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Early Diagnosis of Diabetes (Type I and Type II), Using Particular Restriction Enzymes and Specific Responsible Genes to Determine the Suitable Treatment

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ABSTRACT:- Identification of proper prescriptions is a critical role to initiate adequate therapeutic management of type I and type II diabetes using pharmacogenetics to initiate adequate therapeutic management .This study was carried out in the Department of Medical Laboratory Technology, College of Health & Medical Technology / Basra southern technical university and the samples were collect from several hospitals in Basra. six genes were used to distinguish the type 1 and the type 2 Diabetes .Three genes specific to the first type of diabetes ; (DQA1, DQB1 & DRB1 genes) which had genes size (940bp ,552 bp and 627 bp) respectively and (TP53 ,TCF7L2 & CDKN2A genes) for type 2 with genes size (655bp , 973bp and 658 bp) respectively . 2 different restriction enzymes digested of Nucleic acid DNA :(BccI) and (HPhl) were used to digest these genes in specific sites for purpose early diagnosis and determine suitable treatment. Bccl restriction enzyme gave (3 , 2 and 1) fragments for (DQA1 ,DQB1 and DRB1 genes) respectively , whereas same restriction enzyme(Bccl) digested (1 , 3 and uncut) fragments which special of (TP53 ,TCF7L2 & CDKN2A genes) respectively. HPhl was digest (DQA1) gene into 3 fragments whereas uncut (DQB1) gene , DRB1gene was digest into 3 fragments for type I diabetes . Digested fragments of type II genes were 3 fragments for TP53 gene, **3** fragments for TCF7L2 gene whereas HPhI enzyme was cut CDKN2A gene into only 1 fragment.

Key words:- pharmacogenetics , Bccl , HPhl restriction enzymes , optimal therapeutic intervention , Inherited genetic susceptibility and diabetic nephropathy , microalbuminuria , genetic markers , mapping of genetic disease.

I. INTRODUCTION

Diagnosis and treatment are closely intertwined concepts which have significant implication in combating diabetes, both Type 1 and Type 2. Diagnosis and treatment of type 1 and type 2 diabetes based on signs and symptoms rather than the basis of genetic profiles might yield poor results and consequently wrong treatment, which negatively affect health of the patients [4].

Objectives of my research are early diagnosis by using pharmacogenetics to determine the suitable treatment in more accurate and to choose and determine the exact restriction enzyme to digest the responsible genes of diabetes type I & type II .

Diagnostic techniques should integrate restrictive enzymes and specific responsible genes to give individualized optimal treatment. Utilizing sophisticated biotechnology tools has impacted safe and successful treatment diabetic cases. Shifting to modern technologies for more elaborate diagnostics will present an opportunity as well as a challenge to the healthcare industry. Evidence from laboratory and clinical trials reveal that diabetic complication can progress unopposed through the metabolic memory regardless whether the glycemic control is adequately achieved. This paper discussed how Type 1 as well as Type 2 diabetic disorders, can be diagnosed adequately by altering diabetic genes expressions involving these disorders and using the results to give the correct prescription. The role of restrictive enzymes in enhancing useful diagnostics of the diseases examined in this overview to suggest the optimal therapeutic intervention for these conditions.

Type-1 diabetes

Type1diabetes results from failure of the pancreatic β-cells to stimulate sufficient insulin to metabolize excess glucose in the blood into energy. As a result, these cells are deprived of energy, and excess glucose will remain the blood stream. A series of adverse situations would then arise from Hypoglycemia, lower sugar levels in the blood and high blood sugar (hyperglycemia).Hypoglycemia denies cells enough glucose and more often patient suffer from confusion, unconsciousness, and coma. At times, death can occur in case the glucose content in the brain is very low for a long time. Prolonged low insulin levels and hyperglycemia may cause ketoacidosis which concentrates ketones in the blood when excess fat is metabolized rather than glucose. Since fatty acids

cannot easily convert to glucose at unfavorable conditions, the blood becomes too acidic, and functionality lowers. Consequently, a coma develops which eventually results in death.

Type-2 diabetes (T2D)

Diabetes (Type 2), results from metabolic failure which involves a complex endocrine When genetic factors interact with environmental aspects, a heterogeneous disorder which is progressive develops with different extents of insulin resistance and cause the dysfunction of pancreatic β-cells. Major causes of insulin resistance and intolerance would be attributed to overweight and obesity as well as impaired glucose tolerance [2]**.** If the amount of insulin secreted by the β-cells is not enough to counter insulin resistance, the glucose tolerance impairment progresses to Type-2 diabetes. Hormonal abnormalities, for instance, lower incretin glucagon secretions such as peptide 1(GLP), hyperglucagonemia and increased concentrations of counter-regulatory hormones leads to insulin resistance as well as hyper glycemia in T2D. Obesity impacts on insulin resistance in several ways which include hormonal concentration imbalances (reduced adiponectin, increased leptin, and increased glucagon), suppressing cytokine signaling, increasing cytokine levels, and inflammation and through the retinol-binding protein [3]. B-cell concurrent alterations included hyperinsulinemia compensatory periods characterized with abnormal secretions .reduced insulin secretions will usually translate in T2D.Furthermore when β-cells functionality often results from chronic hyperglycemia, oxidative stress, and high exposure to fatty acids and inflammation. T2D patients experience pancreatic α-cell dysfunction as result of increased glucagon secretions in hyperglycemia and reduced GLP-1 secretions.

