type 2 diabetes

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Abstract

Type 2 diabetes (T2D) mellitus is a common complex disease that currently affects more than 400 million people worldwide and has become a global health problem. High-throughput sequencing technologies such as whole-genome and whole-exome sequencing approaches have provided numerous new insights into the molecular bases of T2D. Recent advances in the application of sequencing technologies to T2D research include, but are not limited to: (1) Fine mapping of causal rare and common genetic variants; (2) Identification of confident gene-level associations; (3) Identification of novel candidate genes by specific scoring approaches; (4) Interrogation of disease-relevant genes and pathways by transcriptional profiling and epigenome mapping techniques; and (5) Investigation of microbial community alterations in patients with T2D. In this work we review these advances in application of next-generation sequencing methods for elucidation of T2D pathogenesis, as well as progress and challenges in implementation of this new knowledge about T2D genetics in diagnosis, prevention, and treatment of the disease.

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Core tip: Next-generation sequencing (NGS) technologies have a broad range of applications in studying the genetic causes of type 2 diabetes (T2D), such as: (1) Identification of rare and common genetic variants, associated with disease; (2) Functional studies for describing role of genes in disease pathogenesis; and (3) Evaluation of environmental contribution to the disease by using microbiome profiling methods. This review of NGS application to the genetic research of T2D presents the advances and challenges related with sequencing analysis-based studies and implementation of this knowledge in clinical practice.

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INTRODUCTION

Type 2 diabetes (T2D) mellitus is a common complex disease that currently affects more than 400 million people throughout the world, and it is projected 552 million cases of T2D by the year 2030[1]. The disease is characterized by insulin resistance and beta-cell dysfunction and can seriously impair overall quality of life[2]. T2D may lead to increased risk of cardiovascular disease, stroke, kidney failure and can result in lower life expectancy by 5-10 years[3-5]. T2D etiology is known to have a significant genetic component that is confirmed by family- and twin-based studies. The risk of the disease developing is approximately 70% when both parents have T2D and approximately 40% when one parent has disease[6]. Twin studies have shown that the heritability of T2D ranges from 26% to 73%, and the concordance rate for T2D in monozygotic twins can reach 76%[7]. Early identification of individuals at high T2D risk enables delay or prevention of T2D onset through effective lifestyle and/or pharma-cological interventions and has been shown to reduce costs of healthcare that causes continuing strong interest in revealing risk markers of T2D[8,9].

The development of high-throughput and affordable genotyping technologies, statistical tools and computational software has allowed remarkable progress over the past decade in the search for genetic associations. Since the first genome-wide association study (GWAS) for T2D identified novel susceptibility loci in 2007, more than 100 T2D susceptibility loci have been discovered[10]. Next-generation sequencing (NGS) technologies have a broad range of applications in studying the genetic causes of T2D, such as: (1) Identification of rare and common genetic variants, associated with disease; (2) Functional studies for describing role of genes in disease patho-genesis; and (3) Evaluation of environmental contribution to the disease by using microbiome profiling methods. However, it remains uncertain if and to what extent our increasing knowledge of genetic and epigenetic T2D risk factors gained by NGS methods will translate into clinical practice.

The aim of this article is to summarize recent progress and discoveries for T2D genetics focusing on the sequencing analysis-based studies and review the challenges in studying the genetic basis of T2D in order to improve diagnosis, prevention, and treatment.

T2D SUSCEPTIBILITY LOCI IDENTIFIED BEFORE THE ERA OF GWAS

The earliest genetic studies of T2D susceptibility focused on family-based linkage analysis and analysis of candidate genes in small-size groups of patients. This approach was successful in identifying familial genetic variants with large effects such as those involved in monogenic forms of the disease. In the past two decades,

numerous candidate gene studies have been performed to identify genetic variants for T2D. However only 4 genetic markers identified in these studies have been confirmed later by GWAS. The first genetic variant for T2D was the P12A polymorphism (rs1801282) in peroxisomal proliferator activated-receptor gamma gene (*PPARG*)[11]. Then, in 2003, in a large-scale association study the previously identified association between the E23K (rs5219) polymorphism in a gene encoding inwardly rectifying potassium channel subfamily J, member 11 (*KCNJ11*) and T2D was replicated[12]. E23K can alter function by inducing spontaneous over-activity of pancreatic β-cells, thus increasing the threshold ATP concentration for insulin release[13]. In previous studies a polymorphism in this genes (KCNJ11 E23K) has been reported to be associated with T2D in several populations, although the data was inconsistent[14-18]. Transcription factor 7-like 2 (T-cell specific, HMG-box) (*TCF7L2*) was shown to be associated with T2D[19]. *TCF7L2* gene product is a member of the high mobility group box family of transcription factors, activated by the WNT signaling pathway and may play a master role in regulating insulin biosynthesis, secretion, and processing. Subsequently, two single nucleotide polymorphisms (SNPs) within intron 3 of *TCF7L2*, rs7903146 and rs12255372, were confirmed to be strongly associated with T2D risk[20-22]. Wolfram syndrome 1 gene (wolframin) (*WFS1*) was reported to be associated with T2D on the basis of in-depth studies of candidate genes[23]. The *WFS1* gene encodes wolframin, endoplasmic reticulum (ER) membrane protein with a role in ER calcium homeostasis. Mutations in *WFS1* are known to be associated with Wolfram syndrome[24].

GENOME-WIDE ASSOCIATION STUDIES ON T2D

Advances in technology of SNP genotyping, implementation of recent genetic knowledge gained from the Human Genome Project, and development of robust statistical methods have allowed GWAS to become the basic method for identification of common genetic variants associated with complex diseases such as T2D. Since the application of GWAS technology the discovery of genetic variants associated with T2D has developed dramatically.

In 2007, the first GWAS performed for T2D has identified three novel susceptibility loci related to pancreatic β-cells: (1) Solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*), which is expressed exclusively in insulin-producing β-cells; (2) Insulin-degrading enzyme (*IDE*)–kinesin-interacting factor 11 (*KIF11*)–hematopoietically expressed homeobox (*HHEX*); and (3) Exostosin glycosyltransferase 2 (*EXT2*)–ALX homeobox 4 (*ALX4*)[25]. Subsequent GWAS revealed four additional loci associated with T2D, namely CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), cyclin-dependent kinase inhibitor 2A (*CDKN2A/B*), insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), and fat mass and obesity associated (*FTO*)[26-30]. In addition, HNF1 homeobox B (*HNF1B/ TCF2*), a gene related to maturity-onset diabetes of the young type 5 (MODY5), was shown to be associated with T2D[31]. One important finding from the initial GWAS results was that effect sizes for common variants involved in T2D were likely to be modest. The statistical power to detect associations between genetic variants and a trait depends on the sample size, the distribution of effect sizes of (unknown) causal genetic variants, the frequency of those variants, and the linkage disequilibrium (LD) between observed genotyped DNA variants and the unknown causal variants[32]. This led to an innovative data merging strategy now known as GWAS meta-analysis and resulted in multiple waves of GWAS studies for T2D.

In 2008, six new T2D loci including *JAZF1*, *CDC123*/calcium/*CAMK1D*, *TSPAN8*/*LGR5*, *THADA*, *ADAMTS9*, and *NOTCH2* were reported by a meta-analysis combining three previous GWAS [Diabetes Genetic Initiative (DGI), Finland–United States Investigation of NIDDM Genetics (FUSION), and Wellcome Trust Case Control Consortium (WTCCC)][33]. In 2009, two loci, namely insulin receptor substrate 1 (*IRS1*) and melatonin receptor 1B (*MTNR1B*) were identified to be associated with T2D by GWAS[34-36]. The *IRS1* gene is related to insulin resistance and hyperinsulinemia, whereas *MTNR1B* is involved in impaired early insulin response to glucose[35].

In 2010 the second wave of the GWAS identified 17 new loci associated with T2D which was made possible because of improved efficiency of GWAS genotyping technology, enabling interrogation of larger numbers of SNPs that better cover common genetic variation across populations in increased sample sizes, as well as because of methodological innovations, such as imputation (described below), which allows prediction of genotypes at SNPs not typed on GWAS arrays[37].

In the past year a leap forward has occurred from smaller, cumulative advances to the description of up to around 250 genome-wide significant loci of T2D[10]. In this work, a large meta-analysis of GWAS in sample of T2D including 62892 cases and

596424 controls was performed by combining 3 GWAS data sets of European ancestry: DIAbetes Genetics Replication and Meta-analysis (DIAGRAM), Genetic Epidemiology Research on Aging, and the full cohort release of the UK Biobank 39 previously unknown loci have been identified[38]. This study highlighted the benefits of integrating multiple omics data to identify functional genes and putative regulatory mechanisms caused by genetic variation. Future applications of integrative omics data analyses are expected to improve our understanding of the biological mechanisms underlying common diseases such as T2D[38].

MAPPING OF CAUSAL VARIANTS AND DISEASE GENES BY NGS METHODS

While conventional genome-wide association studies allow to identify associated loci, GWAS alone cannot be used to map causal variants (many of which are expectedly rare in population), as the method strictly focuses on pre-selected common variants identified by the HapMap project in the beginning of the century[39]. On the other hand, NGS presents a reasonable alternative to the chip-based methods. For genotyping purposes, NGS reads are aligned to a reference genome, and a set of statistical procedures is performed to identify variant sites[40]. Thus, NGS directly identifies most of the genetic variants present in an individual's genome irrespective of their frequency, which enables testing of all variants' association. In this section, we will focus on how NGS datasets might be used for identification of novel causal variants for T2D, and which loci have been identified by these methods.

Fine mapping of GWAS signal using NGS-based reference panels

Large genome and exome sequencing and aggregation consortia, such as the 1000 Genomes project or UK10K provide valuable insights into linkage disequilibrium, *i.e.*, co-occurrence rates, between different variants, enabling probabilistic reconstruction of individual genome sequences from fixed number of genotyped loci (such as in traditional GWAS). This in turn enables testing for the role of rare variants without sequencing *per se*[37,41,42]. Large reference panels for such genotype imputation have been constructed from sequencing data[43]. Genotype imputation has been widely used in the studies of the genetic architecture of T2D[44]. An interesting example is a 2014 study of Icelandic population[45]. In this work, whole-genome sequencing study of a cohort of 2630 Icelanders was performed; and the identified SNPs and indels were imputed into 98721 controls and T2D patients genotyped with Illumina SNP chips. As a result of this study a rare variant in *HNF1A* gene, encoding for a transcription factor required for the expression of several liver-specific genes was identified. Moreover, a new signal with association $P < 1 \times 10^{-8}$ at rs76895963, located within the first intron of cyclin D2 (*CCND2*) was observed[45]. Two of the most recent and comprehensive research efforts aimed at fine mapping of association signal using imputation and islet-specific epigenome maps identified multiple previously unreported loci for T2D, including *PNPLA3, LPL, TPCN2*, *DENND2C*, and *KIF2B*[46,47]. Apart from using NGS datasets for rare variant imputation, different approaches based on combined SNP and exome chip methods have been developed, enhancing the power of imputation-based analyses[48].

Association of single rare variants with T2D in NGS-based studies

As previously stated, many new genetic associations relevant to T2D have been revealed by GWASs, but these findings represent common and mid-frequency genetic variants with small effect sizes and explain only a small proportion of heritability of the disease. Sequencing approach enables more complete assessments of low-frequency and rare genetic variants that can be promising in investigation of complex traits.

Many published studies have focused on identification of T2D susceptibility loci from NGS data. In Danish study, the exomes of 1974 Danes were sequenced to a depth of 8 × and subsequently a two-stage follow-up in 15989 Danes and in a further 63896 Europeans were performed. A low-frequency coding variant in CD300LG associated with fasting HDL-cholesterol and two common coding variants in COBLL1 and MACF1 have been shown to be associated with T2D[49]. CD300LG encodes a protein proposed to serve multiple functions, including endocytosis of various immunoglobulins and mediation of L-selectin-dependent lymphocyte rolling[50,51]. Non-coding SNPs in COBLL1 and MACF1 have previously been associated with other metabolic phenotypes[52-54].

