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Clinical Case Studies and Propensity of Diabetes in Delhi Population and Gene Interactomics

A Major Project dissertation submitted

In partial fulfilment of the requirement for the degree of

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In

Biomedical Engineering

Submitted by

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Under the supervision of

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CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"*Clinical case studies and propensity of diabetes in Delhi Population and gene interactomics*"**, submitted by **Ankit Tripathi (2K13/BME/18)** in partial fulfilment of the requirement for the award of the degree of Master of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by him under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

Date: 08/07/2015

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NAME: ANKIT TRIPATHI
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LIST OF ABBREVIATIONS

DM	**:**	**Diabetes Mellitus**
MA	**:**	**Microalbuminuria**
PCT	**:**	**Proximal Convoluted Tubule**
IDDM	**:**	**Insulin Dependent Diabetes Mellitus**
NIDDM	**:**	**Non- Insulin Dependent Diabetes Mellitus**
CVR	**:**	**Cardio-Vascular Risk**
PPAR-ϒ	**:**	**Peroxisome Proliferation Activated Receptor Gamma**
ACE	**:**	**Angiotensin Converting Enzyme**
MTHFR	**:**	**Methylene Tetrahydrofolate Reductase**
FABP2	**:**	**Fatty Acid Binding Protein 2**
FAT	**:**	**Fat Mass and Obesity Associated protein**
GLU (F)	**:**	**Glucose fasting**
GLU (PP)	**:**	**Glucose Post Prepare**
ALK	**:**	**Alkaline Phosphate**
CH	**:**	**Cholesterol**
HDL	**:**	**High Density Lipoproteins**
LDL	**:**	**Low Density Lipoproteins**
TGS	**:**	**Triglycerides**
VLDL	**:**	**Very Low Density Lipoproteins**
CRET	**:**	**Creatinine**

ABSTRACT

Diabetes mellitus (DM) popularly known as Diabetes is a group of metabolic diseases in which the blood glucose level shoots up for a prolonged period of time. This hiked blood sugar level produces a range of symptoms including glycosuria, polyuria, acedosis and polyphagia. Today, the world has come a long way from Type I diabetes but still remains akin to a tight rope between hyperglycemia and hypoglycaemic episodes. The incidence of Type I diabetes mellitus alter from nation to nation which suggests the role played by genetic constitution and environmental factors in the ultimate expression of the disease. Urinary albumin excretion also called Microalbuminuria (MA) is a well known marker of endothelial dysfunction. Patients with diabetes pose an extra risk of cardiovascular mortality due to low levels of MA. The HbA1c level is altered in diabetics from that of the normal individuals. Similarly, the renal and liver filteration rates are up-regulated as is also true for lipid profile in patients with diabetes. We are studying the properties pertaining to these various profiles in case study of diabetes I and II in a particular population of Delhi.

1) INTRODUCTION

One of the leading causes of many abnormalities including cardio-vascular diseases (like-coronary artery disease, coronary heart disease, or coronary heart risk), kidney and liver complications is diabetes (Geulayov et al; 2010). People belonging to any group are suffering from diabetes throughout the world (Phanse et al 2012; Zohra et al. 2012; Anbreen et al. 2012) and it is for this reason that the diagnosis remains under control (Chou et al. 1997). Various studies have pointed towards the prevalence of Type II diabetes to the level of an epidemic in Asian nations (Yoon et al. 2006). The leading causes of diabetes are sedentary lifestyle and altering environment or human-environment interaction. Apart from this, daily activities and everyday schedule can also be an unexplored factor in fluctuating normal blood sugar level (Doherty, 2011). The occurrence of diabetes can also be age and sex dependent, with males being more prone to the disease than females and aged more susceptible to diabetes.

Diabetes can be categorised into two major categories:

1) Type I – This type of diabetes results from inability of body to produce enough insulin. It is commonly called 'Insulin dependent diabetes mellitus (IDDM).

2) Type II- In this type, Insulin is present in optimum concentration in the body, β-cells of pancreas also have normal functioning but receptors do not provide response to Insulin. This is Insulin resistance disease and known as 'Non Insulin dependent diabetes mellitus (NIDDM)'.

The symptoms which can be observed in a patient suffering from diabetes include the following; these can also be called indicative markers of diabetes.

1. Glycosuria- The Glut1 receptor can absorb only 180mg/100 ml of glucose. When the blood glucose level becomes higher than this concentration, only the given level is absorbed by Proximal Convoluted Tubule (PCT) and the remaining is excreted in urine.
2. Polyuria- It is increase in the frequency of urination and is derived from gycosuria. As the glucose concentration rises in PCT, water from epithelial cells of the membrane surrounding PCT will start water input from outside i.e. more osmosis leading to more frequent urination.
3. Acedosis- When the expression of Glut-1 is negligible that is blood is having increased concentration of glucose by cells, so the normal body cells will switch onto fats as their energy source and fats will release their by-product ketone bodies (β hydroxybutyrate, acetone) resulting into acidic environment in the blood.
4. Polyphagia- Nerve cells and cardiac muscles utilize only glucose as their energy source. If glucose is not reaching nerve cells, they will activate hunger centre of body.

LIVER FUNCTION TEST (LFT)

In order to assess the proper functioning of liver, LFT is routinely done. This test evaluates various parameters such as SGOT and SGPT. The values obtained are matched against control values to arrive at any indication of pathology as diabetes (**Table 1**).

KIDNEY FUNCTION TEST (KFT)

In order to assess the proper functioning of Kidneys, KFT is routinely done. This test evaluates various parameters such as Urea, Creatinine and uric acid. The values obtained are matched against control values to arrive at any indication of pathology as diabetes (**Table 1**).

S.No.	Protein	Diabetic	Normal
1	S. Bilirubin	≥ 1.2	≤ 1.2
2	SGOT, serum	41- 42	≤ 40
3	SGPT, serum	41- 42	≤ 35
4	Alkaline Phosphatase, serum	≥ 131	40-125
5	Urea, serum	≥ 43	12.8 - 40.8
6	Globulin	>5	3.5 - 4
7	Creatinine, serum	1.2	0.7 - 1.0
8	Uric acid, serum	>7	3.4 - 6

Table 1: LFT and KFT of Diabetic vs. Control

LIPID PROFILE TEST

This test aims to evaluate the levels of various biomarkers such as cholesterol, HDL and LDL in serum. The values obtained are matched against control values to arrive at any indication of pathology as diabetes (**Table 2**).