Types 2 diabetes disease is a result of highly prevalent chronic metabolism which has an intense morbidity with cardiovascular disease and obesity. Growing evidence supports a positive correlation between mitochondria and insulin levels which involves the insulin resistance and etiology of T2D. Precisely, molecular cross-talk basis is determined through systems of biology approaches [5]. Combined functional interaction data, including amino-acid interactions, signaling, and metabolic dependencies. Co-expression analyses are filtered and interrogated to construct a mitochondria-insulin (MTIN) network with 286 genes which act as candidate functional linkers between the underlying systems. Internal gene expression analysis of perturbations of mitochondria and insulin suggest the genes have connecting roles. Genomic regions share common variants which imply an association of genes with T2D. Meta-analyses have also confirmed genetic associations with glycemic traits , glycemic traits.

Advanced molecular research and genetic testing have made it possible to classify T2D using pathophysiological clinical sub-phenotypes [8] . Some mature form of diabetes affecting the young, usually referred to as MOYD, results from genetic mutations which impact pancreatic β-cells' ability to secrete insulin. Additionally, T2D can manifest in the presence of a certain disease or exogenous factors. The exogenous factors include medications, viral infections, for example, hepatitis C, and hormonal factors. Most medications which accelerate the occurrence of diabetes disease consist of thiazide diuretics, calcineurin inhibitors, corticosteroids and HIV protease inhibitors [5] . The vulnerability of individuals to develop type 2 diabetes is brought by some factors such as host genetic susceptibility, variable roads due to unfavorable environmental factors and reduced mass of pancreatic beta-cells. Notably, T2D consists of the highest prevalence of diabetic disease and accounts for an estimated 80 percent of all diagnosed cases. T2D is more of a multifactorial disease caused by elevated levels of risk alleles associated with multiple genes as well as disease favoring conditions.

Environmental conditions which promote genetic risk modulation speed up the rate at which T2D develops. Inherited genetic susceptibility is a critical risk factor for T2D. Parents with T2D risk 40 percent likelihood of transferring the condition to their offspring and the risk escalates to 70 percent if both parents are victims. Unlike monogenic diabetes, the common T2D genetic transmission does utilize simple Mendelian patterns [20] . Heredity and the environment have impacted significantly on the etiology of T2D. Heredity accounts for highest rate of liability for T2D at 46 percent, followed by unshared environmental effects at 38 percent and shared environmental effects at 15 percent.

Diabetic Nephropathy (DN)

Research has indicated the presence of genetic susceptibility to diabetic nephropathy. While chronic kidney disease (KCD) commonly referred to as microalbuminuria, as well as end-stage renal disease (ESRD), have been found to segregate in families with diabetic cases, DN established in subsets containing T2D populations across all race groups. Additionally, racial affinity which supports presence of genetic factors with diabetic nephropathy has been established [10] . Iraqi people suffering from T2D have 40 percent chances of developing DN and their conditions are three times likely to progress to ESRD in comparison to Europeans and Americans. Some genes have recorded high susceptibility with DN. Some of those genes include erythropoietin (EPO), carnosinase 1 (CNDP1), carnosinase 2 (CNDP2) and ribosomal protein S12 (RPS12).

Renin system with angiotensin and aldosterone signal linkages can effectively regulate blood sugar and blood pressure and is preferred in renal complication pathophysiology including DN Polymorphisms involving

renin genes, especially angiotensinogen (AGT) genes and angiotensin converting enzyme (ACE) plays a significant role in developing and progressing nephropathy [11] . ACE dipeptidase enzyme contains Zinc which catalyzes angiotensin conversion into a more stable form [23] . In most cases, ACE gene facilitates DN pathogenesis and progresses to overt proteinuria. M235T polymorphism associated with AGT gene is used in encoding threonine rather than methionine at interval 235 long a particular exon. Variant M235T T-allele positively correlates with higher plasma levels of AGT [8]. The treatment of this condition can be achieved by prescribing drugs that render RAAS infective for instance ACE inhibitors as well as angiotensin receptor blockers to control hypertension. These drugs can also adequately keep proteinuria at check whether used alone or some combinations in patients suffering from DN.

