Chapter-7
Conclusions & Perspectives

7.1 Conclusions

Diabetic macrovascular complications (DMC), represents a persistent problem in clinical medicine. Despite significant improvements in therapeutics, the mortality and morbidity associated with DMC remain high. A major reason for this is the lack of early identification of these complications and an unacceptable delay in initiating therapy. In this present work a bioinformatic attempt has made for identification of important proteins, which may be useful as biomarkers are targets for new therapeutic interventions of DMC.

While the identification of these candidate proteins of DMC is encouraging, follow up studies are required for validation in a larger population of individuals and for determination of laboratory-defined sensitivity and specificity values using novel proteomic and metabolomic tools. The combination of proteomic and bioinformatic studies are useful for more accurate prediction of biomarkers/new therapeutic targets.
Proteomic Analysis in Diabetic Cardiomyopathy using Bioinformatics Approach

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Abstract: Diabetic cardiomyopathy is a distinct clinical entity that produces asymptomatic heart failure in diabetic patients without evidence of coronary artery disease and hypertension. Abnormalities in diabetic cardiomyopathy include: myocardial hypertrophy, impairment of contractile proteins, accumulation of extracellular matrix proteins, formation of advanced glycation end products, and decreased left ventricular compliance. These abnormalities lead to the most common clinical presentation of diabetic cardiomyopathy in the form of diastolic dysfunction.

We evaluated the role of various proteins that are likely to be involved in diabetic cardiomyopathy by employing multiple sequence alignment using ClustalW tool and constructed a Phylogenetic tree using functional protein sequences extracted from NCBI. Phylogenetic tree was constructed using Neighbour—Joining Algorithm in bioinformatics approach. These results suggest a causal relationship between altered calcium homeostasis and diabetic cardiomyopathy that implies that efforts directed to normalize calcium homeostasis could form a novel therapeutic approach.

Keywords: diabetes, cardiomyopathy, protein kinase C, calcium, metallomatrix proteins, advanced glycation end products

Introduction

Patients with diabetes mellitus are at increased risk of cardiovascular mortality (1). Diabetic cardiomyopathy is a distinct clinical entity that produces asymptomatic heart failure without evidence of coronary artery disease (CAD) and hypertension and manifests itself as diastolic dysfunction that could eventually lead to left ventricular hypertrophy and failure. Although diabetic cardiomyopathy entity has been well described for quite sometime, its precise molecular basis is still debated. Some of the pathological abnormalities described in diabetic cardiomyopathy are: presence of myocardial hypertrophy, impairment of contractile proteins, accumulation of extracellular matrix proteins, formation of advanced glycation end products, and decreased left ventricular compliance (2). Majority, if not all, of these abnormalities could be attributed to defects in the regulation of calcium homeostasis. In view of this, in the present bioinformatics approach we tried to identify key functional proteins that are closely associated with diabetic cardiomyopathy.

Materials and Methods

We have collected those proteins that are believed to be involved in the pathogenesis of diabetic cardiomyopathy based on literature survey and reports. For instance, both Na\(^+\)-Ca\(^{2+}\)- exchanger and Na\(^+\)-K\(^+\)-ATPase are considered as the key proteins that are closely associated with diabetic cardiomyopathy (2, 3). Similarly, glucose transporter-4 (GLUT-4), MMP-2 (matrix metalloproteinase-2), protein kinase C (PKC), p38 mitogen-activated protein kinase, and CD36 are thought to play a significant role in diabetic cardiomyopathy. Hence, these proteins were selected for the present study (see Table 1). The functional protein sequences in FASTA format for these proteins were collected from NCBI (National
Table 1. Table showing the genes/proteins that have been selected for the current study that are thought to be involved in diabetic cardiomyopathy as evident from literature survey.

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<th>Tissue type</th>
<th>Reference</th>
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</table>

Center for Biotechnology Information, (http\www.ncbi.nih.nlm.gov). These sequences were given to ClustalW (http\www.ebi.ac.uk\clustalw) for the Multiple Sequence Alignment. (which calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen). Based on these results, the scores table and phylogenetic tree that shows the distance between the selected protein sequences was constructed.