To investigate the hypothesis of "missing heritability", the Genetics of Type 2 Diabetes and Type 2 Diabetes Genetic Exploration by Next-generation sequencing in

multi-Ethnic Samples Consortium (GoT2D/T2D-GENES Consortium) undertook whole genome sequencing in 2657 Europeans with and without diabetes, and exome sequencing in a total of 12940 subjects from five ancestral groups. Results of this study showed that the variants associated with T2D were overwhelmingly common and most located within regions previously identified by GWAS. A few coding variant associations outside established common variant GWAS regions have been identified (rs41278853 in MTMR3 gene; rs11549795, rs28265, rs36571 in *ASCC2* gene). A coding variant reached genome-wide significance that was common in East Asian ancestry population (*PAX4* Arg192His, rs2233580)[55]. *PAX4* gene encodes a transcription factor involved in islet differentiation and function. Some *PAX4* variants have been associated with early-onset monogenic diabetes[56,57].

Specific statistical approaches for rare variant associations on NGS data

Despite decreasing costs of NGS-based analyses, there still remain certain notable limitations of such studies. The most evident limitation of all the rare variant-based tests on both whole-genome and imputed SNP array datasets is the difficulty of obtaining enough observations to make confident statistical inference. For example, if a causal variant occurs at a rate of 10^{-4} in a population, one would require many hundreds of thousands of individuals to test its association with the disease. To allow testing for the association of rare variants, especially in smaller samples, a group of techniques were developed, called Rare Variant Association tests. Most of rare-variant tests are designed to identify candidate disease genes through aggregation of all rare variants inside the coding sequence of each gene. Numerous strategies for gene-level testing of rare-variant association have been developed[58]. The two main groups of such methods test either the imbalance of rare allele counts between cases and controls (burden tests) or the proportion of phenotypic variance explained by rare variant genotypes (variance-component tests). However, for T2D almost few significant gene-level associations have been found even in the largest NGS-based population cohorts[55,59]. Only the largest study performed by whole-exome sequencing to date, which included 20791 T2D cases and 24440 controls of multiple ancestries (Hispanic/Latino, European, African-American, East-Asian, South-Asian), identified several gene-level associations: in 3 genes at exome-wide significance, including a T2D protective series of > 30 *SLC30A8* alleles, and within 12 gene sets, including those corresponding to T2D drug targets and candidate genes from knockout mice. The strongest T2D rare variant gene-level signals was shown to explain at most 25% of the heritability of the strongest common single variant signals[60].

Several alternative techniques have been developed to overcome the limitations of rare variant testing. In samples of limited size based on exome sequencing or targeted resequencing, contribution of rare variants might be assessed using tests for case-specificity conditioned on true population minor allele frequency[61]. Such strategy may help to identify variants that serve as the candidate causal markers for the pathology. In a recent study by our group, we identified potential association for the *VAV3, ADAMTS13, HBQ1*, and *DBH* genes with T2D and obesity. While these genes have not been previously implicated in the disease, they are reasonable targets for further clinical investigation.

Another approach to counteract statistical power limitation of rare-variant based tests in small NGS-based datasets is the usage of pedigrees. The biggest advantage of familial studies is that cohorts of related individuals would have higher frequency of alleles that are rare in the general population. One recent example of pedigree-based analysis is a study of 20 Mexican-American families comprising 1034 highly related individuals[62]. While this study still did not identify any significant associations for individual rare variants, it has shown gene-level association for the *CYP3A4* and *OR2T11* genes with glycemic traits, such as fasting glucose levels and 2h insulin levels.

Overall, there are several ways in which NGS might be used to assist identification of causal genes and variants for T2D pathology. These associations are of ultimate relevance for genomic risk prediction of T2D and clinical decision making[63]. Some of the inherent limitation of the technology, however, still do not allow thorough analysis of chromosome- and genome-level genetic variation and/or complex genome regions that are poorly accessible to short read sequencing[64-66]. The spread of third-generation sequencing technologies, such as the Oxford Nanopore Technologies single-molecule sequencing, as well as modifications to the existing laboratory and/or bioinformatic practices would shed light on the roles of higher-level genetic variants in T2D pathology.

NGS IN FUNCTIONAL GENOMIC STUDIES OF T2D

Apart from methods aimed at genotyping, NGS can also be used to dissect functional genome elements rather than sequence variants. NGS techniques for these purposes include transcriptional profiling approaches (RNA-Seq), epigenome mapping techniques (positional methods), and other[67,68]. These methods are commonly used to both identify candidate disease genes and understand pathological mechanisms behind the observed phenotype. Below, we will provide several recent examples of application of these methods to the research of T2D (Figure 1).

Transcriptional profiling of whole tissues and single cells by RNA-Seq

Transcriptional profiling methods, such as RNA-Seq, are used to study activity patterns of genes. In the recent decade, transcriptomic technologies were frequently used to decipher the molecular pathology behind human disease[69]. T2D, being one of the most common pathologies, has also been extensively studied by transcriptional profiling techniques in the recent decade[70]. Traditional way to analyze RNA-Seq data is to align the reads to a reference genome and count the numbers of reads or fragments mapped to each gene or transcript. These counts are then used to search for genes which significantly change their expression in case vs controls (differentially expressed genes, DEGs) using conventional statistical tests or linear regression models, and identify biological processes which are dysregulated in one of the conditions. The latter task is solved by a family of gene set enrichment tests that analyze overrepresentation of genes from a certain pathway among the identified DEGs. Multiple downstream analyses can be performed to identify disease genes and pathways from both bulk and single-cell RNA-Seq data[71]. Below, we will focus on several notable examples of how both bulk and single-cell technologies can be used to identify genes involved in pathological mechanisms of T2D.

One example of a conventional bulk RNA-Seq approach used to identify disease-relevant pathways can be found in a recent work that studied transcriptional profiles of diabetic keratinocytes[72]. This study showed extensive dysregulation of immunity-related genes in these cells compared to controls, with as many as 420 differentially expressed genes identified in total. Moreover, this study has suggested a causal role of miR-340-3p-*DTX3L* interaction in the pathological processes occurring in diabetic skin.

Multiple studies have also focused on the roles of microRNA (miRNA) in the pathology of T2D[73]. microRNAs are a separate class of RNA molecules which play an important role in gene regulation via post-transcriptional gene silencing. One of the most recent studies aiming at systematic analysis of microRNA involvement in T2D by aggregation of published data identified as many 158 microRNAs reported to be differentially expressed in T2D. One example of an important microRNA identified in this study is the miR-375 RNA which affects expression of several disease-relevant genes in islets and other tissues.

Many studies suggest that the alterations in miRNA levels are associated with T2D development and its complications. miRNA may play a key role in regulation of the processes of carbohydrate and lipid metabolisms, adipocytokine and insulin signaling pathways involved in T2D development. It was shown that the dysregulated in the islets miR-7-5p, -129-3p, -136-5p,-187-3p, -224-5p, -369-5p, -375 -495-3p, -589-5p, -655-3p affect the expression of important genes involved in insulin signaling pathway. The altered level of miRNA miR-17-5p, -155-5p, -125b-5p, -30e-5p, -27a-5p, -221-3p, -199a-5p, -130b-3p, -181a-5p, -29a, -29b can cause the dysregulation of lipid and glucose metabolisms. For miR-130b-3p, -140-5p, -147a, -199a-5p, -27b, -221-3p and -30e-5p) their involvement in the regulation of adipogenesis was identified[74]. Stability of miRNAs, their presence in various body fluids and significant changes of specific circulating miRNAs' concentrations associated with diseases allow studying them as potential reliable biomarkers for complex diseases such as T2D and related complications. However, there are some obstacles for straightforward clinical application of circulating miRNAs. The biggest difficulty is due to the composition of circulating miRNA that are sum of many different tissues and cell types in the body. At the same time, it is well known that the expression of miRNAs varies considerably between different tissues.

Another important branch of NGS-based transcriptional profiling techniques is the single-cell RNA sequencing (scRNA-Seq) which allows researchers to study transcriptional responses of individual cells and cell-types. scRNA-Seq techniques are also being extensively used to identify key disease genes for T2D in pancreas cells. For example, scRNA-Seq of pancreatic islets suggested a role of *FXYD2* and *GPD2* genes in pathological processes behind T2D in certain islet cell types, with as many as 245 dysregulated genes in total[75,76].

Identification of epigenetic disease markers

Another widely used group of NGS methods is aimed at understanding the language

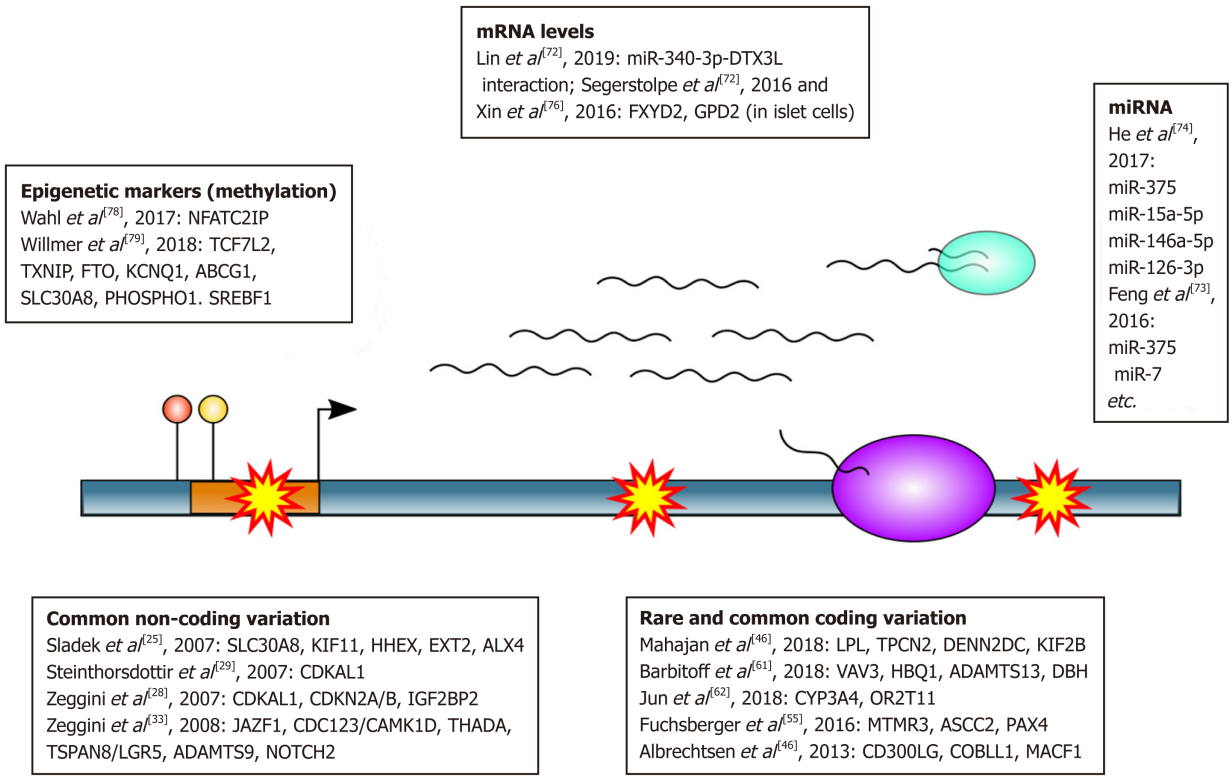

Figure 1 Schematic representation of main type 2 diabetes loci identified recently by high-throughput (mostly, next-generation sequencing-based) technologies. Each box represents certain type of genetic and epigenetic markers of type 2 diabetes.

of epigenetic marks, *i.e.*, non-DNA based units of genetic information. NGS technologies for epigenome studies include but are not limited to: (1) Methods for detection of specific DNA-protein interaction (*e.g.*, Chromatin Immuno Precipitation followed by Sequencing); (2) Methods for identification of DNA methylation sites (such as reduced representation bisulfite sequencing); and (3) Open chromatin mapping technologies (*e.g.*, DNAse-Seq or ATAC-Seq)[67]. All of these methods provide valuable insights into dysregulation of cellular processes, which is of ultimate importance for T2D pathology[77]. Epigenetic marks, as the dynamic features of the cell, are frequently considered as convenient biomarkers for disease risk prediction and prognosis in the clinic. A large-scale survey on the adverse outcome of adiposity showed that methylation pattern at certain loci predicts development of T2D in overweight people[78]. A recent analysis of published data identified 8 differentially methylated genes as potential blood biomarkers of T2D (*TCF7L2*, *KCNQ1*, *ABCG1*, *TXNIP*, *PHOSPHO1*, *SREBF1*, *SLC30A8*, and *FTO*)[79]. Epigenome profiles might also be used to enhance identification of causal variants at complex GWAS loci[80].