S.No.	Protein	Diabetic	Normal
1	Cholestrol, serum	≈ 215	< 200
2	HDL cholesterol, serum	65 -70	40-60
3	LDL cholesterol, serum	100 - 115	90-100
4	VLDL cholestrol	> 50	10-50
5	Triglycerides	151.55 - 157.00	< 150.52

Table 2: Lipid Profile in Diabetic vs. Control

1.1) GENETICS BEHIND DIABETES

After a number of scientific studies and analysis done over diabetic population of different hemispheres of the world, the fact that came into the existence that both Type 1 and Type 2 diabetes are consequences of polymorphism occurred at certain specific locus of specific genes or genetic variants. The literature study of such surveys revealed the roles of certain genes and their translated products in onset and proliferation of diabetes in people such as-PPAR-ϒ, FTO, TCF7L2, KCNJ11, NOTCH-2, SLC3OAB, HHEX, JAZF-1, IGF-2BP2. Out of these genes mostly studied ones are PPAR-ϒ, ACE, MTHFR and FTO genes.

1.1.1) Peroxisome Proliferation Activated Receptor Gamma Gene (PPAR-ϒ)

PPARs (isoforms-α, δ and ϒ) are ligand activated transcription factors having in association Retinoid X Receptor(RXR) as receptor where they get bound for their activation. PPAR-ϒ has antidiabetogenic, anti-inflammatory, and anti-oxidant effects. The genes for PPAR-ϒ are located on chromosome 3p25 which codes for nuclear transcription factors which are responsible for the expression of hundereds of genes (Fajas et al. 1997; Deeb et al. 1998).

To study its role as a diabetogenic factor, a study has also been conducted on Caucasian population which came up with the outcome that a common nucleotide polymorphism (SNP) of the PPAR-ϒ gene (rs8192673) and PPAR-ϒ gene (rs1801282) are concerned with obesity and onset of Type 2 Diabetes Mellitus (T2DM).

Figure 1: 3D view of PPAR-ϒ (as derived from RCSB PDB)

Figure 2: Protein view of PPAR-ϒ (as derived from RCSB PDB)

1.1.2) Angiotensin Converting Enzyme (ACE)

Although ACE is concerned with the cleavage of angiotensin I into the angiotensin II which is a potent vasodilator. It can hydrolyze inactive angiotensin peptide(Snehalatha 2009; Hoskote and Joshi 2008; Das 2006; Barroso et al. 1999; Altshuler et al. 2000; Agostini et al. 2006; Stephens et al. 2005; Jayapalan et al. 2010; Nikzamir et al. 2008) into metabolic angiotensin and it is thousht to inactivate the vasodilators such as- kallidin and Bradykinin (Bor et al. 2000; Ohno et al. 2005; Feng et al. 2002; Arzu et al. 2004). It has also been demonstrated from the studies conducted on diabetic and non-diabetic nephropathies that the deletion polymorphism in ACE gene especially the homozygote is found to be a risk factor for an enhanced loss of kidney function (Lewis et al. 2001), which has shown to be associated with the T2DM (Stephens et al. 2005; Nikzamir et al. 2008; Hsieh et al. 2000; Daimon et al. 2003; Degirmenci et al. 2005; Grammer et al. 2006).

Figure 3: 3D view of ACE (as derived from RCSB PDB)

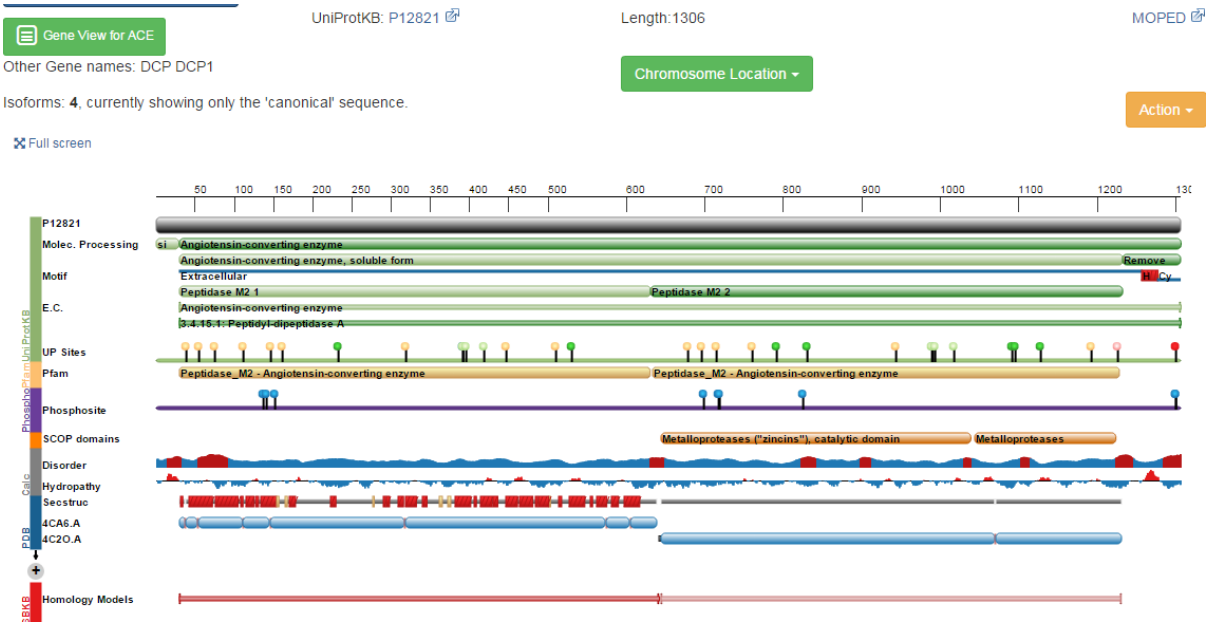


Figure 4: Protein view of ACE (as derived from RCSB PDB)

Polymorphism In ACE I/D Gene Enhances The Chances OF Diabetic Nephropathy:

The knowledge that genetic polymorphism in ACE gene may mediate diabetic nephropathy was already existing but conformation was being done later on, when the researchers conducted a study on type2 diabetes population of Kutch region. This study revealed that association between three polymorphic variants of ACE (I/D, D/D and I/I) and diabetic nephropathy in homogenous T2DM population. Deletion/Insertion polymorphism in 16 number intron of the ACE gene was examined by using the PCR and conducted on 309 different T2DM people, as a result of which genetic variation at the ACE locus as D/D variant in 16 number intron contributed to enhanced risk of diabetic nephropathy but not severely, while genetic variation at I/D variant contributed to diabetic nephropathy severely.