Diabetic Retinopathy (DR)

The chances of contracting this diabetic condition are higher when diabetes persists for a long time and when glycemia is poorly managed [14] . Clustering of families of DR cases from different ethnic background suggests that genetic susceptibility is an attribute of the inherent risk. RD accounts for 21 percent of new T2D cases and 60 percent of T2D patients within two decades of diagnosis [23] . A strong relationship between DR and microalbuminuria in T2D individuals has been confirmed. Likewise, patients with microalbuminuria have double prevalence rates of DR, and this condition can deteriorate faster in the presence of microalbuminuria. Consequently, such a relationship suggests common risk factors between DN and DR pathophysiology is expensive and usually involves interplaying considerable molecular mediation agents including advanced glycation end components neoangiogenesis, pathway activation and oxidative stress [15] .

Determining the suitable treatment

Testing for particular gene variants in individuals has significantly influenced drug prescription and development [15] . Pharmacogenomics is more concerned about important genetic differences that make drugs to work effectively in some people, or they cause reactions in others. Experts' opinions is that medical specialists will increase reliance on genetic tests while administering drugs to individual patients, such that only the most effective medicines and right dosages would be described [18] . When genotypes have been identified based on different responses to certain drugs, pharmaceutical companies will opt to set new and highly customized drugs, while at the same time reviving older ones, which could be ignored in the past as lacking clinical value.

Diagnosis and Treatment of Diabetes

Since most vascular consequences associated with insulin resistance arise from unchecked hyperglycemia as witnessed in Type 2 diabetic cases, reducing glucose levels in circulation will be the primary therapeutic intervention [16] . Pharmacologic strategies suggest a range of drugs which are effective in control this condition. Some of these drugs are discussed in the following paragraphs

Thiazolidinediones (TZDs)

TZDs are efficient, and they work by making molecular targets active [17] . Besides binding specificity with receptors, they diffuse nuclear transcription factors in humans and enhances fatty acid uptake. Through reducing the concentrations of circulating fatty acids and the available lipids in muscles and the liver, these drugs can significantly improve the patient response to insulin while at the same time reducing hyperglycemia [21] . Other the therapeutic effects of TZDs include anti-inflammatory effects, in addition to amelioration of hypertension, hepatic steatosis, and macroalbuminuria .

Sulphonylureas

Apart from insulin resistance, alterations the functionality of β-cell facilitates pathogenesis of T2D [18] . Administering secretagogue drugs can lead reduce insulin production by integrating sulphonylurea receptor components. By doing so, cells become depolarized, and stimulation of insulin is enhanced. The entire channel is essential during insulin secretion process of pancreatic β-cells, modulation of glucose uptake by the skeletal muscles, production of glucose and liver excretion [8] . Lower plasma glucose levels result in lower metabolic rate, thus suppressing electrical activity along with insulin production of insulin.

Biguanides

These drugs are recommended during the initial therapy of T2D patients [19].The core purpose of these drugs to suppress hepatic gluconeogenesis. Also, biguanides increase glucose uptake and assimilation, improves insulin sensitivity and reduce the rate at which glucose is absorbed in the intestines. However, molecular mechanisms associated with drugs are not well established [17] .

II. MATERIAL AND METHODS

45 Infected patient of type 1 diabetes and 55 patients who are suffered from h type 2 diabetes with different ages, range of(3-75) years old .Were Blood samples drawout from them veins then DNA(S) were extracted using DNeasy blood kit , The DNA isolated is quantified by using Nanodrop (260/280 ratio). The contamination of sample with RNA or Phenolic compounds was detected , after separating DNA(s) fragments, Staining the gel with a DNA- binding dye and subsequently illuminating it under UV light l caused the glowing of the DNA fragments , . Therefore, the resultant glow was enable visibility of the DNA strands occupying different points along the gel length [9] . Agarose gel electrophoresis effectively separated DNA fragments with varying sizes within the range of 50 bp to 1kb [22]. Isolation of agarose from seaweed genera Gracilaria and Gelidiumcontain repeated subunits of agarobiose (L- and D-galactose). During gelation, polymers of agarose associate non-covalently forming a network of bundles. The molecular sieving properties of the gel were determined using the pore sizes of the networked bundles Agarose gel electrophoresis revolutionizes DNA separation, before the agarose gels are adopted, sucrose density gradient centrifugation is used to separate DNA to approximate the size.

Separation of DNA during agarose gel electrophoresis requires loading the DNA into the gel's pre-cast wells where a current is applied. Since the phosphate backbone of both the DNA and RNA are negatively y charged, DNA fragments migrate to the anode which is positively charged once an electric current is switched on. Additionally, the mass/charge ratio of the DNA molecule is uniform, which makes the DNA molecules to separate according to their sizes within an agarose gel [7]. DNA movements via agarose gel assumes a leading model where the leading edge is thrashed forward, pulling along the rest of the molecule.