Results and Discussion

The results of the present bioinformatics analysis given in Figure 1 showed that Na⁺⁻Ca²⁺⁻exchanger, protein kinase C-β, and Na⁺⁻K⁺⁻ATPase as the key proteins that could have a causal role in diabetic cardiomyopathy. This is based in the present observation that when phylogenetic tree was constructed based on the alignment scores of all the protein sequences selected showed that proteins with minimum distance are Na⁺⁻Ca²⁺⁻exchanger, protein kinase C-β isomer and Na⁺⁻K⁺⁻ATPase. This suggests that these proteins are not only closely linked to each other but also play a significant role in the pathobiology of diabetic cardiomyopathy. Both Na⁺⁻Ca²⁺⁻exchanger and Na⁺⁻K⁺⁻ATPase are involved in action potential generation and myocardial contractile function. Hence, any abnormality in their function could lead to myocardial dysfunction.

Long standing diabetes could produce myocardial dysfunction due to abnormalities in Na⁺, K⁺, and Ca²⁺ influx and efflux, in part, due to impaired Na⁺⁻Ca²⁺⁻exchanger and Na⁺⁻K⁺⁻ATPase activity. Such impairment in Na⁺⁻Ca²⁺⁻exchanger and Na⁺⁻K⁺⁻ATPase has the potential to cause abnormalities in myocardial function especially in the form of impaired diastolic relaxation and systolic contraction. These abnormalities could occur even in the absence of CAD and hypertension (3). The most typical feature of diabetic cardiomyopathy is the abnormal filling pattern of left ventricle with reduced compliance or prolonged relaxation (4). The shift in the faster V₁ form of myosin to slower V₂ form leads to delayed relaxation of the ventricles. Predominance of myosin subtype V₁, reduces the Ca²⁺⁻ATPase activity (5). Impaired sarcoplasmic Na⁺⁻Ca²⁺⁻exchanger activity and depressed Na⁺⁻K⁺⁻ATPase activity causes retention of calcium that could render myocardial contractile dysfunction.

In addition, diabetic cardiomyopathy may be associated with aberrations in glucose and lipid metabolism (6), which are known to occur in patients with diabetes mellitus, that could lead to secondary to disturbances in carbohydrate, lipid and adenine nucleotide metabolism in the diabetic heart (2). In particular, depletion of glucose transporter-4 (GLUT-4), increase in fatty acids, changes in calcium homeostasis, and associated small vessel disease, cardiac autonomic neuropathy, and insulin resistance may all play a significant role in the onset of diabetic cardiomyopathy.

It was reported that decreased sarcoplasmic reticulum Ca²⁺⁻ATPase activity results in decreased calcium transport in isolated sarcoplasmic reticulum obtained from animals and humans with diabetes mellitus. Furthermore, other abnormalities of Ca²⁺ homeostasis that could occur in these patients include: i) decreased Ca²⁺ uptake, ii) decreased Ca²⁺ binding, and iii) decreased Na⁺⁻K⁺⁻ATPase activity of sarcolemma (2). This is supported by the observation that sarcolemmal and the sarcoplasmic reticular calcium transporters are depressed in diabetic cardiomyopathy. This
Figure 1. Phylogenetic tree that was constructed based on the alignment scores of all the protein sequences involved in diabetic cardiomyopathy. Proteins with minimum distance are Na⁺-Ca²⁺-exchanger, protein kinase C-β isomer and Na⁺-K⁺-ATPase.

results in an increase in cytoplasmic calcium content and a decrease in calcium outward current resulting in prolonged action potential duration and increased myocardial stiffness. This altered intracellular calcium handling eventually leads to decreased myocardial function and failure (3).

Transgenic mice overexpressing PKC-β isoform developed cardiac hypertrophy and myocardial fibrosis, whereas PKC-β isoform inhibitor prevented several of the functional abnormalities seen in diabetic cardiomyopathy (7). This is supported by the observation that activation of PKC can modulate the gene expression of the myocardium that results in myocardial hypertrophy and myocardial fibrosis and eventually causes myocardial failure (8). Since PKC appears to be involved in the pathobiology of development of several complications seen in diabetes mellitus and as diabetic state itself induces the activation of PKC-β isoform that can produce cardiac abnormalities (as evidenced from the studies done with Transgenic mice overexpressing PKC-β isoform), the involvement of PKC in diabetic cardiomyopathy appears persuasive.