1.1.3) Methylene Tetrahydrofolate Reductase (MTHFR) gene

It is a major gene which plays pivotal role in regulating folic acid pathway, which catalyzes the conversion of 5, 10-Methylene Tetrahydrofolate (a Methylene donor) into 5-methyl tetrahydrofolate irreversibly. The gene is located on 1p36.3 (Outinen et al. 1998). MTHFR gene has been found to is also found to up regulate the glucose level and is also found to be associated with increasing risk of type 2 diabetes mellitus (Agullo Ortuno et al. 2002; Frosst et al. 1995; Russo et al. 2006; Di Renzo et al. 2007; Ozmen et al. 2002; Maeda et al. 2003).

From several studies this has also came into the existence that most of the east asian population with diabetes are at higher risk of renal complication and strokes than the European population. And when examined, the evaluation that came forward is that the reason of these strokes is the polymorphism in methyl tetrahydrofolate reductase (MTHFR) gene 677L->T which is also a potent mediator of New-Onset Diabetes (NOD).

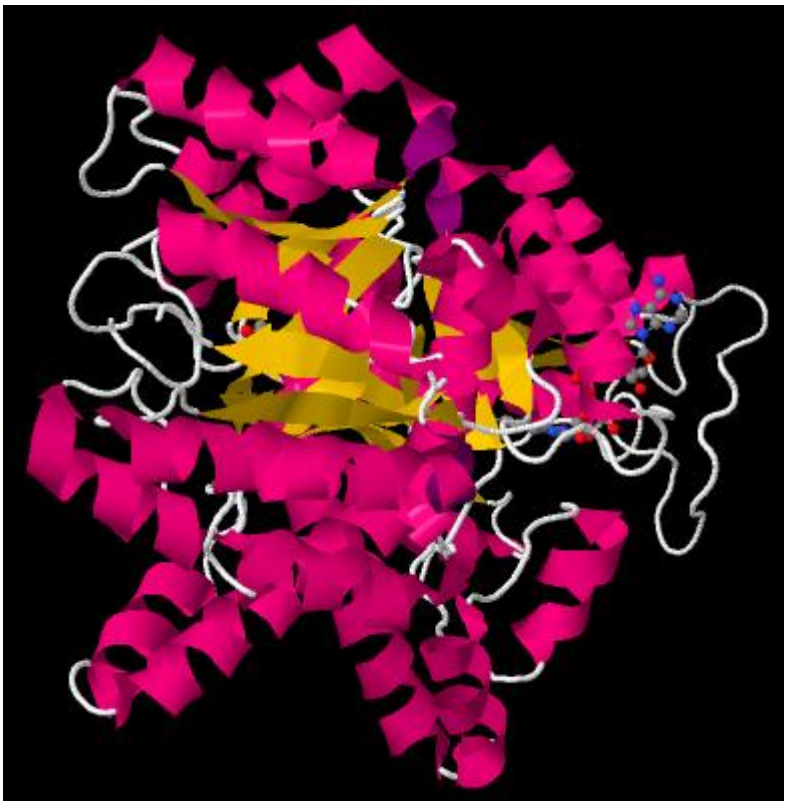


Figure 5: 3D view of MTHFR (as derived from RCSB PDB)

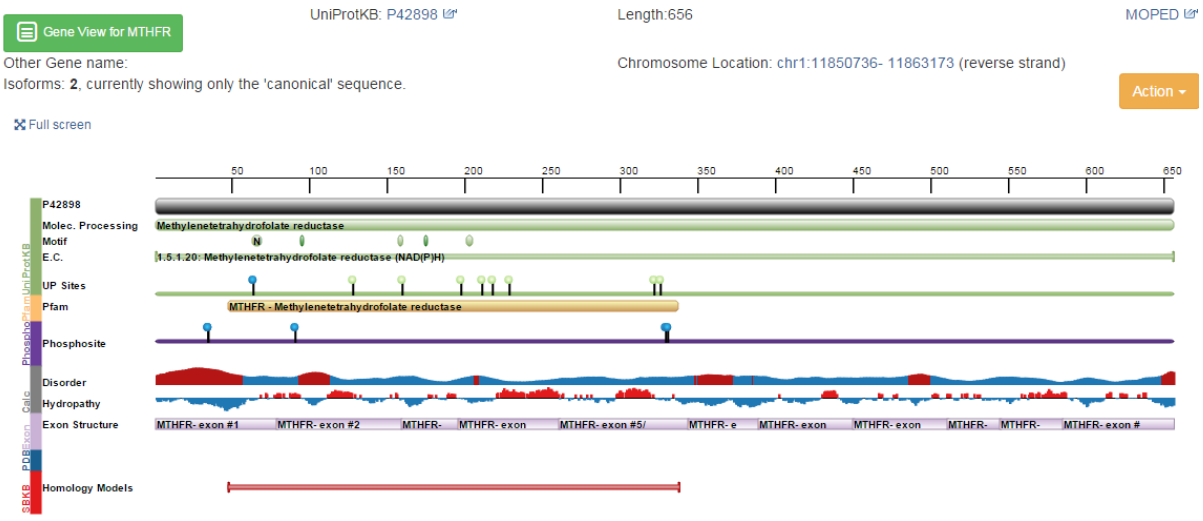


Figure 6: Protein view of MTHFR (as derived from RCSB PDB)

1.1.4) Fatty Acid Binding Protein 2 (FABP2) gene

Since glucose and fatty acid metabolism are co-related phenomenon. FABP2 is found to play pivotal role in the absorption of long chain dietary fatty acids. On doing molecular scanning of demonstrated a mis-sense mutation of Ala54Thr, which is associated with insulin resistance (Baier et al. 1995).