Overall, RNA-Seq and positional NGS techniques provide a very useful framework to investigate cellular processes that are affected during disease pathogenesis. These data may in turn be used for both prediction of diabetes risk and for designing clinical treatment of the disease; furthermore, simultaneous consideration of genotype, expression profile and epigenetic factors might assist efficient personalized treatment of T2D. Further integration of multiple omics datasets would allow researchers and clinicians to have a comprehensive look into the molecular pathology behind T2D.

NGS STUDIES OF HUMAN GUT MICROBIOME AND T2D ASSOCIATIONS

Rapid progress of NGS technologies and bioinformatic data processing methods led to the advent of metagenome studies, *i.e.*, investigation of the microbial composition of natural inhabitants. A decade of advances in the field of intestinal microbiome analysis demonstrated that alterations of gut bacteria composition is implicated in a few medical conditions, including diabetes and obesity[81-83]. Such progress can be attributed to a number of factors, for example, stable decrease of price per single run for NGS platforms, continuous development of bioinformatic tool/pipelines[84-87], creation of specialized gut microbiome 16s rRNA databases and use of metaproteo-

mics, metabolomics and metatranscriptomics in conjuncture with genetic profiling[88-92]. Still, there is no consensus concerning optimal conditions for conducting microbiome research. Choice between 16S RNA profiling/shotgun sequencing methods , differences in effective coverage between V1-V9 hypervariable regions , more precise quantitative analysis for microbiota constituents[93], and generalized protocols for sample acquisition are still in discussion, with main emphasise often being put on low reproducibility of results, partly due to the unstable nature of samples' bacterial composition[81,86,87,92,94]. Overall, intestinal microbiome genetic profiling may find use in clinical practice with development of presently elusive "golden standard" for this research field, leading to better understanding of gut microbiota's role in human homeostasis and associations with diseases[95].

General overview of microorganisms involved at least partially with T2D

As of 2014, microbial community of human gut was estimated to contain at least 957 bacterial genera with phyla Actinobacteria, Bacteroides, Firmicutes, Proteobacteria and Verrucomicrobia demonstrating most diversity and abundance[96]. While both types of diabetes mellitus are known to cause significant changes in gastrointestinal microbial composition, underlying mechanisms for dysbiosis and roles of all microbiome constituents, including bacteria, archaea, eukaryota and fungi, are still not fully understood. *Roseburia intestinalis*, *Faecalibacterium prausnitzii*, and families *Ruminococcaceae/Lachnospiraceae*, all known as butyrate producers, were detected to be lower during T2D[97,98]. Abundance of *Akkermansia muciniphila*, a mucin-degrading primarily mucosal bacteria, had been connected to lower insulin resistance, while their low concentrations were associated with obesity, diabetes, IBD, ulcerative colitis and appendicitis, suggesting future use of this bacteria as a biomarker[99]. However, such broad spectrum of diseases makes effective clinical usage questionable. *Prevotellacopri* and *Bacteroides vulgatus* were mentioned as possible promoters for insulin resistance due to active branched chain amino acids (BCAA) production[100]. Data on general *Firmicutes/Bacteroidetes* ratio changes during prediabetes and T2D are contradicting, which may be explained by differences in sequencing methods and bioinformatics approaches[100,101]. Recent 16S/18S/ITS microbiome profiling study of T2D with 49 adult participants in India showed interesting correlation for archaea, where concentration of *Methanobrevibacter* increased in direction from healthy subjects to fully developed T2D, while *Methanosphaera* concentration gradually decreased. Fungal component demonstrated overall abundance growth with inclusion of pathogenic *Aspergillus* and *Candida* phyla[98]. Most of aforementioned microorganisms were proposed as possible indicators for prediabetes, T1D (type 1 diabetes) and T2D, but their use in clinical practice is not recommended at the moment due to low amount of data and contradictory nature of results between studies, which may be solved in the future[86].

Linkage of microbiome to diabetes through obesity and metabolic syndrome.

Both T2D and obesity demonstrate a growing trend across the globe, with subjects suffering from the latter being often viewed as possible T2D risk group[102,103]. Recent findings in the field of microbiome variation during diabetes and obesity had reaffirmed earlier theories concerning microbiota's participation in adipose tissue function and insulin resistance. Network-based gene expression association studies of host's genome underline digestive metabolism, immunization, and signal transduction as the most prominent mechanisms in development of obesity/T2D[104], while the data on gastrointestional microbiome role is yet to be unified in coherent system. Gut microbiota had been shown to regulate body mass in a set of fecal transplantation experiments conducted on lean, obese and germ-free mice. Transplantation of gastrointestional microbiota from lean to obese mice led to lower insulin resistance, while transfer of microbiota from obese to lean mice led to body mass increase by 60% and higher insulin resistance[83,104,105]. Low grade inflammation, acquired through activation of TLR4/MyD88/NF-κB pathway by lipopolysaccharides from gram-negative bacterial walls, had been connected to insulin resistance through insulin receptor substrate serine phosphorylation by participants of inflammatory cascade[106]. Inhibition of NF-κB led to increase of Akkermansia/Lactobacillus, reduced body mass and lower insulin tolerance[100,107]. Short chain fatty acids (SCFA), obtained by bacteria through fermentation of non-digestible fibers, serve as signaling molecules in a broad list of processes, including proliferation of pancreatic β cells and insulin biosynthesis. This partially explains prebiotic treatment effectiveness and changes in abundance of *Roseburia intestinalis* and *Faecalibacterium prausnitzii*, but further research is required, as results from different studies often contradict each other[100,108,109]. High serum levels of BCAAare attributed to both obesity and T2D with steady increase of *Prevotella copri* and *Bacteroides vulgatus* during the onset of the diseases[110]. Both probiotics and prebiotics tend to increase insulin sensitivity and lower body mass, although studies

have small sample sizes and require longitudinal research[111,112].

Metformin effects on microbiome composition

Recent findings demonstrate that effectiveness of metformin, most prescribed antidiabetic drug whose pharmacodynamics mainly involve activation of hepatic AMP-activated protein kinase in liver, may be partially attributed to mediation of diabetic dysbiosis. Increase of *Akkermansia muciniphila* abundance after metformin treatment was detected in both human and animal studies, while in vitro conditions in gut simulator demonstrated metformin as a growth factor for both *Akkermansia muciniphila* and *Bifidobacterium adolescentis*[113,114]. Metformin therapy was found to promote growth of SCFA-producing bacteria in rats (Allobaculum, Bacteroides, Blautia, Butyricoccus, Lactobacillus, Akkermansia and Phascolarctobacterium) and humans (Akkermansia, Lactobacillus, Bifidobacterium, Prevotella, Megasphaera, Shewanella, Blautia or Butyrivibrio)[113].

NEW APPROACHES FOR CLINICIAN INTERPRETATIONS OF NGS DATA

The identification of multiple loci by GWAS and sequencing technologies has given a considerable impetus to the disclosure of pathogenesis of T2D and provides a tempting opportunity to translate genetic information to clinical practice. This knowledge may have potential role in disease risk prediction including identification of subjects at risk of developing disease at an early-stage, and in clinical management of individuals to modify treatment regimens so that affected individuals would benefit most by their therapy and avoid the occurrence of complications[63]. The emerging availability of genomic and electronic health data in large populations is a powerful tool for research that has drawn interest in bringing precision medicine to diabetes[115].

Can a genetic test motivate lifestyle changes?

According to the latest polls people are interested in genetic testing for T2D risk since this allows them to evaluate the individual feature of pathology state[116]. However, several studies have shown that some factors contribute to the failure of individuals to conduct a genetic test. The main factors that influence refusal include distrust of medical researchers, religious prejudices and lower levels of education[117,118]. Some have argued that the clinical significance of genetic markers of T2D have only a minor role in predicting the risk with careful clinical risk assessment, the predictive value increases[116,119].

Until recently, it has been assumed that genetic predisposition awareness can motivate healthy behavior[120]. According to some authors, it is considered that the patient does not appear motivated to a healthy lifestyle after identifying his genetic predisposition[121-123]. At the same time, research on the molecular basis of the development of T2D is absolutely necessary when making a diagnosis, since young individuals with T1D can also be obese[124,125]. Misdiagnosis of diabetes can lead to misuse of medical treatment[126].

Studies of genetic biomarkers: Prediction, and diagnosis of T2D

Many studies have analyzed the utility of genetic variants in T2D risk prediction for undiagnosed individuals with T2D using cross-sectional studies and incident T2D using longitudinal studies. Early studies provided much optimism and showed that common variants at the *TCF7L2* locus predict the progression to diabetes in subjects with impaired glucose tolerance[63,127]. Unfortunately, diabetes mellitus is diagnosed on the basis of its biochemical effects (increased glucose), and the absence of detection of the main defect, which indicates the absence of the disease[128]. However, at present, aggregated available data do not provide robust evidence to support the utility of genetic testing for T2D predictions and indicate a modest contribution of genetic variants[129-131]. Several large population-based follow-up studies have been published aiming to investigate the predictive power of common genetic variants on the risk of incident T2D. The results of these studies were similar to those from cross-sectional case-control studies. It was shown that risk variants did not essentially increase the AUC to predict T2D when combined with clinical risk factors[132]. However, it seems possible to improve T2D risk prediction and overcome factors limiting predictive power, such as: (1) Modest effect sizes of common variants, (2) Insufficient knowledge of rare and coding variants missed by GWAS; (3) Heterogeneous nature of the disease; and (4) Genetic diversity between ethnic groups (detailed below).

The limitations related with modest effect sizes of common alleles and necessity of

further investigation aimed to identify rare and coding variants involved in T2D pathogenesis have been reviewed above. T2D seemingly encompasses a group of several subtypes of diseases, which makes it rather difficult to distinguish it from other types, as it may be the result of defects in various metabolic pathways. The accuracy of prediction models may be affected by the fact that latent autoimmune diabetes in adults has been identified and the number of monogenic forms of diabetes is increasing, which can also indicate the level of misclassification[133].

In different populations, heterogeneity in association of genetic variants with the disease was demonstrated, apparently related to the design of the study, in particular the results of a large meta-analysis that combines cases of T2D with different origins or signs and evaluates them with a generalized intermediate hyperglycemia phenotype, despite the fact that the phenotype may differ due to a multitude of unrelated causes within the physiology of the body or the environment[134]. In recent years, a large number of projects have been carried out to study the causes of diabetes, large-scale studies have been created and huge biobanks of samples of these patients have been collected. In addition, some variants were found that are important in the prevention and treatment of T2D, found in individual population isolates, demonstrating the value of studying genetically isolated populations[128]. Because of genetic drift, deleterious variants with large phenotypic effects could rise randomly to higher allele frequencies. Which makes investigation of such variants' association easier in isolated populations compared to the admixed ones, in which these variants might not be present or might be very rare[10].

Circulating miRNAs in plasma or serum have several features that make them ideal candidate biomarkers of complex diseases such as T2D[135]. Hundreds of miRNAs are actively or passively released to the blood circulation to regulate specific gene function[136]. Current studies demonstrate that changes in expression miRNAs involve in dysfunction of insulin and progression of T2D. Many studies confirmed that some miRNAs have been identified and found to be associated with T2D[137]. miR-21, miR-126 and miR146a have been shown to have potential to be biomarkers of early diagnosis of T2D disease[138-140]. Thus, the above mentioned miRNAs and a number of other miRNAs may be candidates for testing the effectiveness of therapy but further studies are needed to identify them[137].

Genetic tests of T2D: Implications for therapy

T2D commonly develops with insulin resistance, a disorder in which cells located primarily within the muscles, liver, and fat tissue do not use insulin properly, and progresses to pancreatic beta-cell failure. T2D trigger are insulin resistance and inadequate insulin secretion[141].

Selection of drug therapy based on the genetic features of the individual can be a huge breakthrough because there are individual drug idiosyncrasy and many patients eventually fail to achieve recommended levels of glycemic control due to their genetic characteristics[142,143]. Currently, only half of patients initiating therapy with metformin or sulfonylurea, reached a level of hemoglobin A1c in 7%[144]. It should be emphasized that sulfonylureas and metformin are the most studied classes of drugs used to treat T2D[115].