PCR genes size in gel (agarose) electrophoresis

Primers were designed using Primer3, and the genomic DNA(s) were then PCR amplified . Six genes were used to distinguish between type 1 & type 2 diabetes include [DQA1 , DQB1 and DRB1 genes] which are special for type 1 diabetes with gene size [940bp , 552bp & 627bp] respectively and [TP53 , TCF7L2 and CDKN2A genes] for type 2 diabetes with size of gene [655bp ,973 bp & 658 bp] respectively .

Sequence of primers for type 1 and type 2 diabetes PCR products

The primers for Type 1 & Type 2 diabetes were sequenced based on two factors namely; the amplicon size and Oligonucleotide sequence [6] , Tables (1&2) .

Table 1: Primer sequencing for type I diabetes PCR products

Target group		**sequence of oligonucleotides (5′-3′)**	**Amplicon size (bp)**
DQA1 GENE	Left primer	CCCAACACCCTCATCTGTCT	940
	Right primer	CATCAGCAGAAGGGAGGAAG	
DQB1 GENE	Left primer	AGGGCATGTGCTACTTCACC	552
	Right primer	CTTCCTTCTGGCTGTTCCAG	
DRB1 GENE	Left primer	CCGCTTCAGAAAGACAGAGG	627
	Right primer	TATACTTACCCCGCCACAGG	

Table 2: Primer sequencing for type 2 diabetes PCR products

Target group		**sequence of oligonucleotides (5′-3′)**	**Amplicon size (bp)**
TP53 GENE	Left primer	GTGCCAGGAGCTGTTCTAGG	655
	Right primer	CTGGCCGGAAATGTTTTCTA	
TCF7L2 GENE	Left primer	CTCACGCCTCTCATCACGTA	973
	Right primer	TCCTGTCGTGATTGGGTACA	
CDKN2A GENE	Left primer	CTTTGGGGAGCAGTATGGAA	658
	Right primer	CTGGCCAACCTTTTACTCCA	

Amplicons were visualized on 1.2% agarose Gel, before using restriction enzymes were mentioned in Fig: (1) .

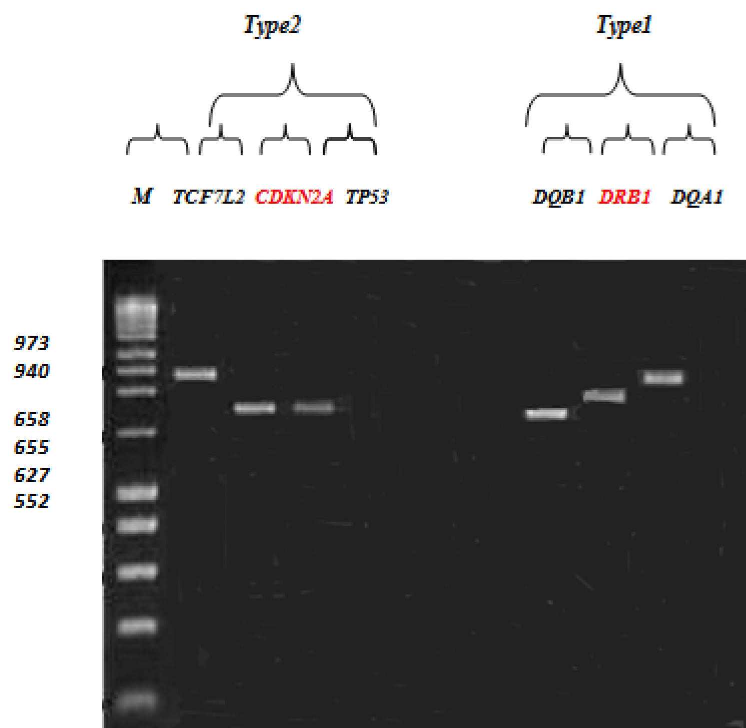


Fig. (1): Bands of PCR genes size in gel (agarose) electrophoresis.

Then RFLP markers were appropriately genotypes using Restriction enzymes to digested PCR fragments. PCR products were subjected to two types of restriction enzymes (BccI & Hphl) which were digested the six genes into different sizes and numbers of fragments , BccI restriction enzyme was digest (DQA1) gene into 2 fragments with (374 bp & 69 bp) size , (DQB1) gene cut into 3 fragments with (543bp, 302bp 7 294bp), for DRB1gene was digest into 1 fragment with (223bp) size for type I diabetes . whereas digested fragments of type II genes were , 1 fragment for TP53 gene with (209 bp) , **3** fragments for TCF7L2 gene with (791bp , 788 bp ,& 355bp) size whereas BccI enzyme was uncut CDKN2A gene were as mentioned in Fig: (2) .