It is known that accumulation of myocardial collagen resulting in interstitial and perivascular fibrosis that occurs in long-standing diabetes can be correlated with early diastolic and systolic left ventricular dysfunction (9). Non-enzymatic glycosylation of collagen and diminished activity of collagen degrading enzymes such as matrix metalloproteinases (MMP) are considered to be pathognomonic of myocardial fibrosis (9) that could eventually lead to myocardial dysfunction in diabetes. There is also general consensus that formation of advanced glycation end products that occurs as a result of persistent hyperglycemia can also cause cellular and myocardial dysfunction in diabetes (10, 11). In addition, there is evidence to suggest that activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) contributes to the development of endothelial and myocardial dysfunction in diabetes. It was reported that hyperglycemia, cardiac PARP activation, a selective loss of endothelium-dependent vasodilation in the thoracic aorta, and an early diastolic dysfunction of the heart accompanied development of diabetes in experimental animals (12, 13). Treatment with PARP inhibitor, starting 1 week after the onset of diabetes, restored normal vascular responsiveness and significantly improved cardiac dysfunction, despite the persistence of severe hyperglycemia. The beneficial effect of PARP inhibition persisted even after several weeks of discontinuation of the treatment. Thus, PARP activation plays a central role in the pathogenesis of diabetic cardiovascular (cardiac as well as endothelial) dysfunction and PARP inhibitors may exert beneficial effects against the development of cardiovascular complications in diabetes.

It is evident from the preceding discussion that several pathological processes are proposed and seems to be involved in the development of diabetic cardiomyopathy. Of all these mechanisms, the earliest pathological event appears to be altered calcium homeostasis. The results of the present bioinformatics study also support the role of PKC in the development of cardiac abnormalities seen in diabetic cardiomyopathy. Thus, our bioinformatics study highlights the involvement of deranged calcium homeostasis and impairment of contractile proteins as the major responsible for the development of diabetic cardiomyopathy.
References


Bioinformatics Analysis of Functional Protein Sequences Reveals a Role for Tumor Necrosis Factor-α and Nitric Oxide in Insulin Resistance Syndrome

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Abstract: Using bioinformatics techniques and sequence analyses algorithms, we identified that tumor necrosis factor-α (TNF-α) and nitric oxide (NO) have a significant role in the pathobiology of insulin resistance syndrome, a condition that is common in subjects with abdominal obesity, hypertension, dyslipidemia, atherosclerosis, and coronary heart disease and are accompanied by endothelial dysfunction due to reduced endothelial nitric oxide generation. TNF-α has neurotoxic actions, stimulates inducible NO synthase activity, and modulates the expression of neurotransmitters involved in the control of feeding and thermogenesis. NO is a neurotransmitter and influences secretion and actions of various hypothalamic peptides and neuropeptides. Insulin suppresses the production of TNF-α but stimulates that of endothelial NO. This close interaction between TNF-α, NO, hypothalamic peptides, and insulin suggests that regulation of TNF-α and NO production and action could be critical in the management of insulin resistance syndrome and its associated conditions.

Keywords: Insulin resistance, tumor necrosis factor, nitric oxide, hypothalamus, neurotransmission, insulin, endothelium, inflammation.

INTRODUCTION

Insulin resistance syndrome also called as metabolic syndrome X is characterized by diminished ability of tissues to respond to insulin that leads to compensatory hyperinsulinemia. Insulin resistance enhances the risk for type 2 diabetes mellitus, coronary heart disease (CHD), and stroke and is often associated with abdominal obesity, hypertension, dyslipidemia, low high-density lipoprotein (HDL) cholesterol, and high glycemia [1]. Although insulin resistance is equated with impaired whole-body insulin-mediated glucose disposal, defective regulation of non-esterified fatty acid and glycerol metabolism occurs even in those in whom glucose tolerance is either normal or only marginally impaired [2].