Sulphonylureas (SUs) are widely used drugs in the clinical practice however, different side effects, such as weight gain and increased risk of hypoglycemia, have been frequently[145]. Studies have shown that these drugs can act effectively in response to a defect induced by variants in *KCNJ11* (rs5219, rs5215) and *ABCC8* (rs757110) in patients with T2D[146,147]. Also important in the selection of SUs play role *CYP2C9* (rs1799853, rs1057910), *TCF7L2* (rs12255372, rs7903146), *IRS*1 (rs2943641, rs1801278) and *CAPN10* (rs3842570, rs3792267, rs5030952)[148-151]. It should also be noted rs7754840 in the gene *CDKAL1*, which is significantly associated with the response to treatment with sulfonylurea and in combination with other clinical and pathological data will help move to individual therapy of patients with T2D[152].

Metformin is the most commonly used drug in the treatment of T2D, which is not metabolized in the liver, therefore, the effect of reducing the level of metformin is not affected by genetic variants in the genes encoding metabolizing enzymes[153]. *SLC22A1* (rs12208357, rs34130495, rs35167514, rs34059508) is the most studied gene that is involved in the response to metformin[154]. However, other genes involved in the metabolism of metformin have been identified, for example, *SLC22A2* (rs316019), *PPARG* (rs1801282)[145,155]. It should also be noted that the T2D–associated variant rs7903146 in *TCF7L2* influences the acute response to both glipizide and metformin in persons free of overt diabetes[156].

CONCLUSION

The growing power and reducing cost sparked an enormous range of applications of NGS technology that gave us the excellent instrument for solving various problems in molecular biology. Rational usage of this instrument, taking into account all of its benefits and limitations, is the next step on the way to elucidation of pathogenesis of complex diseases such as T2D. Results obtained in sequencing-based studies combined with earlier findings from GWAS and candidate genes studies allow ordering and improving our knowledge about T2D and give us an opportunity to translate genetic information to clinical practice. The increasing knowledge provides a fascinating opportunity to use this information to predict the occurrence of disease and to identify subgroups of patients for whom therapies will have the greatest efficacy or the least adverse effect. However, this new knowledge should be treated with caution. Unfortunately, the accuracy of risk prediction models based on genetic information of T2D is not remarkable to date. Hence, further research and technological improvement is needed in studying the individual and aggregate contribution of genetic markers for the development of diabetes for widespread use in clinical practice.

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ORIGINAL ARTICLE

Basic Study

Epidermal growth factor receptor rs17337023 polymorphism in hypertensive gestational diabetic women: A pilot study

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Abstract

BACKGROUND

Women with gestational diabetes mellitus have an increased risk of developing gestational hypertension, which can increase fetal and neonatal morbidity and mortality. In the past decade, single nucleotide polymorphisms in several genes have been identified as risk factors for development of gestational hypertension. The epidermal growth factor receptor activates tyrosine kinase mediated blood vessels contractility; and inflammatory cascades. Abnormalities in these mechanism are known to contribute towards hypertension. It is thus plausible that polymorphisms in the epidermal growth factor receptor gene would be associated with the development of hypertension in women with gestational diabetes.

AIM

To determine whether the epidermal growth factor receptor rs17337023 SNP is associated with the occurrence of hypertension in gestational diabetic women.

METHODS

This pilot case-control study was conducted at two tertiary care hospitals in Karachi, from January 2017-August 2018. Two hundred and two women at 28 week of gestation with gestational diabetes were recruited and classified into normotensive (n = 80) and hypertensive (n = 122) groups. Their blood samples were genotyped for epidermal growth factor receptor polymorphism rs17337023 using tetra-ARMS polymerase chain reaction. Descriptive analysis was applied on baseline data. Polymorphism data was analyzed for genotype and allele frequency determination using chi-squared statistics. In all cases, a P value of < 0.05 was considered significant.

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RESULTS

Subjects were age-matched and thus no difference was observed in relation to age of the study subjects (P >0.05). Body fat percentage was significantly higher in hypertensive females as compared to normotensive subjects (35.138 ± 4.29 Case *vs* 25.01 ± 8.28 Control; P < 0.05). Similarly, systolic and diastolic blood pressures among groups were significantly higher in hypertensive group than the normotensive group (P < 0.05). Overall epidermal growth factor receptor rs17337023 polymorphism genotype frequency was similar in both groups, with the heterozygous AT genotype (56 in Case *vs* 48 in Control; P = 0. 079) showing predominance in both groups. Furthermore, the odds ratio for A allele was 1.282 (P = 0.219) and for T allele was 0.780 (P = 0.221) in this study.

CONCLUSION

This pilot study indicates that polymorphisms in rs17337023 may not be involved in the pathophysiology of gestational hypertension in gestational diabetes *via* inflammatory cascade mechanism. Further large-scale studies should explore polymorphism in epidermal growth factor receptor and other genes in this regard.

Key words: Gestational diabetes mellitus; Gestational hypertension; Epidermal growth factor receptor; rs17337023; Single nucleotide polymorphism; Polymorphism; Case-control

Core tip: Gestational Hypertension (GHTN) can increase risk of fetal and neonatal morbidity and mortality. Many environmental, nutritional and genetic factors are related to the development of GHTN. Among them, Epidermal Growth Factor Receptor (EGFR) has been found to contribute to arterial hypertension. It is thus plausible that Single nucleotide polymorphisms (SNPs) in EGFR gene would be associated with the development of GHTN in women with GDM. This pilot study indicated that EGFR rs17337023 polymorphism may not be involved in the pathophysiology of GHTN in GDM positive females in a local population. Further large-scale studies should explore SNPs in EGFR and other genes in this regard.

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INTRODUCTION

Gestational diabetes mellitus (GDM), defined as any degree of glucose intolerance with onset or first recognition during pregnancy[1,2], is a significant risk factor for maternal development of a hypertensive pregnancy disorder (HPD)[3-5]. Up to 10% of all pregnancies are complicated by HPDs[6,7], especially in cases with pre-existing GDM[8]. One type of HPD, Gestational Hypertension (GHTN) occurs in 1.8%-4.4% of pregnancies[9]. GHTN is defined as blood pressure (BP) that reaches ≥ 140/90 mmHg for the first-time during pregnancy (after 20 wk gestation), without proteinuria. BP normalizes by 12 week postpartum[10]. Complications of GHTN include increased risk for fetal death and severe neonatal morbidity and mortality[11]. Hypothesized mechanisms of HPD development include dysfunction of the placenta, endothelium or lipid metabolism, as well as inflammatory states[12]. However, it is being increasingly established that genetic factors also contribute towards HPDs[13].

Single nucleotide polymorphisms (SNPs) have been a particular focus in genetic mechanisms leading to HPD[14]. SNPs such as NOS SNP rs2070744[15], APM1 SNP rs1501299[16], CYP19A1 SNP rs700158[17], KDR SNP rs2071559[18] and HSD11B1 rs846910[19], have been found to be associated with HPDs. The epidermal growth factor receptor (EGFR) is a single chain transmembrane protein of the ErbB family of

receptor tyrosine kinases, which is activated following binding with peptide growth factors of the EGF-family of proteins[20]. The functions of EGFR include inducing cell growth and differentiation[21]. EGFR is abundantly expressed in the vascular wall and myocardium, and is thought to be linked to arterial hypertension, possibly by producing vasoconstriction and renal Na^+ retention[22]. Apart from its normal EGF Ligands, EGFR also undergoes transactivation by vasoactive substances such as catecholamines[23] and aldosterone[24]. The EGFR SNP rs17337023 (T > A), located on Exon 16 with a global variant allele frequency of 0.456[25], is associated with chronic inflammation, which may lead to vascular damage and hypertension[26,27]. Given the mechanistic link of EGFR to BP regulation, we decided to conduct a pilot study to explore any association of SNP rs17337023 with the development of GHTN in pregnant females with GDM.

MATERIALS AND METHODS

In a case-control study, n = 202 pregnant women at 28 wk of gestation with GDM were recruited. The study was conducted at Aga Khan University and Jinnah Postgraduate Medical Center during the period of January 2017 till August 2018. The sample size was calculated using the Open-Epi website[28], with a confidence level of 95%, power of 80%, least extreme odds ratio (OR) of 2 and a pregnancy hypertension prevalence of 8% taken according to previously published data sources[29]. The minimum sample size calculated for this research was n = 106. The institutional ethics committee approved the research protocol (Ref # 4523-BBS-ERC-16) (REF: No.F.2-81/GENL-2017-IRB/15107/JPMC). GDM was diagnosed by means of a 75-g 2-h oral glucose tolerance test, as per the criteria set by the IADPSG[30]. All study subjects gave a written informed consent followed by weight and body mass index (BMI) assessment based on South Asian criteria for BMI values [normal weight (BMI 18-22.9 kg/m^2), and obese (BMI ≥ $26kg/m^2$)][31]. BP assessment was done following the latest European Society of Cardiology and the European Society of Hypertension task force guidelines[32,33], (Normal BP < 139/85 mmHg and Hypertension > 139/85 mmHg). Subjects diagnosed with GHTN were subsequently being treated by antihypertensive medication. Any individual with a history of pre-existing diabetes, or any inflammatory condition, taking oral contraception or hormonal support, was not included in this study. Based on these measurements, grouping of study subjects was done as follow: (A) Normotensive (n = 80); (B) Hypertensive (n = 122) (on diet or medication).

Ten milliliters of venous blood were collected from each subject. DNA was extracted from whole blood by Qiagen DNA extraction kit (Cat. #51185, Valencia, CA, United States). The quantification of extracted DNA was performed by measuring the ultraviolet absorbance of the samples using a Nanodrop-ND1000 (Thermo Fisher Scientific, Waltham, MA). The absorbance ratio (A280/A260) was determined for 2 μL samples using ND-1000 V3.8.1 software (Thermo Fisher Scientific, Waltham, MA). A ratio of approximately 1.8 was considered acceptable for confirming the purity of extracted DNA. Furthermore, around 10% of samples were confirmed on gel electrophoresis by running 1 μL of sample in a 1% agarose gel against a 1 kb ladder. Tetra arms polymerase chain reaction (PCR) was performed using the Ruby Taq PCR Master mix 2X (Cat. #71191, Affymetrix, United States) as per the manufacturer's instructions. PCR products were electrophoresed in a 2% agarose gel. Genotyping quality control was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates was > 99 %). The following primer set was used for gene amplification: Statistical analyses were conducted using the IBM Statistical Package for the Social, Sciences (IBM SPSS version 21; IBM Corp Inc, Armonk, NY). Descriptive analysis was applied, and data was expressed either as mean ± standard deviation or absolute number and percentage. SNP data was analysed for genotype and allele frequency determination by applying chi-squared statistics. In all situations a P value of < 0.05 was considered significant. The statistical analyses for this study were performed and reviewed by Syed Adnan Ali (PhD. Statistics) of the University of Karachi.

RESULTS

The detailed results are shown in Table 1, 2 and Figure 1. All study subjects were age-matched and therefore, no difference was observed in relation to age of the study subjects (P > 0.05). Body fat percentage was significantly higher in hypertensive females as compared to normotensive subjects (P < 0.05). Similarly, systolic and diastolic BP among were significantly higher in hypertensive group than the normo-

tensive group ($P < 0.05$). 90% of the hypertensive females practiced sedentary lifestyle versus 17.7% normotensive females ($P < 0.05$).

Overall EGFR rs17337023 polymorphism genotype frequency was similar in both the normotensive and hypertensive groups, with the heterozygous AT genotype showing predominance in both groups. Furthermore, the OR for A allele was 1.282 ($P = 0.219$) and for T allele was 0.780 ($P = 0.221$) in this study.

DISCUSSION

The developmental causes of GHTN in women with pre-existing GDM is poorly understood and it is possible that genetic factors such as SNPs may play a role. Many studies have demonstrated associations of certain SNPs with development of HPDs. Our objective was to investigate whether the EGFR SNP rs17337023 displayed any significant association with the occurrence of GHTN in pregnant women with GDM. However, the findings of our study showed that the frequency of the rs17337023 genotype was not significantly different in the two groups. Furthermore, the OR for the A and T alleles were also non-significant. These results suggest that the SNP rs17337023 does not play any major role in the pathophysiology of GHTN in GDM.

There are possible explanations for the lack of any significant association. The study proposing mechanisms linking EGFR to arterial hypertension does so primarily on the basis of results obtained from experimenting using animal models, and voices uncertainties about its applicability to humans[22]. Moreover, apart from Rheumatoid Arthritis, the SNP rs17337023 has been shown to have no significant association with pathologies such as Gastric Carcinoma[34] and Nasopharyngeal Carcinoma[35]. This suggests that the function of EGFR is not altered significantly enough due to the SNP rs17337023 mutation to cause any major pathological state. It is possible, however, that other EGFR SNPs may indeed be associated with GHTN in women with GDM.