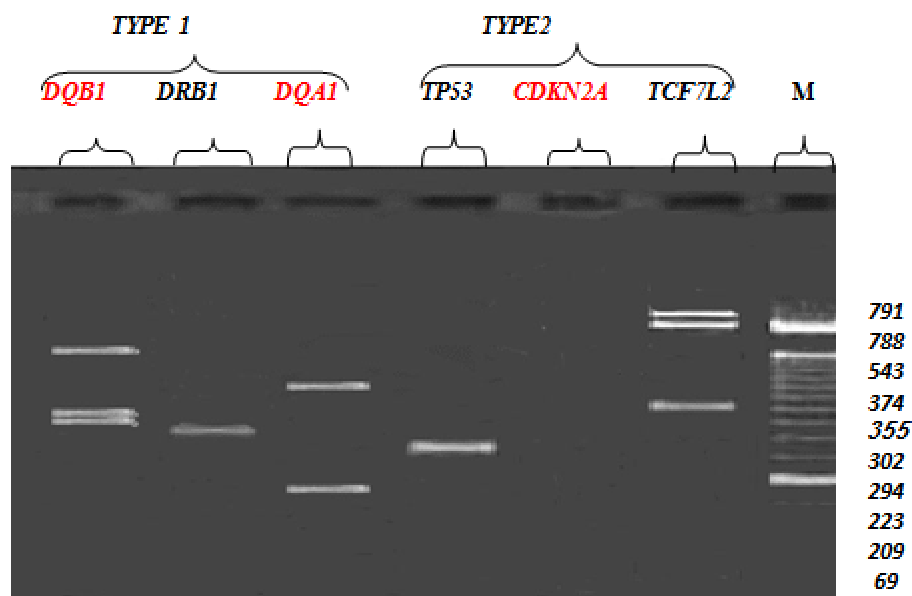
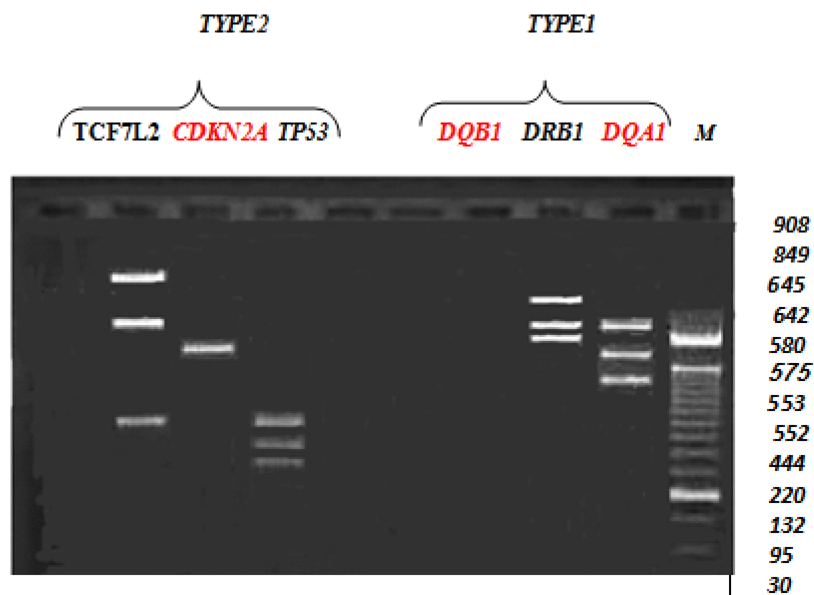


Fig. (2) : BccI restriction enzyme digested (DQA1, DQB1 & DRB1) genes of type1 diabetes into(2 , 3 &1) fragments respectively with different size and (1, 3 & uncut) fragments for type 2 Diabetes (TP53 , TCF7L2 & CDKN2A) genes as well as respectively .

The second restriction enzyme **HPhl** was digest (DQA1) gene into 3 fragments with (849 bp & 642 bp & 575BP) size whereas uncut (DQB1) gene , DRB1gene was digest into 3 fragments with (580 bp , 553bp , & 444bp) size for type I diabetes . Digested fragments of type II genes were 3 fragments for TP53 gene with (220 bp , 132bp , & 30 bp)size , **3** fragments for TCF7L2 gene with (908 bp , 645 bp ,& 95 bp) size whereas HPhI enzyme was cut CDKN2A gene into only 1 fragment (522bp) as mentioned in Fig: (3) .