INSULIN RESISTANCE SYNDROME IS A LOW-GRADE SYSTEMIC INFLAMMATORY CONDITION

Plasma levels of C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), markers of inflammation, are elevated in subjects with obesity, insulin resistance, essential hypertension, type 2 diabetes, and CHD [3-8]. Higher plasma CRP, IL-6, and TNF-α concentrations are associated with increased risk of CHD, ischemic stroke, and peripheral arterial disease [4]. Similarly, a strong correlation between elevated CRP levels and cardiovascular risk factors, fibrinogen, and HDL cholesterol has also been reported, suggesting that inflammation occurs throughout life and that it participates in the development of atherosclerosis and cardiovascular disease. These evidences suggest that insulin resistance syndrome and its associated conditions can be regarded as low-grade systemic inflammatory conditions [3-10].

INSULIN RESISTANCE SYNDROME AND NITRIC OXIDE

Insulin resistance is accompanied by increase in peripheral vascular resistance that is due to decreased endothelial nitric oxide (eNO) generation that is also responsible for endothelial dysfunction. High-fructose-fed rats showed decrease in metabolic clearance rate of glucose compared to control that was reverted to normal by sodium nitroprusside infusion, a donor of NO [11], suggesting that NO improves insulin resistance. Furthermore, sustained hyperinsulinemia causes impairment of NO production that contributes to insulin resistance and hypertension [12]. In insulin resistant experimental animals, depletion of tetrahydrobiopterin (H4B) and elevation of 7,8-dihydrobiopterin (7, 8 H4B) (activating and inactivating cofactors of nitric oxide synthase respectively) contributes to impairment of NO-dependent vasodilation through reduction of NO synthase activity as well as increased superoxide anion generation. A stepwise decrease in the maximal acetylcholine-induced vasodilation (that is known to be due to eNO) and plasma H4B/7,8-H4B ratio, and increase in coronary lipid peroxide production as insulin sensitivity decreased was reported. The acetylcholine-induced vasodilation was positively correlated with insulin sensitivity, whereas H4B/7,8-H4B ratio was inversely correlated with insulin sensitivity, indicating that both abnormal peroxide metabolism and vascular oxidative stress are linked to coronary endothelial dysfunction and reduced NO generation in insulin-resistant subjects [13]. In addition, subjects with insulin resistance showed elevated plasma concentrations of asymmetrical dimethyl arginine (ADMA), an endogenous inhibitor of NO [14]. These results emphasize that insulin resistance is accompanied by decrease in eNO production that could be due to increase in ADMA levels and oxidant stress.

HYPOTHALAMUS AND INSULIN RESISTANCE SYNDROME

Ventromedial hypothalamic (VMH) lesion in rats induces hyperphagia and excessive weight gain, fasting hyperglycemia, hyperinsulinemia, hypertriglyceridemia and impaired glucose tolerance [15, 16]. Intraventricular administration of antibodies to neuropeptide Y (NPY) abolished the hyperphagia and ob mRNA (leptin mRNA) in these animals, suggesting a role for NPY in hyperphagia and obesity seen in VMH lesioned animals and that ob gene is up regulated even in non-genetically obese animals [17, 18]. Increased NPY concentrations were noted in the paraventricular, ventromedial (VMH), and lateral hypothalamic areas of streptozotocin-induced diabetic rats [19]. Thus, dysfunction of VMH impairs pancreatic β cell function and induces metabolic abnormalities similar to those seen in type 2 diabetes.
In this context, it is important to note that TNF-α and NO are produced by neurons in the brain and that insulin receptors exist in the specific areas of brain. TNF-α produced by glial cells enhances synaptic efficacy by increasing surface expression of AMPA receptors. Continued presence of TNF-α is required for preservation of synaptic strength at excitatory synapses [20, 21]. In contrast, excess TNF-α induces apoptosis of neurons [22, 23], whereas insulin is needed for neuronal growth and differentiation and synaptic plasticity in the CNS [24, 25], suppresses TNF-α production [26, 27], and antagonizes neuronal death induced by TNF-α [22, 23]. These results suggest that a close interaction exists between insulin, TNF-α, and NO with regard to the survival and function of hypothalamic neurons.