Additionally, since the sample for our pilot study consisted of 202 women from Pakistan, it is possible that the results may show greater significance if the study were replicated in another population with a larger sample size. The lack of association between the EGFR SNP rs17337023 served to suggest that the EGFR gene may not be involved in the pathophysiology of GHTN in the case of pre-existing GDM. Our study was limited by the inability to recruit a larger sample size due to cultural beliefs and barriers towards participation in genetic studies. Moreover, the group of women with GHTN were not managed uniformly in terms of diet and antihypertensive medications. Nevertheless, the rs17337023 polymorphism was in Hardy-Weinberg Equilibrium for cases and controls, suggesting the randomness of the sample as a strength of our study.

This pilot study indicates that polymorphisms in rs17337023 may not be involved in the pathophysiology of gestational hypertension in gestational diabetes *via* inflammatory cascade mechanism. Further large-scale studies should explore polymorphism in epidermal growth factor receptor and other genes in this regard.

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Table 1 Polymerase chain reaction primers details

Gene	Primers	Base pairs	PCR cycle	Amplicon size
Epidermal growth factor receptor (EGFR) rs17337023	Forward outer: ATTAACCACCAATCCAACATCCAGAC	26	67 °C; 30 s; 30 cycles	T allele 180; C allele 271; Control 406
	Reverse outer: CTTTCCCTCCACTGAGGACAAAGTT	25		
	Forward inner (A allele): TCTCTTTCACTTCCTACAGATGCTCA	26		
	Reverse inner (T allele): AGCCTTCAAGACCTGGCGCA	20		

PCR: Polymerase chain reaction.

Table 2 Descriptive statistics and genotype frequency of study subjects

	Hypertensive pregnant case (*n* = 122)	Normotensive pregnant control (*n* = 80)	*P*-value
Age (Yr)	30.55 ± 8.05	29.13 ± 10.19	0.054
Weight (kg)	77.56 ± 16.88	69.24 ± 11.07	0.025
Body Fat %	35.138 ± 4.29	25.01 ± 8.28	0.000
Waist circumference (cm)	104.50 ± 12.09	86.92 ± 12.03	0.000
Systolic blood pressure (mmHg)	131.76 ± 13.04	122.02 ± 8.27	0.000
Diastolic blood pressure (mmHg)	85.88 ± 8.45	73.31 ± 11.27	0.000
Walk			
None	90.1%	17.7%	0.000
30 min/3 days week	7.9%	74.2%	
30 min/5 days week	2.0%	8.1%	
Genotype frequency			
EGFR rs17337023 polymorphism			
AA	30	19	0.079
AT	56	48	
TT	36	13	
Allele Odds Ratio			
Allele A	1.282 [0.860-1.912]		0.219
Allele T	0.780 [0.523-1.163]		0.221

Data presented as Mean ± SD and percentages. In all cases a *P* value of < 0.05 was considered significant. Hardy-Weinberg Equilibrium (HWE) for case *P* = 0.378 and control *P* = 0.064, Where EGFR is epidermal growth factor receptor.

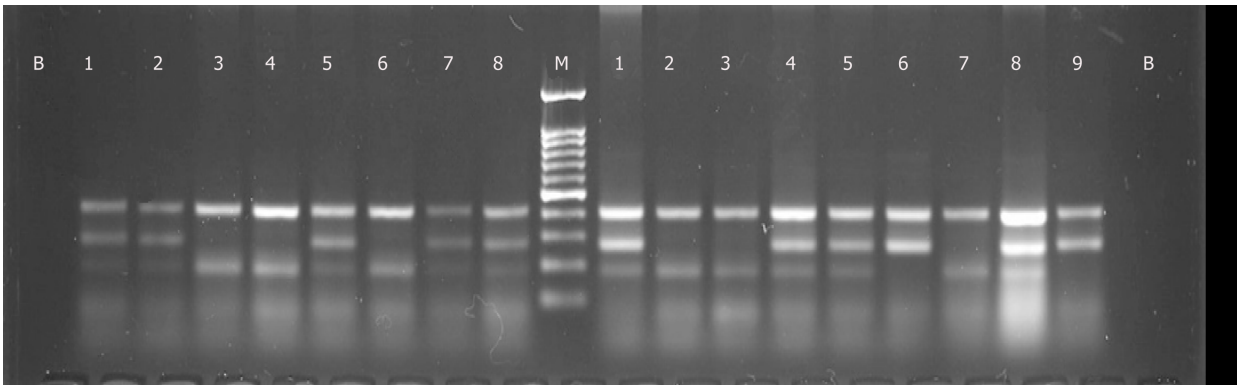

Figure 1 Gel Electrophoresis of epidermal growth factor receptor samples 1 to 8 cases and 1 to 9 Controls. B is blank, and M is the 100bp ladder. Tetra arms control band is visible at 406bp; C allele at 271bp and T allele at 180bp.

ARTICLE HIGHLIGHTS

Research background

Pregnancy induced hypertension and diabetes are an increasing threat to the wellbeing of both mother and the baby. The basic pathophysiological link to disease predisposition is attributed to the functionality of epidermis and angiogenesis. Several genetic studies have provided evidence that epidermal growth factor dysfunction can lead to hypertension and its complications in pregnancy.

Research motivation

Materno-fetal mortality is on the rise in lower middle income countries; predominantly due to lack of primary prevention of non-communicable diseases. This led us to investigate one of the route cause *i.e.* genetic modification as a risk for development of disease.

Research objectives

Explore any association of SNP rs17337023 with the development of gestational hypertension in pregnant females with gestational diabetes.

Research methods

A case-control study was conducted recruiting 202 pregnant women at 28 wk of gestation. Their blood pressure, blood glucose levels were measures and genotyping of EGFR SNP rs17337023 was performed via tetra arms PCR.

Research results

No difference was seen in the EGFR rs17337023 polymorphism genotype frequency among both normotensive and hypertensive groups in this study.

Research conclusions

This pilot study indicates that polymorphisms in rs17337023 may not be involved in the pathophysiology of gestational hypertension in gestational diabetes. Further large-scale studies should explore polymorphism in epidermal growth factor receptor and other genes in this regard.

Research perspectives

This study has shown some negative results linking a specific area of the gene EGFR; however, it should be noted that other factors may also be in play such as obesity and family history that can be a contributing factor along with genetic predisposition for hypertension. This opens up new avenues for researchers to perform prospective studies to identify the causal link between genetic and environmental factors.

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ORIGINAL ARTICLE

Observational Study

Diabetes empowerment scores among type 2 diabetes mellitus patients and its correlated factors: A cross-sectional study in a primary care setting in Malaysia

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Abstract

BACKGROUND

There are limited studies on diabetes empowerment among type 2 diabetes patients, particularly in the primary care setting.

AIM

To assess the diabetes empowerment scores and its correlated factors among type 2 diabetes patients in a primary care clinic in Malaysia.

METHODS

This is a cross sectional study involving 322 patients with type 2 diabetes mellitus (DM) followed up in a primary care clinic. Systematic sampling method was used for patient recruitment. The Diabetes Empowerment Scale (DES) questionnaire was used to measure patient empowerment. It consists of three domains: (1) Managing the psychosocial aspect of diabetes (9 items); (2) Assessing dissatisfaction and readiness to change (9 items); and (3) Setting and achieving diabetes goal (10 items). A score was considered high if it ranged from 100 to 140. Data analysis was performed using SPSS version 25 and multiple linear regressions was used to identify the predictors of total diabetes empowerment scores.

RESULTS

The median age of the study population was 55 years old. 56% were male and the mean duration of diabetes was 4 years. The total median score of the DES was 110 [interquartile range (IQR) = 10]. The median scores of the three subscales

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were 40 with (IQR = 4) for "Managing the psychosocial aspect of diabetes"; 36 with (IQR = 3) for "Assessing dissatisfaction and readiness to change"; and 34 with (IQR = 5) for "Setting and achieving diabetes goal". According to multiple linear regressions, factors that had significant correlation with higher empowerment scores among type 2 diabetes patients included an above secondary education level ($P < 0.001$), diabetes education exposure ($P = 0.003$), lack of ischemic heart disease ($P = 0.017$), and lower glycated hemoglobin (HbA1c) levels ($P < 0.001$).

CONCLUSION

Diabetes empowerment scores were high among type 2 diabetes patients in this study population. Predictors for high empowerment scores included above secondary education level, diabetes education exposure, lack of ischemic heart disease status and lower HbA1c.

Key words: Diabetes; Empowerment; Scores; Diabetes Empowerment Scale; Type 2 diabetes; Primary care; Malaysia

Core tip: This study aims to assess the diabetes empowerment scores and its correlated factors among type 2 diabetes patients in a primary care clinic in Malaysia. Median age of the study population was 55 years old, 56% were male and mean duration of diabetes was 4 years. The total median score of the Diabetes Empowerment Scale was 110 (interquartile range = 10). Diabetes empowerment scores were high among type 2 diabetes patients in this study population. The predictors for high empowerment score were those who had above secondary education level, diabetes education exposure, no ischemic heart disease status and lower glycated hemoglobin.

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INTRODUCTION

Diabetes has become a global epidemic of the 21st century. Over 70% of known cases of diabetes occur in developing countries. Four hundred and fifteen million adults were estimated to have diabetes globally in 2015, or 1 in 11 adults. This is estimated to rise to 642 million by 2040[1]. The Southeast Asian region has seen a recent dramatic increase in diabetes. An estimated 96 million people have diabetes in the region, 90% of whom have type 2, which is preventable[2]. In Malaysia, the incidence rate has also significantly increased from 8.3% in 1996 to 17.5% (3.5million) in 2015[3]. The Federal Territory of Putrajaya was noted to have the highest increment in the prevalence of diabetes in adults, from 2011 to 2015, that is 8.8% to 19.2%[4]. Primary care was identified as the backbone in managing diabetes. A majority sought treatment at government health clinics (59.3%) and private clinics (15.1%)[3]. Therefore, the population attending government health clinics would provide a better picture of overall diabetes management.

Evidence shows that self-empowerment is important in managing chronic diseases, especially diabetes[5,6]. Self-empowerment is an approach that can improve the ability of the patients with diabetes to understand the disease process better, involve themsel-ves actively in self-care, and practise healthy lifestyles for better disease control[6,7]. The process of empowerment improves diabetes control by helping patients in making decisions in regards to diabetes care and self-realization of their responsi-bilities in managing type 2 DM[8]. Tol *et al*[9] and Liu *et al*[10] showed that self-efficacy and self-esteem have a strong relationship with empowerment. However, there are few studies on diabetes empowerment among type 2 diabetes patients in Malaysia. Therefore, this study was conducted to examine diabetes empowerment and its correlated factors among patients with type 2 diabetes in a primary care

setting in Malaysia.

MATERIALS AND METHODS

Setting

This is a cross-sectional study of patients registered with a primary health care clinic located in Putrajaya, a Federal Territory and the administrative capital of Malaysia. It has a total population of 91900[11]. The study was conducted over a 3-month period from January 2019 to March 2019.

Inclusion criteria

The inclusion criterion for this research included patients aged 18 years and above diagnosed with type 2 diabetes mellitus (DM) and following up for at least 6 months in the primary care clinic. The exclusion criteria included intellectual disability or dependence for activities of daily living, being bed ridden, requiring nursing care to carry out daily activities or being clinically unstable during the study period. The sample size was calculated using the Lemeshow formula based on the prevalence of high DES scores of 36.9% for married and 3.8% for unmarried . The calculated sample size was 322 after taking account of non-respondent rate of 30%, 80% power and significance level of 0.05.

Data collection

Face-to-face interviews were conducted using an adapted structured questionnaire. After obtaining ethical approval, we approached the participants and explained the nature of the study before obtaining written consent to participate in the study. Systematic sampling was used to recruit respondents. The estimated number of diabetic patients attending follow up in the primary care clinic is about 15 patients per day, and 900 patients over the three-month duration of data collection. The estimated sample size for this study was 322; therefore, a sampling interval of 3 was used as the constant during study recruitment. The starting number of 1 was selected randomly from the health clinic registration counter using a dice.

Data collection instrument

The questionnaire was initially prepared in English by the author. Then, forward and backward translations into Malay and English languages were performed by two certified translators. The questionnaire was a self-administered type divided into two sections. The first section includes the patients' sociodemographic information. The second section explores the clinical profiles, clinical outcome and total diabetes empowerment scores.