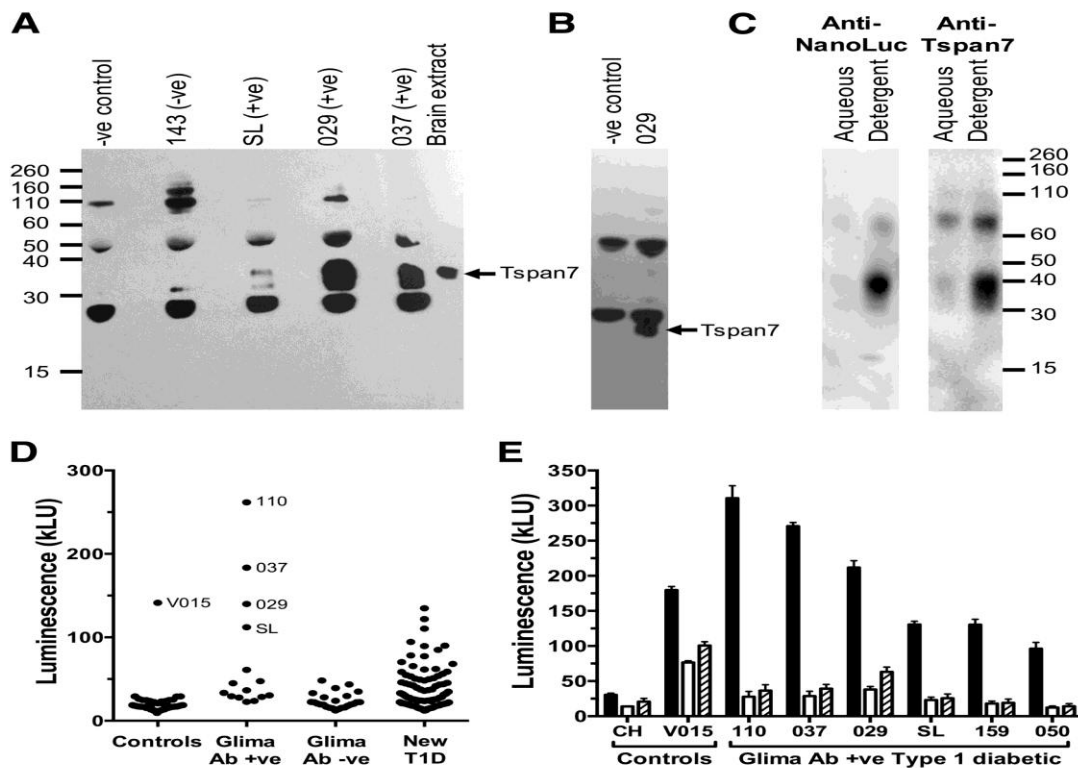


Fig(3): HPhl restriction enzyme digested (DQA1, DQB1 & DRB1) genes of type1 diabetes into(3, uncut &3) fragments respectively with different size and (3, 3 &1) fragments for type 2 Diabetes (TP53, TCF7L2 & CDKN2A) genes as well as respectively.

The standard genetic markers comprise of short genetic repetition, and their detection and analysis form valuable tools in the mapping of genetic disease. They are also critical in retrieving genetic polymorphism data of a given population. Electrophoretic techniques which involve agarose or acrylamide can effectively detect STRs. Use of sophisticated sequencing technology had resulted in the detailed characterization of numerous short tandem repeats (STRs), leading adequate identification of genes and alleles during the pathogenesis of type 2 diabetes. Advanced molecular genotyping utilized automated genetic systems which were fluorescent gel-based, such as Applied Biosystems (ABI), ALF express DNA sequence from Pharmacia Biotech and LI-COR Model 4000. In these systems, detection of DNA fragments occurs through laser induced fluorescence integrated into the fluorescent tags.

During electrophoresis, the fragments passed over a laser that scans the gel, by use of an internal lane fluorescing at varying wavelength to allow for comparison of each sample using the same scale [1]. Genomic revolution illustrates that big data sets can yield clinical and biological insight. Improved genetics study, aided by new and improved technology had significantly contributed to diagnosis and treatment of diabetes type 1 and type 2. Expertise of medical specialists is crucial when handling some delicate cases involving diabetes Fig.(4, 5 & 6) .