Figure 7: 3D view of FABP (as derived from RCSB PDB)

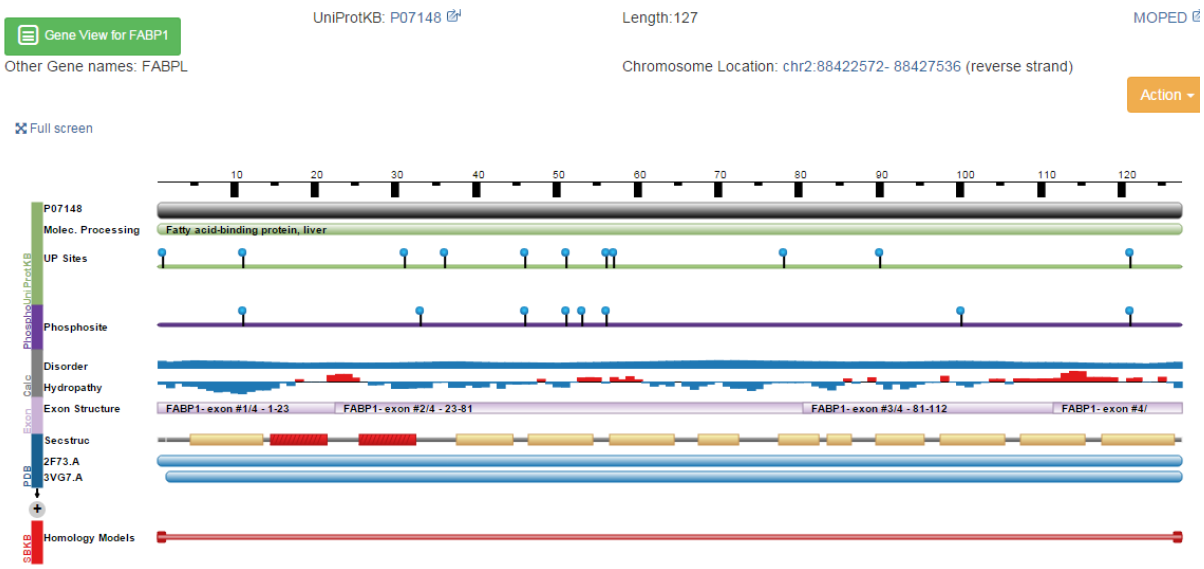


Figure 8: Protein view of FABP (as derived from RCSB PDB)

1.1.5) Fat Mass And Obesity Associated (FAT) gene

FTO gene expresses primarily in the hypothalamus dependent and encodes 2-oxygluterate dependent nucleic acid demethylase. FTO gene in genome-wide association (GWA) is found to predispose people towards diabetes through an impact on body mass index (BMI). FTO is strongly related with obesity and promoting type 2 diabetes mellitus.

Figure 9: 3D view of FAT (as derived from RCSB PDB)

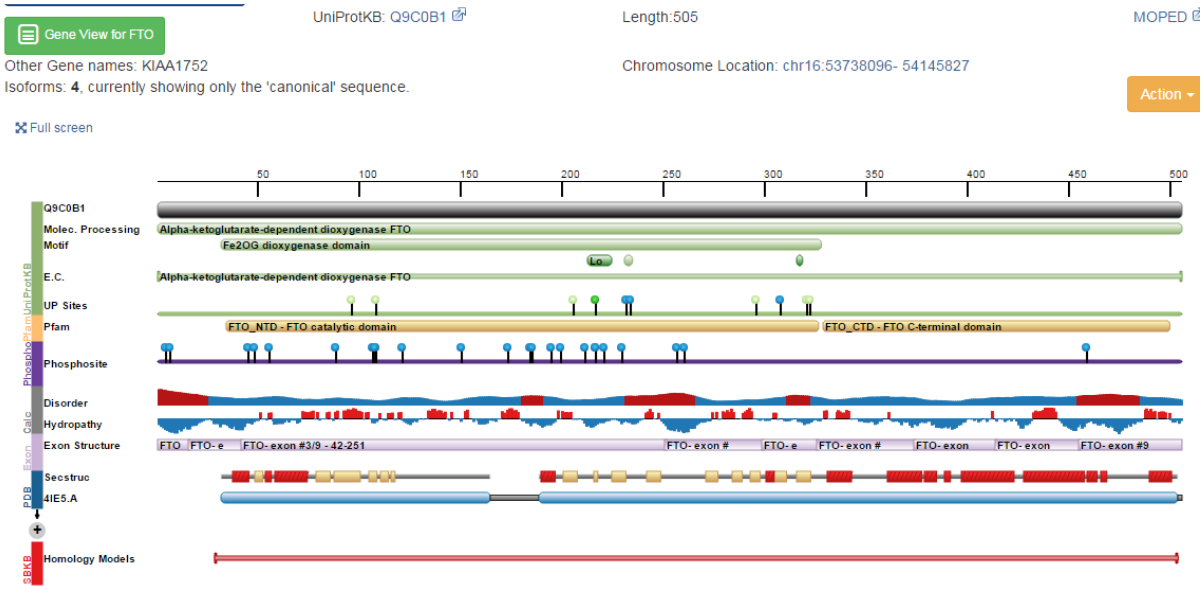


Figure 10: Protein view of FAT (as derived from RCSB PDB)

1.1.6) ***GLUCOSE TRANSPORTERS***

GLUT 1: The receptor is distributed widely in fetal tissues. But in adults it is highly expressible in RBCs as well as in endothelial cells of tissue barriers like- blood -brain barrier. It's role is to maintain low-basal glucose uptake levels for the sustenance of cellular respiration. But this is very renowned fact that concentration of GLUT 1 is inversely proportional to the amount of blood glucose.

GLUT 2: It is a bi-directional transporter which enables blood glucose to flow in two directions. It is expressed in renal tubular cells, beta cells of langerhans and liver cells. GLUT 2 expression has also been seen in the basolateral membrane of the small intestinal epithelium. GLUT 2 receptors are responsible for the transport of glucose from blood to hepatocytes.

GLUT 3: Highly expressible in placenta and neurons.

GLUT 4: Expression seen in especially in striated muscles like- cardiac, skeletal muscle and adipose tissue. GLUT 4 is responsible for translocation of glucose from hepatocytes to blood.

1.1.7) ***INSULIN RECEPTORS (IR)***

Insulin receptors are members of the tyrosine kinase family. They are transmembrane proteins and can be activated by insulin, IGF-I and IGF-II binding upon phosphorylation. IR regulates glucose levels in specialized cells of the body.

2) MATERIALS AND METHODS

2.1) Materials

To test blood sugar level, we need following materials:

1. Blood Glucose Meter
2. Test strips
3. Lancing device.
4. Code chip
5. Battery
6. USB Cable

2.2) Procedure

1. Wash hands (using warm water may help the blood flow) and dry properly.
2. Turn the meter on and prepare test strip.
3. Choose the spot for pricking, it is not recommended to check from the same finger all the time.
4. Prepare the lancing device and get a drop of blood from side of fingertip or other approved site.
5. Check the blood sugar by touching and holding the test strip opening to the drop until it has absorbed enough blood to begin the test.
6. View the test result and take proper consultation if the sugar level is too high or low.
7. Discard the used lancet properly.
8. Record the results in a log book hold them in meter's memory or copy to a computer's memory so that it can be reviewed and analysed later on also.