Diabetes Empowerment Scale

The Diabetes Empowerment Scale (DES-28) was developed by the University of Michigan Diabetes Research and Training Center. The questionnaires consist of 28 items with 3 subscales, with each item rated along a 5-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree). The range of score was divided in three subgroups as low (28-65 scores), middle (66-103) and high (104-140). Cronbach's alpha coefficient is a measure of internal consistency and can be interpreted as the mean of all possible split-half coefficients[12]. By convention, if Cronbach's alpha is greater than or equal to 0.7 to 0.8, there is acceptable agreement[13].

This DES-28 is a reliable tool with good internal consistency (Cronbach's alpha = 0.96)[14]. The Cronbach's alpha of each subscale was 0.93 for "managing the psychosocial aspects of diabetes"; 0.81 for "assessing dissatisfaction and readiness to change"; and 0.91 for "setting and achieving diabetes goals". Each coefficient for the overall DES and three subscales was good[14].

For the DES Malay version, the questionnaire was originally in English by the author from the University of Michigan Diabetes Research and Training Center, then forward and backward translated into Malay and English languages by two certified translators. The questionnaire was a self-administered questionnaire, which was pretested through a pilot study prior to the actual data collection. The Cronbach's alpha coefficient for the Malay version total DES was 0.92.

The pilot study included 30 patients, 10% of the actual sample size of 322. Recruitment was performed via the systematic sampling method, with every one in two patients registered at the health clinic counter for follow-up selected for the pilot study. About five to eight respondents were collected a day for five days. Question 5 had a spelling error, "realitik" which was corrected to "realistis". Two other questions were rephrased for easier understanding, namely questions 1 and 2 "apa bahagian" (What part) to "bahagian apa". The findings from this pilot study were not included

in the data analysis of the actual study.

Operational definitions

Ethnicity was defined as Malay, Chinese, Indian or others. Education level was according to the respondents' self-reported highest attained level of education: No formal education, primary school, secondary school or tertiary (diploma/university). Smoking status was defined as whether the patient is a smoker, non-smoker or ex-smoker who had quit smoking at least 6 months from the quit date[13]. BMI was calculated as the weight in kg divided by the square of height in meter, and classified according to the Asian population[14]. Diabetes duration was defined as the duration of diabetes in years. Compliance to treatment was defined as the patients' self-reported compliance to treatment. The clinical outcomes [systolic and diastolic blood pressure, low-density lipoprotein (LDL) level, high-density lipoprotein (HDL) level, triglycerides (TG) level, glycated hemoglobin (HbA1c) %] in this study were defined in terms of the latest levels measured.

Data analysis

Statistical Package for Social Sciences (SPSS) version 25.0 was used to analyze the data collected from the study. Descriptive analysis was used to describe the characteristics of the respondents in terms of frequencies, percentages, median, and interquartile range (IQR). In this study, we used Chi-square test for the categorical data, Spearson's test, Mann-Whitney *U* test and Kruskal Wallis test for the continuous data to identify the associations between the total diabetes empowerment scores with sociodemographic factors, clinical profiles and clinical outcomes. Multiple linear regressions were used to identify the predictors of total diabetes empowerment score. All variables with a *P* value < 0.25 in the univariate analysis, as well as clinically significant variables, were entered into the multiple linear regression. The dependent variable was total diabetes empowerment score among type 2 diabetes patients. The independent variables are sociodemographic factors (age, gender, ethnicity, level of education, marital status, smoking status) and clinical profiles (DM durations, DM education exposure, compliances to treatment, BMI, hypertension status, dyslipidemia status, ischemic heart disease status, asthma status systolic and diastolic blood pressure, HbA1c, HDL level, LDL level and TG level).

Ethical approval

Ethical approval was obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-17-3085-38099).

RESULTS

A total of 322 participants were recruited into this study, for a response rate of 93.7%. There were no missing data in our study. Table 1 demonstrates the sociodemographic and clinical characteristics of the study population. Median age was 55 years old with IQR of 18. More than half of the participants were male (58.7%, *n* = 189). The majority of the study population were Malay (92.2%), married (92.2%) and had an education above the secondary school level (88.8%). Two-thirds of the participants were non-smokers (66.5%). With regards to the clinical profiles (Table 2), the median diabetic duration for the participants was 4 years (IQR = 7). The mean systolic and diastolic blood pressures are 133.2 ± 15.5 mmHg and 83.7 ± 10.0 mmHg respectively. More than half of respondents were obese (62.7%), had hypertension (64.3%) and dyslipidemia (76.4%). The median for HbA1c was 7.4% with IQR 2.6. The mean for LDL was 3.0 ± 1.0 mmol/L. The median for HDL was 1.1 mmol/L with IQR 0.3, and the median for TG was 1.4 mmol/L with IQR 0.9.

Most of the participants had received diabetes education (82%, *n* = 264). The total diabetes empowerment median score was 110 (IQR = 10) and classified as high. The median scores of the three subscales were 40 (IQR = 4) for "Managing the psychosocial aspect of diabetes"; 36 (IQR = 3) for "Assessing dissatisfaction and readiness to change"; and 34 (IQR = 5) for "Setting and achieving diabetes goal". Spearman Correlation coefficient showed a statistically significant relationship between HbA1c level (r = -0.132, *P* value 0.018) with the total empowerment score as shown in Table 3. Mann Whitney *U* test showed that those with diabetes education exposure (*P* = 0.004), received above secondary school level (*P* < 0.001), and those without ischemic heart disease (*P* = 0.004) were statistically significant correlated with total diabetes empowerment score as shown in Tables 4 and 5.

There is no significant correlation between total diabetes empowerment score with other variables like age, diabetes duration, systolic and diastolic blood pressure, gender, ethnicity, marital status, smoking status, hypertension status, dyslipidemia

Table 1 Sociodemographic profiles of the study participants in primary health care clinic in Putrajaya (*n* = 322), *n* (%)

Variables	Frequency	Median (IQR)
Age (yr)		55 (18)
Gender		
Male	189 (58.7)	
Female	133 (41.3)	
Ethnicity		
Malay	297 (92.2)	
Chinese	6 (1.9)	
Indian	14 (4.3)	
Others	5 (1.6)	
Education level		
No formal education	12 (3.7)	
Primary school	24 (7.5)	
Secondary school	132 (41.0)	
Diploma/University	154 (47.8)	
Marital status		
Single	25 (7.8)	
Married	297 (92.2)	
Smoking status		
Yes	46 (14.3)	
Never	214 (66.5)	
Ex-Smoker	62 (19.2)	

IQR: Interquartile range.

status, asthma status, compliance to treatment, LDL level, HDL level and TG level. According to multiple linear regressions, factors that had significant correlation with higher empowerment scores among type 2 diabetes patients included above secondary education level ($P < 0.001$), diabetes education exposure ($P = 0.003$), lack of ischemic heart disease ($P = 0.017$) and lower HbA1c ($P < 0.001$) as shown in Table 6.

DISCUSSION

In our study, the median score of the total diabetes empowerment was 110. We thus conclude that the empowerment of this study population is high based on the range for high empowerment score range in DES being 104 to 140. The total mean score found by Tol *et al*[9] was 88.13 ± 30.3, which indicated a middle score according to DES score range, lower than that of our study. This is probably due the difference of the education level between the two study populations, as less than half of their study population had a diploma or higher education, and the study was conducted in a diabetes research centre. A majority of our study population had an education above the secondary school level, the study was conducted in an urban primary care clinic setting. This may be due to socio-culture restrictions as well. For example, in Iran, quality diabetes care is not widely available, with a significant knowledge gap in handling diabetes. Diabetes diagnosis, prevention and management are suboptimal[14].

The study findings showed that the subscale of "Setting and achieving diabetes goal" has highest median score among the three subscales. This finding is similar with two previous studies[15,16]. The literature has shown that structured goal setting is the best way to aid diabetes patients to set behavior goals to practice healthy lifestyle and improve HbA1c level[17,18].

This study shows that a higher than secondary school education level is significantly correlated with diabetes empowerment score. This result is similar with other studies. Tol *et al*[18] showed that an education level of diploma or higher had higher empowerment score. Similarly, D'Souza *et al*[19] showed that those with high school and diploma education level had higher diabetes empowerment scores[15,16]. This indicates that patients with a higher education level possibly understand the disease process better and have more awareness towards self-care of diabetes management[19].

Table 2 Clinical profiles of the type 2 diabetes mellitus patients with total diabetes empowerment scores, *n* (%)

Variables	Frequency	Median (IQR)
Diabetes duration (yr)		4.00 (7.0)
Compliance to diabetes treatment		
Yes	310 (96.3)	
No	12 (3.7)	
Diabetes education exposure		
Yes	264 (82.0)	
No	58 (18.0)	
BMI (kg/m^2)		28.70 (7.12)
Underweight (< 18.5)	3 (1.0)	
Normal (18.5-22.9)	29 (9.0)	
Overweight (23-27.4)	88 (27.3)	
Obese (> 27.5)	202 (62.7)	
Hypertension status		
Yes	207 (64.3)	
No	115 (35.7)	
Dyslipidaemia status		
Yes	246 (76.4)	
No	76 (23.6)	
Ischemic heart disease status		
Yes	42 (13.0)	
No	280 (87.0)	
Asthma status		
Yes	30 (9.3)	
No	292 (90.7)	

BMI: Body mass index; IQR: Interquartile range.

This study found that diabetes education exposure had a significant relationship with the total diabetes empowerment score. Those participants who had diabetes education exposure had better empowerment compared to those who had no diabetes education exposure. Diabetes education consists of structured programs, which cover basic information on diabetes, insulin therapy, blood glucose levels and targets, physical exercise, diet management and hypoglycemia[20]. It incorporates practical skills especially using the home blood glucose monitoring and insulin therapy in diabetes management. The education program also emphasizes the importance of achieving targeted glycemic control to prevent complications and it includes foot care[21]. Thus, those who received diabetes education exposure are better skilled in managing their disease, as reported in the literature. Enhancement of patient empowerment is achieved when patients are educated with adequate information on their health conditions[22].

Our study showed a significant correlation between those without ischemic heart disease with total diabetes empowerment score. The majority of the patients without ischemic heart disease had secondary education and above (89.6%), which correlates to higher diabetes empowerment score.

Our study found that HbA1c was 7.4% with an IQR of 2.6. It would be better to compare this to the mean HbA1c for type 2 DM population in Malaysia[23]. According to National Diabetes Registry, the mean HbA1c for type 2 DM from 2009 to 2012 was 8.1. Our study showed that a lower HbA1c level was significantly correlated with higher diabetes empowerment scores. This finding is consistent with those of a previous study[16]. Patients with higher empowerment score were better in self-care and practicing healthy lifestyle contributing to a better HbA1c level[8,24,25]. Age had no significant correlation with diabetes empowerment score in our study. Our study participants were aged between 26 to 84 years old with a median age of 55 (IQR = 18). This finding was not similar compared to the study done previously by D'Souza *et al*[19] in a study in Oman, which reported that higher empowerment levels were seen among those 40-49 years old.

Table 3 The correlation between clinical outcome with total diabetes empowerment scores and subscales and among type 2 diabetes mellitus patients

Variables	Total diabetes empowerment score		Managing the psychosocial aspect of diabetes		Assessing dissatisfaction and readiness to change		Setting and achieving diabetes goal	
	Coefficient correlation	*P* value	Coefficient correlation	*P* value	Coefficient correlation	*P* value	Coefficient correlation	*P* value
Systolic blood pressure[1] (mmHg)	0.046	0.411	0.073	0.192	0.014	0.798	0.013	0.821
Diastolic blood pressure[1] (mmHg)	-0.009	0.867	-0.011	0.849	-0.055	0.323	0.024	0.674
HbA1c[1] (%)	-0.132	0.018	-0.122	0.028	-0.11	0.049	-1.168	0.003
HDL level[1] (mmol/L)	0.022	0.693	0.019	0.734	0.013	0.816	0.104	0.063
LDL level[1] (mmol/L)	-0.087	0.12	-0.064	0.252	-0.044	0.435	-0.062	0.269
TG level[1] (mmol/L)	-0.034	0.538	0.043	0.438	-0.015	0.788	-0.067	0.231

[1]Indicates Spearman's test was used. HbA1c: Glycated hemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides.