Fig.(4) : Images of genomes size bands for Type diabetes



Fig, (5); Images of genomes size bands for Type diabetes

Sequence of gene for type 1 of Diabetes

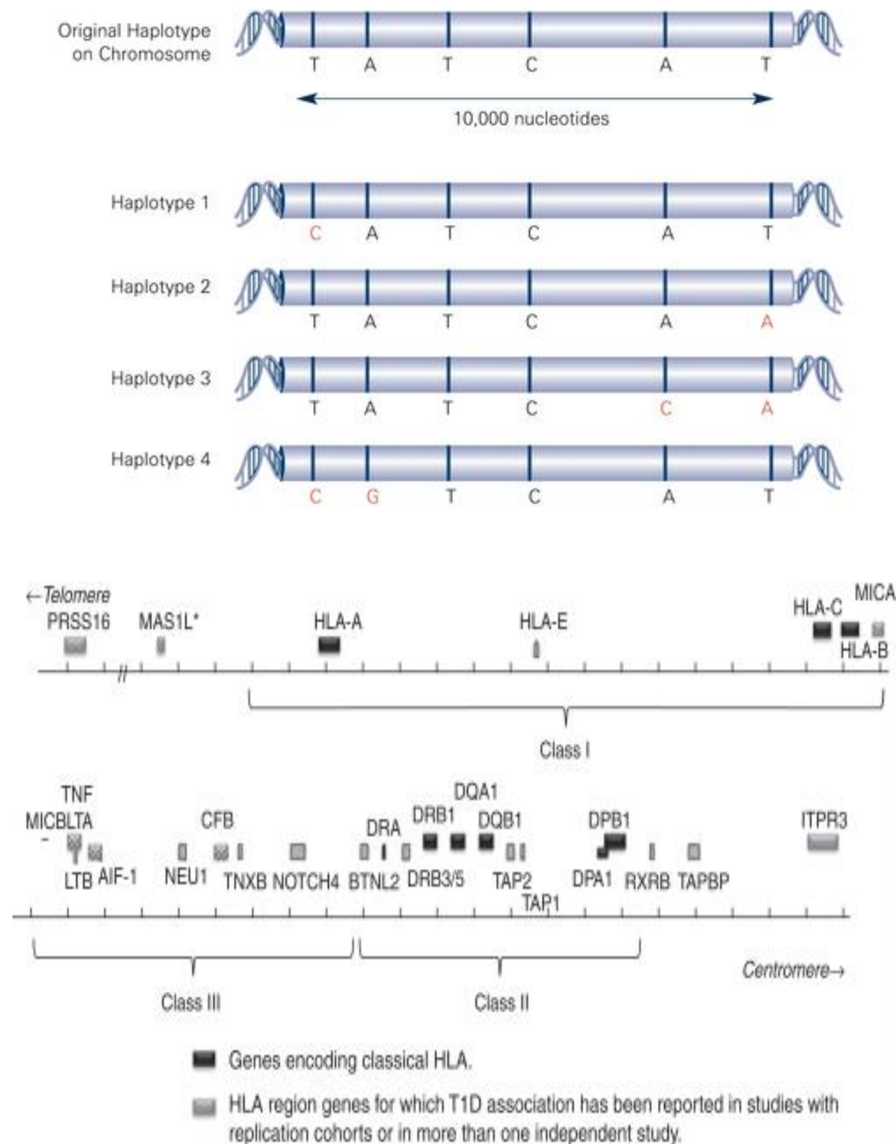
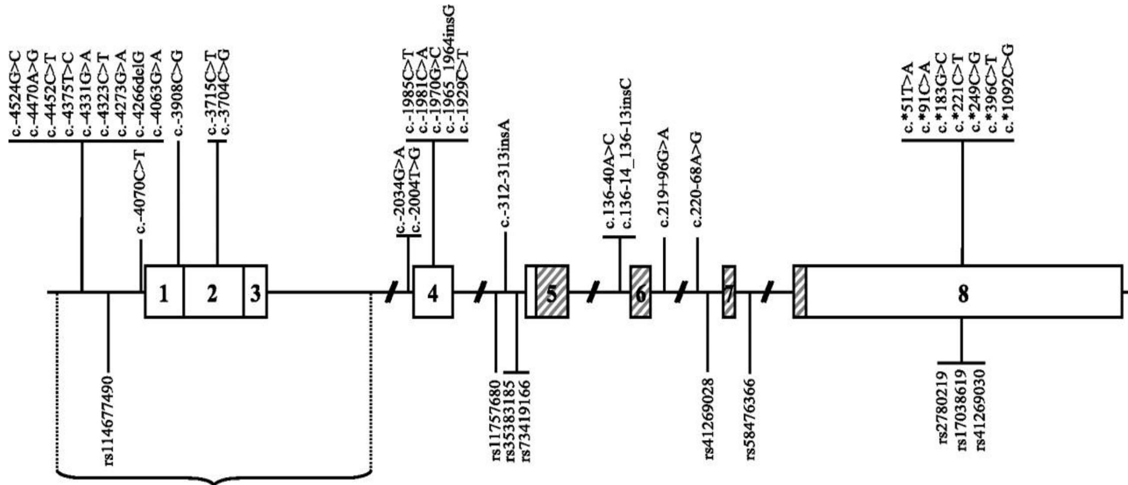


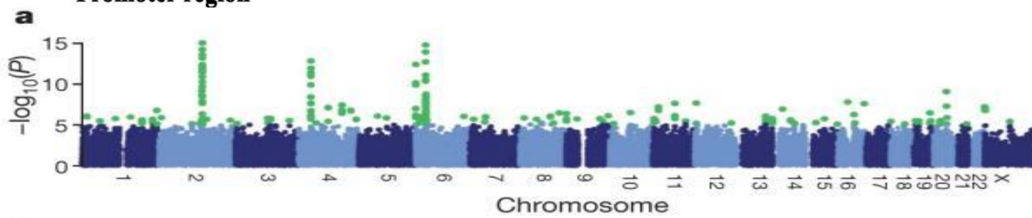
Fig. (6) : As well as the PCR fragments can be also sequenced with an ABI3700 capillary sequencer along with terminator kit. The staden package is then used to align the sequence reads. Many individuals with exons *(CREM* and *SDF1)*, exon-intron boundaries and of gene upstream and downstream up to 3 kb are sequenced.