CROSS TALK BETWEEN GUT AND HYPOTHALAMUS

Leptin, produced by adipose cells and acts on brain, mediates its actions through its receptors that are present in afferent visceral nerves and hypothalamic arcuate nucleus [ARC]. Arcuate neurons express and release neuropeptide Y (NPY) and agouti-related protein (AgRP) that activates the ingestive behaviour through paraventricular nucleus (PVN). Gut hormones: cholecystokinin (CCK), peptides YY (PYY) and oxyntomodulin (OXM) act on afferent nerves and on ARC neurons to inhibit expression and release of NPY and AgRP and inhibit food intake by inhibition of PVN. During fasting, gut produces ghrelin and orexins that act on hypothalamic ARC to stimulate growth hormone release, and promote the expression and release of hypothalamic NPY and and suppressing TNF-α action.

Table 1. Table Showing the Genes/Proteins that have been Studied in the Present Study, which are Believed to be Involved in Insulin Resistance Syndrome

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Bioinformatics Analysis of Functional Protein

AgRP to drive food intake [28]. It is interesting to note that TNF-α, insulin, and NO modulate the release and actions of NPY, AgRP, CCK, and PYY [29, 30].

CCK inhibited LPS-induced TNF-α mRNA expression and NF-κB binding activity in macrophages and thus, brings about its anti-inflammatory activity [31]. Ingestion of diet rich in oleic acid and polyunsaturated fatty acids stimulates CCK receptors, and leads to attenuation of the inflammatory response by way of the efferent vagus nerve and nicotinic receptors. Vagotomy and administration of antagonists for CCK and nicotinic receptors blunted the inhibitory effect of high-fat enteral nutrition on hemorrhagic shock-induced TNF-α and IL-6 release. The beneficial actions of high-fat enteral nutrition on inflammation were abrogated by vagotomy and administration of antagonists for CCK and nicotinic receptors [32], suggesting that there exists a neuro-immunologic pathway, controlled by nutrition connecting brain and the gut.

NPY significantly enhanced NO release from slices of hypothalamus suggesting that NO participates in its signaling pathway [33]. PYY, a gastrointestinal hormone with multiple inhibitory effects on the proximal digestive tract, including suppression of secretion by the exocrine pancreas, significantly reduced mortality due to sepsis and inhibited early IL-6 release suggesting that it has immunomodulatory actions [34]. Furthermore, PYY reduced TNF-α-induced nuclear translocation of the p65 subunit of NF-κB and activation of other transcription factors: Smad3/4 and PPARα/γ [35], and suggested that PYY has immunomodulatory actions. These evidences suggest that gut and hypothalamic hormones/peptides and cytokines interact not only to regulate body weight, appetite, and energy homeostasis but also to modulate immune response. Since insulin resistance syndrome is a low-grade systemic inflammatory condition, it is likely that changes in the gut and hypothalamic hormones/peptides and neurotransmitters and peptides occurs in view of the cross talk between the gut and hypothalamus.

In view of this close interaction between the gut and hypothalamus, we performed bioinformatics analysis of functional protein sequences of genes and related proteins that are involved in insulin resistance syndrome.

MATERIALS AND METHODS

We collected ~ 20 proteins, which are related to type II diabetes mellitus and insulin resistance syndrome that are believed to be involved in their pathogenesis. The functional protein sequences in FASTA forms for these genes are collected from NCBI (National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov). These sequences are given to clustalw (http://www.ebi.ac.uk/)