2.3) Methodology

1. An extensive literature survey was done to arrive at the receptor protein and ligands.
2. The protein structures were derived from Protein Data Bank (www.rcsb.org/pdb/protein).
3. Docking of protein (IR) and ligands was done using Hex 8.0.0 Cuda software.
4. The results obtained were analysed on the basis of total energy, E_{total} of docking.
5. The E_{total} of all the ligands against EGFR was noted in a table and plotted in the form of a graph in descending order of E_{total}.

3) RESULTS

The sample collected throughout the south-west Delhi population was tested for various parameters to ascertain the propensity of given population for diabetics and associated CV risk. The results are presented in the tabular form below. The parameters in the table distinguish the diabetic person from that of the non-diabetic.

		Blood Glucose Levels		Kidney Function Test				Hb A1 c	Lipid Profile				
Age (Y)	Sex	GLU (F)	GLU (PP)	Urea	Uric Acid	CRET	ALK		CH	HDL	LDL	TGS	VLDL
50	F	156	234	18.7	2.6	0.46	43	9.6	168.1	24.6	124.1	151.3	30.26
52	M	148	304	26.2	6.7	0.87	73	8.8	199.8	38.2	141.4	201.2	40.24
57	F	90.91	115.83	29.7	6.3	0.59	76	6	204.2	44.6	42.5	171.3	34.26
67	F	145.96	276.12	29.7	5.2	0.66	64	7.7	246.1	67	168.2	105.1	21.02
62	F	224.41	298.98	22.2	3.5	0.67	90	9.6	94.4	44.5	30.4	141.1	28.22
52	M	147	307	16.3	6.26	0.76	72.46	8.1	207.7	47.79	134.43	155.52	31.1
80	F	120	125	42	6.5	1.22	75	5.9	157.1	65	68.4	82.9	16.58
62	M	101	137	29.3	6.3	0.85	85	6	163.9	35.6	102.4	124.6	24.92
59	F	107	195	25.4	3.1	0.6	111	6.9	178.7	48.3	103.1	133.4	26.68
39	F	241.08	355.62	16.9		0.57		12	129	31.1	63	155.2	31.04
65	F	79.98	107.9	74.6	2.51	1.68	64.08	7.4	180.24	77.11	82.78	80.04	16.01
75	M	112.31	190.09	24		0.97		6.5	173.3	62.49	83.69	169.86	33.97
57	F	98.27	123.16					6.1	156.26	51.67	87.67	86.74	17.35
60	M	85	99	19.4	4.1	0.83	58		186.2	61.7	105.9	71.5	14.3
63	M	246	284	32.9	3	0.58	156	10.5	200.8	39.2	127.4	175.4	35.08
55	M	95	111	25.6	2.8	0.91	70		187.9	40.6	126.8	104.3	20.86
51	M	192		16	2.6	0.87			222.4	44.8	135.7	199.8	39.96
60	F	117		12.9	3.6	0.59	110		195.3	46.1	117.8	167.4	33.48
60	F	107.45	152.29	24.8	5.77	0.84	101.8	7.3	208.95	61.51	132.86	98.47	19.69

53	F	118.93	120.4	27.4	5	0.77	92	6.6	188	65.9	103.3	70.6	14.12
56	M	155.93	201.81	23.4	6.12	0.77	85.91	8.3	175.28	30.56	137.95	123.27	24.65
57	F	82.07		42.5	6.39	1.12	97.54	8.7	115.23	40.33	54.52	100.1	20.02
51	M			28	5.42	0.87	70.37	9.6					
58	F	108.84	147.73	11.7	4.15	0.66	96.82	10.8	117.13	34.46	66.95	108.82	21.76
69	F			99.4	4.97	7.37	143.7						
32	F	104		24.4	4.2	0.61	67		167.2	50.9	112.8	133	26.6
65	M	166.24	209.66	39.7	5.21	0.84	81.17		170.79	36.25	113.84	101.54	20.31
72	F	104	150.18	32.1	6.6	0.96	104		324.9	46.9	226.7	448.5	89.7
50	F	89		28.3	3.92	0.59			171.82	39.1	106.27	145.64	29.13
63	F	109	136.92	19.6		0.8			114.4	56.3	58.1	75.1	15.02
41	F	90.53		24.5	3.88	0.51	94.41		186	45.8	113.76	91.51	18.3
60	F	155.85	248.63	24.6	3.84	0.61	59.1	6.7					
68	M	85		25	5.9	0.9	68		126.9	28.5	86.7	127.9	25.58
49	M	85		33	6.28	0.88	98.3		156.39	35.66	90.24	118.22	23.65
48	M	212.72	286.9	26.6		0.62		12	177.28	36.28	120.3	104.05	20.81
62	F	85.63	164.35	24.9	5.22	0.49	116.4						
71	M	114.15	167.73	32.2	5.12	0.94		6.7	121.47	40.67	69	61.81	12.36
53	F	173.89		23.3	3.43	0.59	97.65	10.7	202.45	68.58	111.25	131.23	26.25
75	M	134.79	180.98	44.4	6.4	1.08	95	7.6	202.2	46.1	136	160.7	32.14
62	M	140.4	154.11	37.9	5.54	1.08	69.91						
67	M	133.72	160.04	24.3	5.7	0.69	123		148.3	45.7	83.7	147.3	29.46

48	F	200.59	259.81	13.8	2.9	0.53	78						
59	M	126.08	134.78		5.69	1.02		7.3	195.77	59.63	117.15	130.08	26.02
54	M	120	143	26.3	6.4	0.94	99		319.5	43.8	226.3	264.8	52.96
50	M	137		26.2	5.4	0.87	56		236.7	45.5	166.5	138.1	27.62
45	F	112.23	120.56			0.67			187.02	33.04	134.56	174.82	34.96

Table 3: Clinical data of Diabetic population under study

The study was further extended to check the interaction of IR with various genes involved in the onset or/and progression of diabetes. The aim was to ascertain protein-protein interaction of all the genes (ligand proteins) with the receptor protein IR and arrive at a stage to predict as to which of these genes could contribute maximum in causing diabetes and associated with CVR. The docking results are represented in the pictorial forms below.