There is no significant relationship between diabetes duration with total diabetes empowerment score in our study. This contradicts a study done in Oman in which the duration of diabetes was significantly correlated with total diabetes empowerment score[16]. The median diabetes duration in our study was 4 years (with IQR = 7), similar to a study in Iran[15]. However, in the study in Oman, 63% of participants had been diagnosed with diabetes for more than 10 years[16]. Patients have better empowerment when they had diabetes for a longer duration, which translates into a longer duration of learning and adopting skills and knowledge through experience and exposure to diabetes education to make better decisions for self-care, set targets and achieve goals[10].

In our study, there was no significant correlation between gender and empowerment score. Tol *et al*[18] showed that females were more empowered than males, probably due to the distribution of their sample, in which more than half of their participants were female. The literature indicates that gender may influence lifestyle modification, as men are more proactive with their health, but women are more likely to change eating habits[26].

Strengths and limitations

To date, this is the first study conducted among type 2 diabetes patients in the primary care setting in Malaysia. Furthermore, the sample size of this study is relatively larger than others in the literature[15,16]. In addition, this study has not only identified socio-demographic factors, but also correlates clinical profiles and outcomes with total diabetes empowerment scores, which has not been reported by any local studies, especially in the primary care setting. The limitations are mainly due to the recruitment of participants at a single clinic, which may not be representative of the country's population. This is due to the short duration of the study and limitation of human resources. Therefore, similar future studies should consider multiple centers. This is a cross sectional study, and only an associational and not causational relation can be inferred in this study.

Our study reported high empowerment scores among type 2 diabetes patients. Potential predictors for total diabetes empowerment scores in our study included higher than secondary education level, diabetes education exposure, lack of ischemic heart disease and lower HbA1c levels.

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Table 4 The correlation between sociodemographic factors with total diabetes empowerment scores and subscales among type 2 diabetes mellitus patients

Variables	Total diabetes empowerment score			Managing the psychosocial aspect of diabetes			Assessing dissatisfaction and readiness to change			Setting and achieving diabetes goal		
	Coefficient correlation	Median rank	*P* value	Coefficient correlation	Median rank	*P* value	Coefficient correlation	Median rank	*P* value	Coefficient correlation	Median rank	*P* value
Age[1]	0.05		0.37	0.095		0.087	0.089		0.111	-0.06		0.919
Gender[2]			0.629			0.692			0.173			0.528
Male		159.4			163.18			155.6			158.79	
Female		164.49			159.11			169.89			165.35	
Ethnicity[2]			0.56			0.2			0.757			0.332
Malay		162.38			163.38			161.04			162.94	
Non-Malay		151.06			139.14			167			144.42	
Education level[2]			< 0.001			< 0.001			< 0.001			< 0.001
Below Secondary		109.36			106.86			110.49			105.69	
Above Secondary		168.06			168.38			167.92			168.52	
Marital Status[2]			0.478			0.119			0.346			0.564
Single		148.82			134.28			144.74			151.34	
Married		162.57			163.79			162.91			162.36	
Smoking status[2]			0.18			0.704			0.008			0.105
Non-smoker		166.42			162.87			171.18			167.38	
Smoker		151.75			158.79			142.32			149.85	

[1]Indicates Spearman's test was used.
[2]Indicates Mann Whitney test was used.

Last but not least, we would like to thank the Director General of Health Malaysia for his permission to publish this article.

Table 5 The correlation between clinical profiles with total diabetes empowerment scores and subscales among type 2 diabetes mellitus patients

Variables	Total diabetes empowerment score			Managing the psychosocial aspect of diabetes			Assessing dissatisfaction and readiness to change			Setting and achieving diabetes goal		
	Coefficient correlation	Median rank	*P* value	Coefficient correlation	Median rank	*P* value	Coefficient correlation	Median rank	*P* value	Coefficient correlation	Median rank	*P* value
Diabetes Duration[1] (yr)	-0.016		0.774	-0.1		0.857	0.011		0.847	-0.055		0.324
Diabetes education exposure[2]			0.004			0.01			0.001			0.05
Yes		168.43			167.59			169.72			168.18	
No		129.97			133.8			126.38			131.1	
Compliance to treatment[2]			0.326			0.538			0.284			0.241
Yes		162.5			162.11			162.59			162.68	
No		135.63			145.67			133.38			131.04	
BMI[3] (kg/m^2)			0.568			0.96			0.605			0.938
Underweight												
Normal												
Overweight												
Obese												
Hypertension status[2]			0.11			0.478			0.707			0.052
Yes		155.53			158.82			160.06			154.11	
No		172.6			166.32			165.1			174.81	
Dyslipidemia status[2]			0.789			0.371			0.679			0.341
Yes		162.27			164.02			162.69			158.8	
No		159.01			153.35			157.66			170.24	
Ischemic heart disease status[2]			0.004			0.011			0.104			0.001
Yes		122.83			128.32			139.83			118.65	
No		167.3			166.48			164.75			167.93	
Asthma status[2]			0.69			0.265			0.829			0.4
Yes		167.95			179.08			158.02			174.92	
No		160.84			159.69			161.86			160.12	

[1]Indicates Spearman's test was used.
[2]Indicates Mann Whitney test was used.
[3]Indicates Kruskal Wallis was used. BMI: Body mass index.

Table 6 Predictor of total empowerment scores among type 2 diabetes mellitus patients using multiple linear regressions

Variables	Unstandardized coefficients	*t*	Sig.	95%CI for B	
	Beta			Lower bound	Upper bound
Those without ischemic heart disease	5.621	2.409	0.017	1.03	10.212
Those with secondary education level and above	16.023	6.263	< 0.001	10.99	21.057
HbA1c level	-1.403	-3.668	< 0.001	-2.155	-0.65
Those received DM education exposure	6.301	3.026	0.003	2.204	10.399
Smoker status	-1.157	-0.685	0.494	-4.481	2.168
Hypertension status	1.866	1.098	0.273	-1.444	5.092

Dependent Variable: Total DES; Beta is coefficient of the gradient of the regression line and the strength of the relationship between a predictor and the outcome variable; *t* is t-statistic tests; Sig is *P* value. DM: Diabetes mellitus; DES: Diabetes empowerment scores; HbA1c: Glycated hemoglobin.

ARTICLE HIGHLIGHTS

Research background

There is a limited study on the diabetes empowerment among type 2 diabetes patients particularly in primary care settings. This study aims to assess the diabetes empowerment scores and its correlated factors among type 2 diabetes patients in a primary care clinic in Malaysia.

Research motivation

Diabetes is becoming a global epidemic of the 21st century and over 70% of known cases of diabetes occur in the developing countries. Evidence shows that self-empowerment is important in managing chronic diseases, especially diabetes. Self-empowerment is an approach that can improve the ability of the patients with diabetes to understand the disease process better, involve actively in self-care and practice healthy lifestyles for better disease control. Therefore, it is very crucial to identify the predictors for diabetes empowerment score among type 2 diabetes patients.

Research objectives

Our objective was to access the diabetes empowerment score among type 2 diabetes patients, also to identify correlated factors with diabetes empowerment scores among type 2 diabetes mellitus (DM) patients in primary care clinic. In addition, we aimed to identify the predictors for diabetes empowerment score among type 2 diabetes patients.

Research methods

This is a cross sectional study involving 322 adults with type 2 DM patients followed up in a primary clinic. Systematic sampling method was used for patients' recruitment. The Diabetes Empowerment Scale (DES) questionnaire was used to measure patients' empowerment. Data analysis was done using SPSS version 25 and multiple linear regressions was used to identify the predictors of total diabetes empowerment scores.

Research results

Median age of the study population was 55 years old, 56% were male and mean duration of diabetes was 4 years. The total median score of the DES was 110 [interquartile range (IQR) = 10]. The median scores of the three subscales were 40 with (IQR = 4) for "Managing the psychosocial aspect of diabetes", 36 with (IQR = 3) for "Assessing dissatisfaction and readiness to change" and 34 with (IQR = 5) for "Setting and achieving diabetes goal". According to multiple linear regressions, factors that had significant correlation with higher empowerment scores among type 2 diabetes patients were those who had above secondary education level ($P < 0.001$), those who had diabetes education exposure ($P = 0.003$), those who had no ischemic heart disease ($P = 0.017$) and those who had lower glycated hemoglobin (HbA1c) level ($P < 0.001$).

Research conclusions

Diabetes empowerment scores were high among type 2 diabetes patients in this study population. The predictors for high empowerment score were those who had above secondary education level, diabetes education exposure, no ischemic heart disease status and lower HbA1c.

Research perspectives

Given the high empowerment score were those who had above secondary education level, diabetes education exposure, no ischemic heart disease status and lower HbA1c, hence all the diabetes patients should be educate and empower on self-care for long-term diabetes management.

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CASE REPORT

HNF1A mutation in a Thai patient with maturity-onset diabetes of the young: A case report

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Abstract

BACKGROUND

Maturity-onset diabetes of the young (MODY) is the most common form of monogenic diabetes. The disease is transmitted in autosomal dominant mode and diabetes is usually diagnosed before age 25 year. MODY 3 is caused by mutation of hepatocyte nuclear factor (*HNF*) *1A* genes and is the most common MODY subtype. Diagnosis of MODY 3 is crucial since glycemic control can be accomplished by very low dose of sulfonylurea. In this report we described a Thai MODY 3 patient who had excellence plasma glucose control by treating with glicazide 20 mg per day and insulin therapy can be discontinued.

CASE SUMMARY

A 31-year-old woman was diagnosed diabetes mellitus at 14 years old. The disease was transmitted from her grandmother and mother compatible with autosomal dominant inheritance. Sanger sequencing of proband's DNA

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identified mutation of *HNF1A* at codon 203 which changed amino acid from arginine to cysteine (R203C). This mutation was carried only by family members who have diabetes. The patient has been treated effectively with a combination of oral hypoglycemic agents and must include a very low dose of glicazide (20 mg/d). Insulin therapy was successfully discontinued.

CONCLUSION
We demonstrated a first case of pharmacogenetics in Thai MODY 3 patient. Our findings underscore the essential role of molecular genetics in diagnosis and guidance of appropriate treatment of diabetes mellitus in particular patient.

Key words: Oral sulfonylureas; Maturity-onset diabetes of the young; *HNF1A*; Case report

Core tip: Maturity-onset diabetes of the young (MODY) is the most common form of diabetes in patients diagnosed under the age of 25. In addition, MODY is characterized by autosomal dominant inheritance. We report a R203C mutation in the *HNF1A* causing MODY type 3. The genetic diagnosis is implicated to alter SU treatment. This study revealed that excellent glycemic control in this patient could be achieved by very low dose SU. Furthermore, this is the first report of exceptional response to treatment with SU in Thai MODY3.

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INTRODUCTION

Maturity-onset diabetes of the young (MODY) is the most common type of monogenic diabetes, it is inherited in an autosomal dominant manner, and it is normally diagnosed before 25 years of age. To date, at least 15 subtypes of MODY caused by mutations of 15 different genes have been identified[1,2]. Thus, the clinical heterogeneity of MODY is explained by its genetic heterogeneity[3]. MODY3 is caused by mutation of hepatocyte nuclear factor 1A (*HNF1A*), which encodes a transcription factor that regulates functions of several proteins, including amylin, insulin, GLUT2, and L-pyruvate kinase, that are important for glucose metabolism and insulin secretion. *HNF1A* dysfunction are leading to Diabetes development and imbalance of insulin in patients. More than 350 mutations of *HNF1A* have been identified, and MODY3 is the most common MODY subtype among Caucasians[4]. In contrast, Asians most commonly have MODY-X or MODY subtype without identified genetic cause[5-10]. Identification of MODY3 is very important, because pancreatic β-cells exhibited hyperexcitability in this subtype in response to treatment with sulfonylurea (SU)[11]. The Siriraj Center of Research Excellence for Diabetes and Obesity (SiCORE-DO) discovered 3 different *HNF1A* mutations, including R203C, G554fsX556, and P475L, in 3 unrelated MODY pedigree[12-14]. Here, we report a Thai MODY3 patient carrying *HNF1A* R203C mutation that exhibited outstanding diabetes control with low-dose glicazide, which is a short-acting second-generation SU. Rapid deterioration of her glycemic control was observed after withdrawal of SU. The purpose of this report is to present alteration of drug treatment in patient by genetic diagnosis.

CASE PRESENTATION

Chief complaints

A 31-year-old Thai woman came to Siriraj Diabetes Center, Siriraj Hospital, Bangkok, Thailand for her diabetes management.