Fig. (1). The phylogenetic tree that was constructed based on the alignment scores of all the protein sequences involved in insulin resistance syndrome and type 2 diabetes mellitus. A high degree of homology was noted between TNFα and NO (nitric oxide synthase).
Bioinformatics analysis of functional protein sequences of genes and related proteins that are involved in insulin resistance syndrome revealed a high degree of homology between TNF-α and NOS (nitric oxide synthase). It is evident from the preceding discussion and results of the present bioinformatics study that NO and TNF-α play a significant role in the pathobiology of insulin resistance syndrome. Both TNF-α and NO have a regulatory role in the secretion and action of various gut hormones and hypothalamic peptides and neurotransmitters. In view of the close interaction between insulin, TNF-α and NO and their role in the regulation of appetite, satiety, body adiposity, and metabolic homeostasis, any imbalance in the feedback regulation among them could lead to abnormalities in the secretion and actions of various gut and hypothalamic hormones/peptides and neurotransmitters. This suggests that changes in gut hormones/peptides and hypothalamic peptides and neurotransmitters observed in insulin resistance, obesity, type 2 diabetes mellitus, and metabolic syndrome X could be secondary to alterations in insulin/TNF-α/NO system (Fig. 2). Hence, methods designed to normalize insulin-NO-TNF-α system is important in the treatment of insulin resistance and its associated conditions. The results of the present bioinformatics study lend support to this view.

REFERENCES

Bioinformatics Analysis of Functional Protein


Bioinformatics analysis of diabetic retinopathy using functional protein sequences

Allam Appa Rao a, Hanuman Thota b, Ramachandra Sridhar Gumpeny c, Annapurna Akula d, Suresh Babu Changalasetty b, Siva Reddy Challa d, Tejaswi Ravavarapu a, Siva Prasad Akula b, Ch. Divakar e, K. Srinivas b, Undurti N. Das f,

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Summary Diabetic retinopathy is the leading cause of blindness among patients with diabetes mellitus. We evaluated the role of several proteins that are likely to be involved in diabetic retinopathy by employing multiple sequence alignment using ClustalW tool and constructed a phylogram tree using functional protein sequences extracted from NCBI. Phylogram was constructed using Neighbor-Joining Algorithm in bioinformatics approach. It was observed that aldose reductase and nitric oxide synthase are closely associated with diabetic retinopathy. It is likely that vascular endothelial growth factor, pro-inflammatory cytokines, advanced glycation end products, and adhesion molecules that also play a role in diabetic retinopathy may do so by modulating the activities of aldose reductase and nitric oxide synthase. These results imply that methods designed to normalize aldose reductase and nitric oxide synthase activities could be of significant benefit in the prevention and treatment of diabetic retinopathy.

Introduction

Diabetic retinopathy, a microvascular complication of hyperglycemia, can result in blindness. Multiple interlinked biochemical mechanisms have been postulated to be involved in diabetic retinopathy. The mechanisms mainly include: increased aldose
Several animal studies revealed that there is strong evidence that aldose reductase, the first and rate-limiting enzyme of the polyol pathway that converts glucose to fructose, plays a key role in the pathogenesis of microvascular complications including diabetic retinopathy [10–15]. Aldose reductase plays an important role in the pathogenesis of diabetic retinopathy by inducing retinal lesions including blood retinal barrier break down, loss of pericytes, neuroretinal apoptosis and glial reactivation and neovascularization-events that are associated with diabetic retinopathy [15]. Animal studies revealed that administration of aldose reductase inhibitors to diabetic rats prevented basement membrane thickening, pericyte loss, and development of microaneurysms in the retinal capillaries [15]. These results emphasize the importance of aldose reductase in the pathogenesis of diabetic retinopathy. However, clinical trials of the aldose reductase inhibitors were disappointing, suggesting that changes in the concentrations and activities of other proteins and enzymes also play a significant role in the pathogenesis of diabetic retinopathy and/or cooperate with aldose reductase to induce the development of diabetic retinopathy. In order to evaluate the role of other such factors in the development of diabetic retinopathy, the present study was performed to identify key protein(s) contributing to diabetic retinopathy using bioinformatics tools. We sought to verify the role of various molecules believed to be involved in the pathogenesis of diabetic retinopathy using bioinformatics approach by employing multiple sequence alignment using ClustalW tool and constructed a phylogram tree employing functional protein sequences extracted from NCBI. Phylogenetic analysis and construction of the scores table (see Table 1) revealed that 28 proteins are closely associated with this disease process. Construction of the phylogeny tree using (see Fig. 1) this data revealed that of all the proteins studied aldose reductase and nitric oxide synthase are the two proteins with minimum distance indicating a dominant role for them in diabetic retinopathy. These results suggest that aldose reductase not only interacts with nitric oxide synthase but also with several other proteins such that various pathogenic events seen in diabetic retinopathy are initiated and allowed to progress.