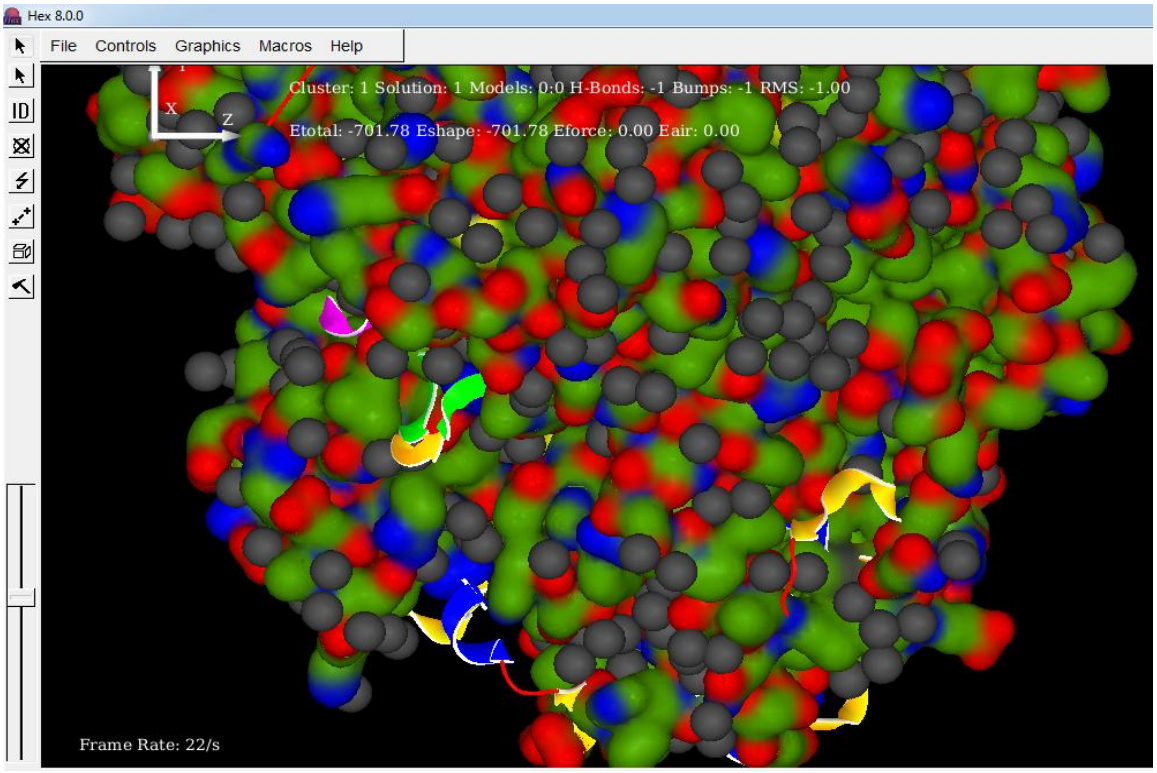


Figure 11: Docking of ACE-IR

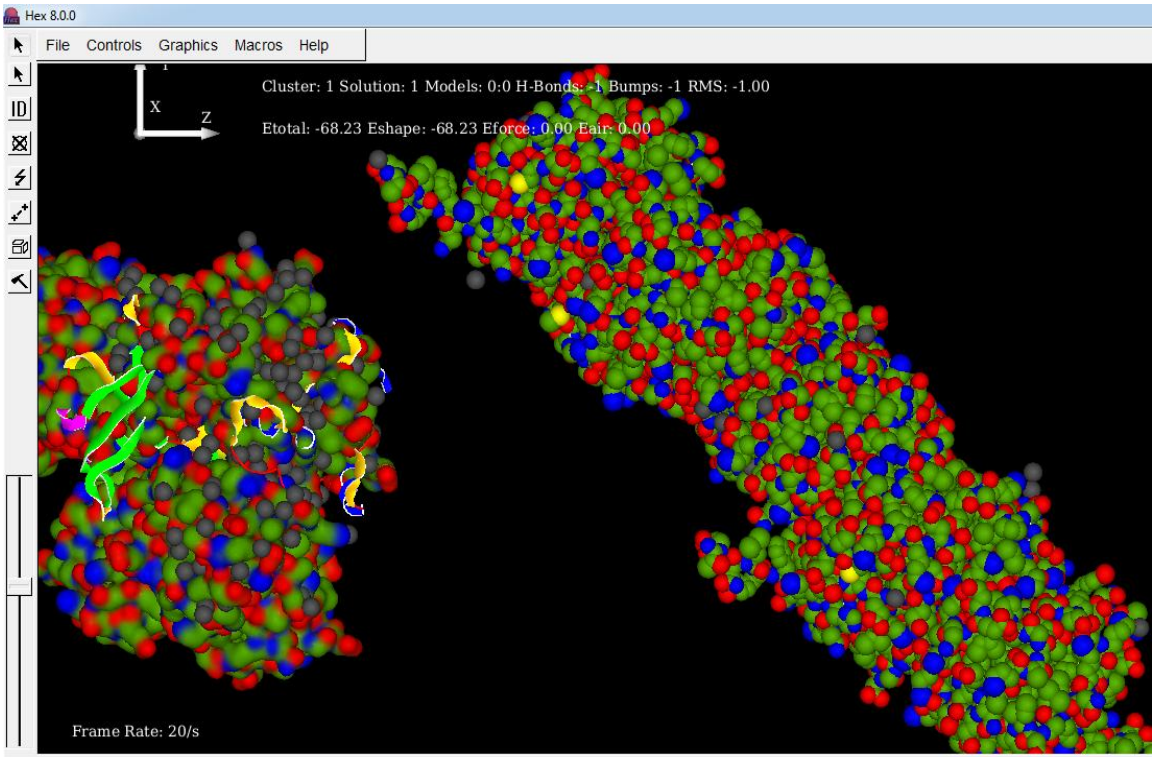


Figure 12: Docking of FABP-IR

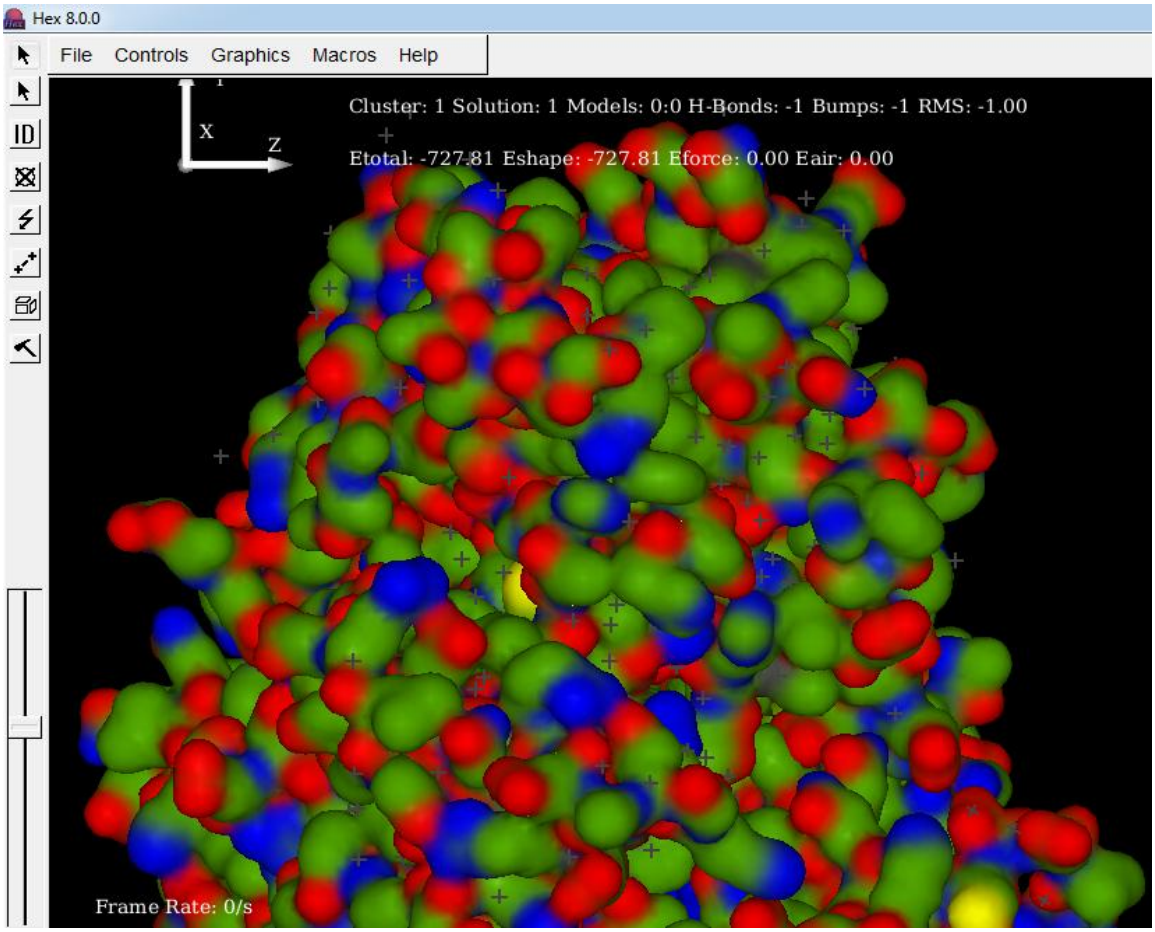


Figure 13: Docking of FTO-IR

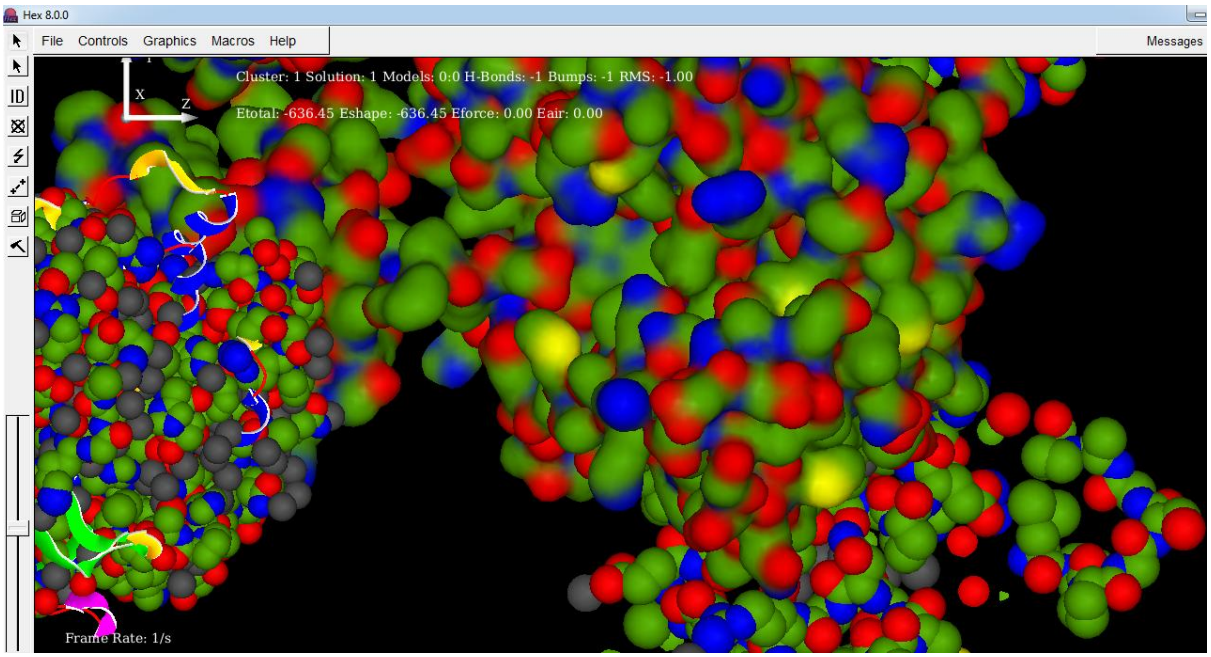


Figure 14: Docking of MTHFR-IR

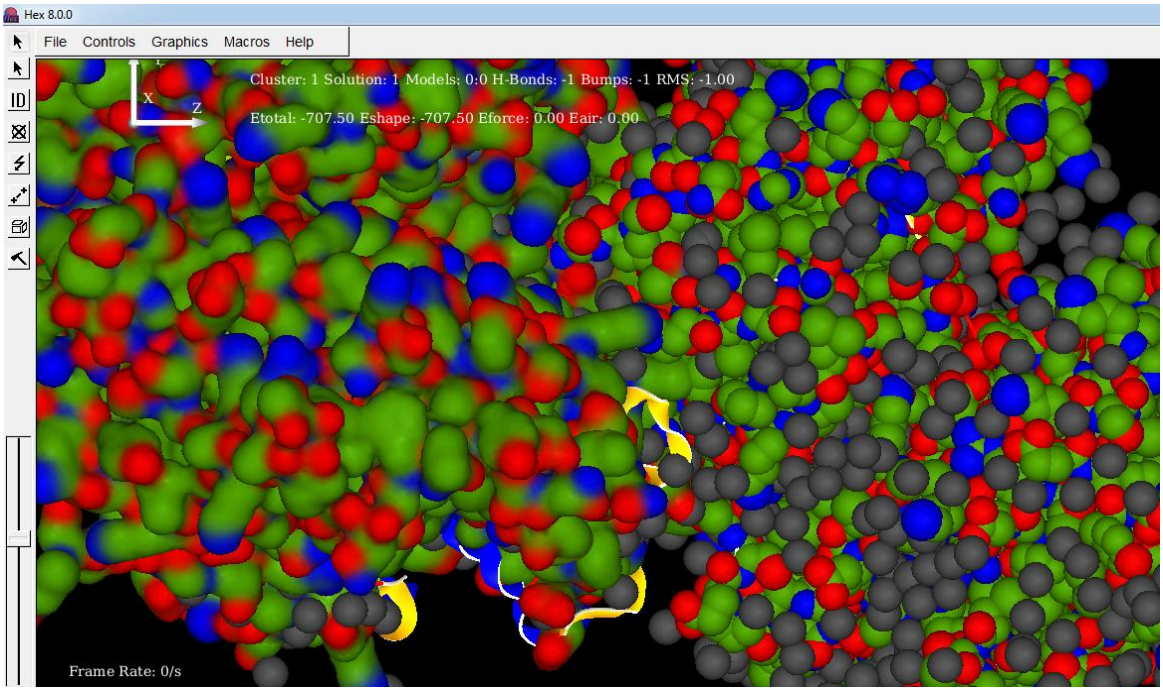
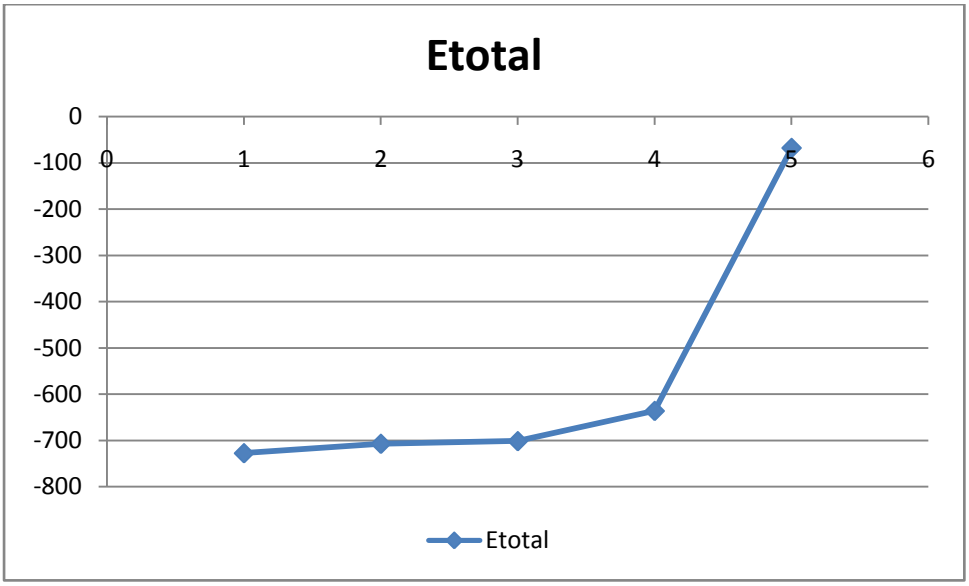


Figure 15: Docking of PPARγ-IR

S.No.	PROTEIN	PROTEIN	Etotal
1	IR	FAT	-727.81
2		PPARγ	-707.5
3		ACE	-701.78
4		MTHFR	-636.45
5		FABP	-68.23

Table 4: Docking results of Proteins with IR (in descending energy order)



Graph 1: E_{total} plotted curve of proteins

On the basis of E_{total} docking, <u>FAT</u> was identified as the most interacting protein showing highest binding with maximum docking energy (-727.81) with IR.

4) DISCUSSION

The major implications of the present study can be summarised as below:

- People aged 25 and below are more prone to Type 1 DM.