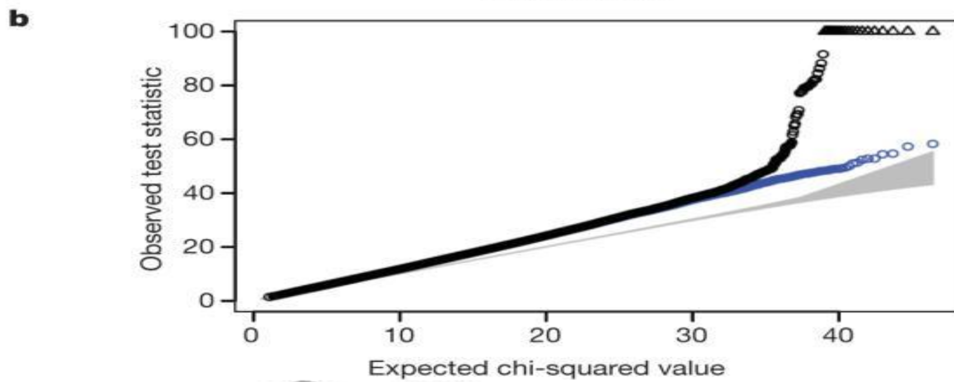
The next step is to submit the identified SNPs to dbSNP to Third Wave Technologies, Madison, WI) Invader to perform SNP genotyping. The microsatellite markers are appropriately genotypes using TaqMan also.

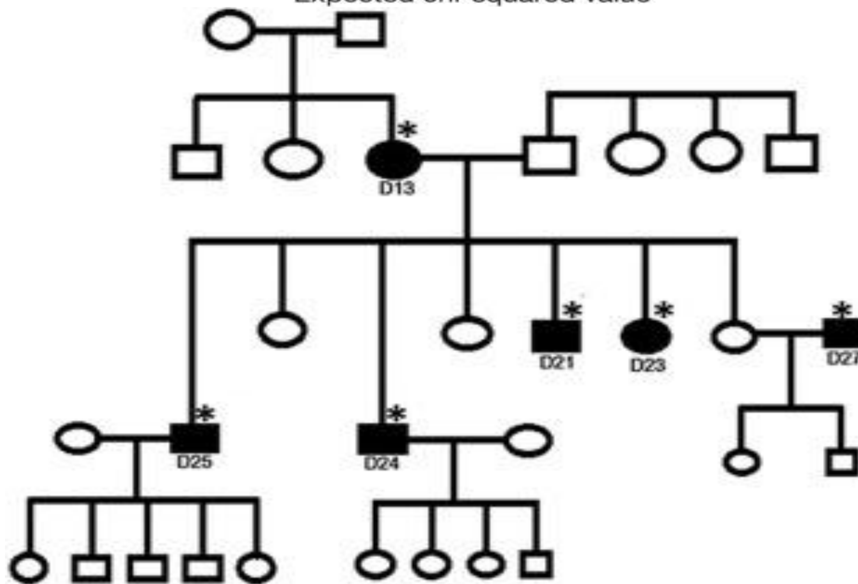
Sequence of gene for Type II of Diabetes

Candidate genes along with linkage-based studies have only identified a small number of T2D risk genes, but genome-wide association studies have helped to identify multiple genes. The multiple genes are assumed to play no role in the transmission of T2D. GWAS studies have provided a deeper insight into highly replicated genes such as TCF7L2, KCNQ1, and KCNJ11.









a maternally inherited diabetes mellitus Type II patients. The filled symbols represent affected members. Numbers represent family members from which sample and the studies informed consent was obtained. The asterisk shows respective probands in the pedigree.

III. CONCLUSION

In conclusion, pharmacogenetics plays a critical role to initiate adequate therapeutic management of type I and type II diabetes by administering proper prescriptions. Differences in oral antidiabetic drugs treatment outcomes are subject to enzymes metabolizing activity, drug-transporters and diabetes related genes[21]. Significant pharmacogenetics evidence suggests an association between patient therapeutic variability and specific gene polymorphisms [20] . Identifying drug-genotype interactions might result in important clinical implications that will see specific personalized therapies in combating diabetes .While the benefits of personalized diabetes care can be traced to patients with particular monogenic diabetic forms, individualized treatment options are anticipated in common polygenic forms. RFLP marker was one of the means to detect the different between the two type of diabetes through the use of molecular biology , two types of restriction enzyme were used to digest six genes responsible for type I and type II diabetes which produed different numbers and sizes of DNA fragments .

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