Discussion

Aldose reductase is the first and rate-limiting enzyme of the polyol pathway. Under normoglycemic conditions, aldose reductase plays a minor role in glucose metabolism. In contrast, persistent hyperglycemia due to uncontrolled diabetes leads to a significant increase in aldose reductase activity. This increase in polyol pathway due to hyperglycemia could lead to complications seen in diabetes such as retinopathy, neuropathy and nephropathy. An increase in aldose reductase activity results in...
Table 1: Genes (28 in number) that are believed to be involved in the pathogenesis of diabetic retinopathy with their respective gene symbol and protein ID that have been analysed in the present study.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Gene symbol</th>
<th>Protein ID</th>
<th>Tissue type</th>
<th>Length</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACE</td>
<td>AAH36375</td>
<td>Testis</td>
<td>739 aa</td>
<td>[32,33]</td>
</tr>
<tr>
<td>2</td>
<td>ADIPOQ</td>
<td>AAH96310</td>
<td>PCR rescued clones</td>
<td>244 aa</td>
<td>[34]</td>
</tr>
<tr>
<td>3</td>
<td>AGER</td>
<td>AAH20669</td>
<td>Lung</td>
<td>404 aa</td>
<td>[35—37]</td>
</tr>
<tr>
<td>4</td>
<td>AKR1B1</td>
<td>AAH00260</td>
<td>Eye, retinoblastoma</td>
<td>316 aa</td>
<td>[14,29]</td>
</tr>
<tr>
<td>5</td>
<td>ALDRL2</td>
<td>AAH92369</td>
<td>No tissue type</td>
<td>325 aa</td>
<td>[14—17]</td>
</tr>
<tr>
<td>6</td>
<td>ANGPT2</td>
<td>AAJ26203</td>
<td>Colon, PCR rescued clones</td>
<td>496 aa</td>
<td>[38]</td>
</tr>
<tr>
<td>7</td>
<td>AOC3</td>
<td>AAH50549</td>
<td>Peripheral nervous system, sympathetic trunk</td>
<td>763 aa</td>
<td>[39]</td>
</tr>
<tr>
<td>8</td>
<td>CORT</td>
<td>AAH40034</td>
<td>Brain, adult, six pooled whole brains</td>
<td>122 aa</td>
<td>[40]</td>
</tr>
<tr>
<td>9</td>
<td>CRP</td>
<td>NP_000558</td>
<td>LIVER</td>
<td>224 aa</td>
<td>[41]</td>
</tr>
<tr>
<td>10</td>
<td>CTGF</td>
<td>AAH87839</td>
<td>Peripheral nervous system, dorsal root ganglion</td>
<td>349 aa</td>
<td>[42—45]</td>
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<tr>
<td>11</td>
<td>GCNT1</td>
<td>AAJ09103</td>
<td>PCR rescued clones</td>
<td>428 aa</td>
<td>[46]</td>
</tr>
<tr>
<td>12</td>
<td>HGF</td>
<td>AAJ30285</td>
<td>Brain, cerebellum, PCR rescued clones</td>
<td>728 aa</td>
<td>[47,48]</td>
</tr>
<tr>
<td>13</td>
<td>IGF1</td>
<td>NP_000609</td>
<td>—No tissue type—</td>
<td>153 aa</td>
<td>[49,50]</td>
</tr>
<tr>
<td>14</td>
<td>ITGA2</td>
<td>AAH34795</td>
<td>—No tissue type—</td>
<td>1181 aa</td>
<td>[51,5]</td>
</tr>
<tr>
<td>15</td>
<td>MTHFR</td>
<td>AAH18766</td>
<td>Eye, normal, pigmented retinal epithelium</td>
<td>73 aa</td>
<td>[52]</td>
</tr>
<tr>
<td>16</td>
<td>NOS2A</td>
<td>AAJ30284</td>
<td>Pool, cerebellum, kidney, placenta, testis, lung, colon, liver, heart, thyroid, bladder, uterus, PCR rescued clones</td>
<td>1153 aa</td>
<td>[53,54]</td>
</tr>
<tr>
<td>17</td>
<td>NOS3</td>
<td>AAH63294</td>
<td>Placenta, normal</td>
<td>1203 aa</td>
<td>[57,58]</td>
</tr>
<tr>
<td>18</td>
<td>PGF</td>
<td>AAH01422</td>
<td>Placenta, chorloidacarcinoma</td>
<td>170 aa</td>
<td>[46,59]</td>
</tr>
<tr>
<td>19</td>
<td>PRKCB1</td>
<td>AAH36472</td>
<td>Brain, hippocampus</td>
<td>673 aa</td>
<td>[60]</td>
</tr>
<tr>
<td>20</td>
<td>RAGE</td>
<td>AAH53536</td>
<td>Brain, lung, testis, adult, pooled whole</td>
<td>231 aa</td>
<td>[61]</td>
</tr>
<tr>
<td>21</td>
<td>SST</td>
<td>AAH32625</td>
<td>Brain, fetal, whole pooled</td>
<td>116 aa</td>
<td>[62]</td>
</tr>
<tr>
<td>22</td>
<td>TFGA</td>
<td>AAH61159</td>
<td>Renal carcinoma</td>
<td>160 aa</td>
<td>[44]</td>
</tr>
<tr>
<td>23</td>
<td>TIMP2</td>
<td>AAH75186</td>
<td>Placenta, pre-eclamptic</td>
<td>220 aa</td>
<td>[63,64]</td>
</tr>
<tr>
<td>24</td>
<td>TNC</td>
<td>CAI15101</td>
<td>—No tissue type—</td>
<td>2201 aa</td>
<td>[65]</td>
</tr>
<tr>
<td>25</td>
<td>TNF</td>
<td>BAE78639</td>
<td>Peripheral blood leukocyte</td>
<td>233 aa</td>
<td>[66]</td>
</tr>
<tr>
<td>26</td>
<td>VDR</td>
<td>AAH33465</td>
<td>Brain, lung, testis, adult, pooled whole</td>
<td>473 aa</td>
<td>[67]</td>
</tr>
<tr>
<td>27</td>
<td>VEGF</td>
<td>CAI19965</td>
<td>—No tissue type—</td>
<td>191 aa</td>
<td>[68,69]</td>
</tr>
</tbody>
</table>