  Type1 DM normally occurs in the children or it occurs in the early stages of life due to which it is called as "Juvenile Diabetes" (Cooke and Plotnick 2008). In this type of diabetes usually the β-pancreatic cells of Langerhans secrets none or very less insulin due to which the glucose absorption gets affected (Roy and Lloyd 2012). Thus, in order to cure this, insulin is required to give exogenously to the patient (Wright 2002).

- People aged 40 or above are found to be more prone Type 2 DM.
  Type2 DM normally occurs in the late stages of life hence it is termed as "Adult type of DM" (Vijan 2010). This type of diabetes is not because of lack of insulin secreted by pancrease as it is insulin independent but due to the inefficiency of insulin receptors to get associated with their ligand i.e., insulin (Smyth 2006). Therefore it is sometimes also called as Non-Insulin Dependent DM (NIDDM) (Urban 2009).

- Further, people with Type 2 Diabetes are more susceptible to CVR due to the enhanced level of LDL and Triglycerides.
  Among the different types of Lipids; Triglycerides, Cholesterol and Low Density Lipoproteins which is also commonly known as "Bad Lipid" are found to be higher in the patient with Type2 DM which is responsible for making such patient susceptible to CVR especially- Strokes, Atherosclerosis and Coronary blockages (Bucula et al. 1993; Tribble 1995; Blatter et al. 1993; Mackness et al. 1991; Mackness et al. 1993; La 1992; Watson et al. 1995) .

- FAT gene demonstrated more binding towards IR.

  Among all the genes that have been discussed in this case study Fat Mass And Obesity Associated (FAT) gene is found to be highly expressive and showed maximum association to IR, thus it can be concluded that because this particular gene blocks the IRs due to which it inables the binding of Glucose to GLUTs as a result of which DM results (Fredriksson et al. 2008; Gerken et al. 2007; Clifton et al. 2007; Hinney 2007; Scuteri et al. 2007).

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