History of present illness

The patient has been following up every 3 months at the Siriraj Diabetes Center, Siriraj Hospital, Bangkok, Thailand. Currently, she was 44 years old and treated with glicazide 20 mg/d. She has excellence glycemic control without diabetic complications. Laboratory assessment included fasting plasma glucose (FPG) 78 mg/dL, hemoglobin A1c (HbA1c) 6.7%, serum creatinine (0.56 mg/dL), total cholesterol (TC) 173 mg/dL, high-density lipoprotein (HDL) 99 mg/dL, low-density lipoprotein (LDL) 62.6 mg/dL, and triglycerides (TG) 57 mg/dL.

History of past illness

The patient was first seen at Siriraj Diabetes Center when she was 31 years old and diabetes was diagnosed at age 14.

Personal and family history

Her mother and brother were diagnosed with diabetes at age 17 and 13, respectively. There was no history of diabetic ketoacidosis, and glycemic control could be achieved without insulin treatment for more than 5 years after diabetes diagnosis in all 3 patients.

Physical examination upon admission

The patient's body mass index (BMI), waist-to-hip ratio, and blood pressure was 19.43 kg/m^2, 0.83, and 120/70 mmHg, respectively (Table 1).

Laboratory examinations

Laboratory assessments at her first visit to Siriraj Diabetes Center included FPG 126 mg/dL, HbA1c 9.5%, serum creatinine (0.6 mg/dL), TC 156 mg/dL, HDL 71 mg/dL, LDL 90 mg/dL, and total TG 55 mg/dL.

Sequencing profile and timeline of patient's glycemic control with and without SU

Sanger sequencing of her DNA revealed heterozygous mutation of *HNF1A* at codon 203 in exon 3 that caused substitution of cysteine for arginine (R203C) (Figure 1). This mutation was also identified in all diabetic family members, but not in non-diabetic family members whose DNA were available for sequencing (Figure 2). The patient's glycemic control profile (with and without SU) is shown in Figure 3. The results of our analysis revealed that excellent glycemic control could only be achieved when our patient was taking SU. Interestingly – when SU treatment was withdrawn, severe hyperglycemia eventually developed, even when insulin was given. The optimal dose of glicazide in this case was 20 mg per day. This patient continues to do well with no observed diabetic complications.

FINAL DIAGNOSIS

SU hyperresponsiveness in MODY subtype 3 due to *HNF1A* mutation.

TREATMENT

The patient has been successfully treated with glicazide 20 mg/d, metformin 2000 mg/d and sitagliptin 100 mg/d.

OUTCOME AND FOLLOW-UP

The patient's glycemic control has been excellence and without hypoglycemic episodes during the last 4 years of follow up. No diabetic complications have developed.

DISCUSSION

MODY3 is one of the best examples of precision medicine in diabetes. Studies in animal models showed that total deletion of *HNF1A* resulted in decreased SU uptake by hepatocytes and decreased excretion[2,12,15,16]. Clinical studies in humans demonstrated that MODY3 patients treated with SU exhibited excellent glycemic control, and withdrawal of SU led to severe hyperglycemia – even with insulin treatment. However, dosage adjustment is essential since inappropriate SU dose can lead to hypoglycemia[11]. The current recommendation for treatment of MODY3 patients is to

Table 1 Demographic, anthropometric, and clinical characteristics of the case profiled in this report

Characteristics	Values
Age (yr)	31
Age at onset (yr)	14
Duration (yr)	17
BMI (kg/m^2)	19.43
Waist circumference (cm)	77
Waist-to-hip ratio	0.83
Systolic BP (mmHg)	120
FPG (mg/dL)	126
HbA1c (%)	9.5
Serum creatinine (mg/dL)	0.6
Total cholesterol (mg/dL)	156
Total triglycerides (mg/dL)	55
LDL (mg/dL)	90
HDL (mg/dL)	71.0

BMI: Body mass index; BP: Blood pressure; FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin; LDL: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein cholesterol.

use a very low dose of SU. Caution should be exercised if SU is to be withdrawn from the treatment plan since a deterioration in the patient's glycemic status can be anticipated. Our MODY3 patient exhibited exceptional plasma glucose control using a very low dose of glicazide, and severe hyperglycemia developed after glicazide was discontinued, even though she was treated with metformin, sitagliptin, and insulin glargine. Moreover, her glicazide dosage was titrated to 20 mg/d to avoid hypoglycemia, even though the usual dose is up to 80 mg/d for treatment of type 2 diabetes. Upon reaching her maintenance dosage and after stabilization of her blood sugar, insulin therapy could be discontinued and the durability of glycemic control has been almost 4 years (Figure 3). To our knowledge, this is the first report of exceptional response to treatment with SU in Thai MODY3. Our findings are in agreement with those from previous reports in MODY3 patients from different ethnicities, including Caucasian, Saudi Arabian, and Tunisian[17-19]. A study from the United Kingdom reported lower HbA_{1c} and lower BMI at genetic diagnosis, and shorter duration of diabetes to be factors that significantly influence treatment success after treatment with SU in MODY3 patients[20]. However, this finding has not yet been investigated or confirmed in Asian population due to the relatively lower prevalence of MODY3 in this ethnicity.

CONCLUSION

In this report, we presented and described a 31-year-old Thai MODY3 patient with a heterozygous mutation of *HNF1A* at R203C who demonstrated excellent diabetic control with a very low dose of SU. To our knowledge, this is the first report of exceptional response to treatment with SU in Thai MODY3. Our findings emphasize the critical role of correct genetic diagnosis, especially in patients with early-onset diabetes.

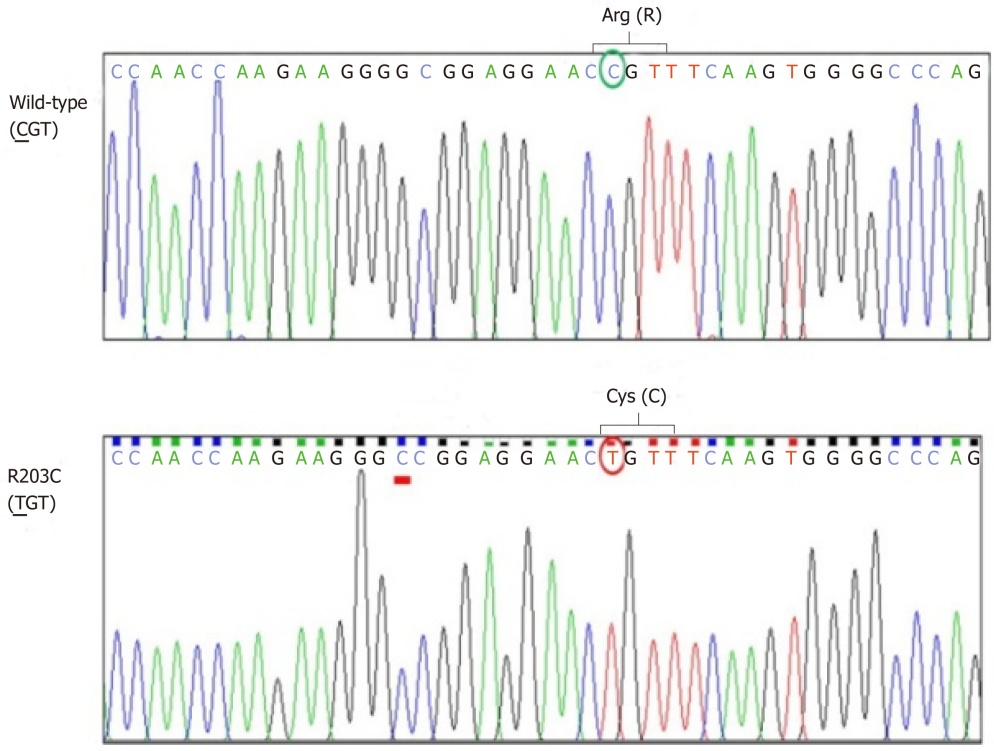

Figure 1 Sequencing profile of exon 3 of *HNF1A* in the mutation region (R203C). The green circle indicates the location of C in wild-type, and the red circle indicates the location of T substitution in heterozygous.

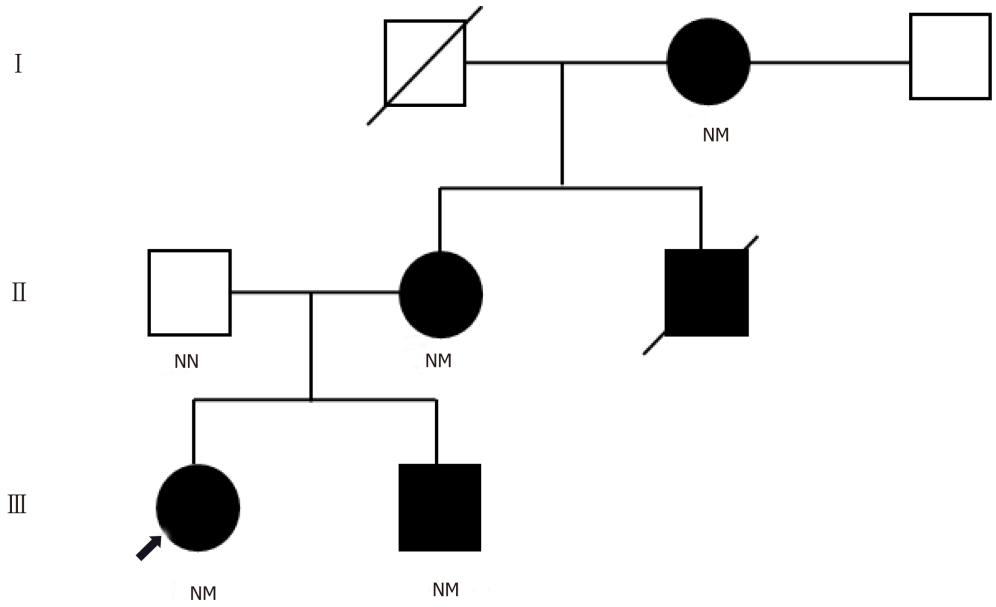

Figure 2 Pedigree showing autosomal dominant inheritance of diabetes associated with a hepatocyte nuclear factor-1-alpha mutation. Symbols and abbreviations: Circles: Females; squares: Males; Darkened circles or squares: Diabetes; NM: Heterozygous *HNF1A* R203C; NN: *HNF1A* wild-type genotype.

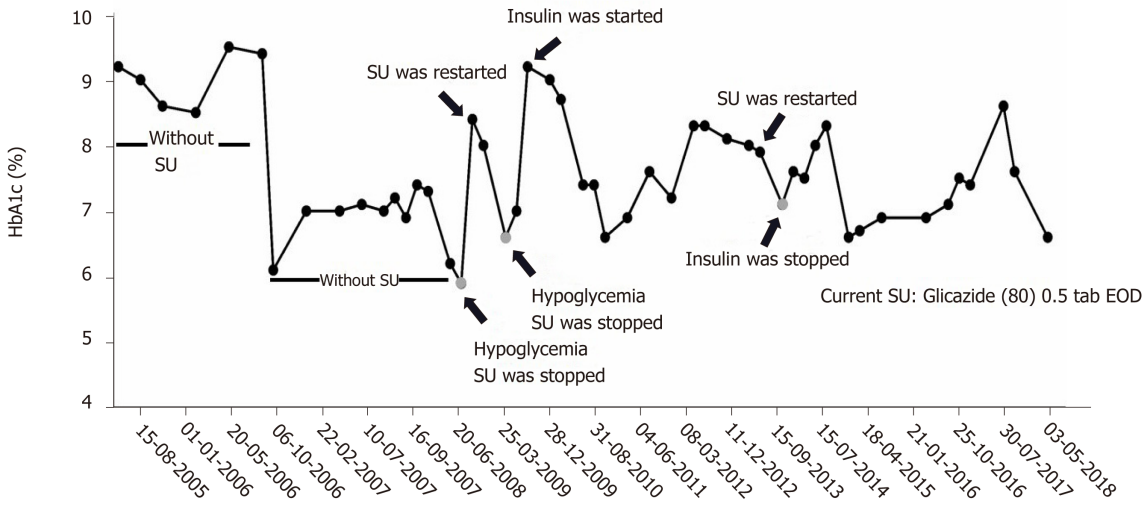

Figure 3 Timeline of patient's glycemic control with and without sulfonylurea.

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