Figure 1: Phylogram constructed using Neighbor-Joining Algorithm in bioinformatics approach.

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sorbitol accumulation that produces osmotic stress leading to loss of cellular integrity and function [10]. This is supported by the observation that increase in the prevalence of diabetic retinopathy is positively associated with an increase in enhanced erythrocyte aldose reductase levels in patients with type 2 diabetes [16]. Several reports suggested that aldose reductase gene polymorphism is associated with development of diabetic retinopathy [12,17–19]. Although treatment with aldose reductase inhibitors has been shown to prevent tissue injury in animal models of diabetes, the clinical efficacy of these drugs remains to be established. Recent studies revealed that glucose might be an incidental substrate of aldose reductase, which appears to be more adept in catalyzing the reduction of a wide range of aldehydes generated from lipid peroxidation. Inhibition of aldose reductase enzyme has been shown to increase inflammation-induced vascular oxidative stress and prevent myocardial protection associated with the late phase of ischemic preconditioning. These studies indicate that aldose reductase enzyme has potent antioxidant actions. Furthermore, aldose reductase is a critical component of intracellular signaling, and its inhibition prevents high glucose-, cytokine-, or growth factor-induced activation of PKC and nuclear factor-kappa-binding (NF-kB) protein. Thus, it is anticipated that aldose reductase inhibitors prevent vascular smooth muscle cell growth and endothelial cell apoptosis and inflammation and thus, aid in the prevention or arrest of retinopathy in type 2 diabetes mellitus. It is likely that the antioxidant and signaling roles of aldose reductase are interlinked and that aldose reductase regulates PKC and NF-kB via redox-sensitive mechanisms [20]. These results emphasize the need for development of drugs that selectively inhibit aldose reductase-mediated glucose metabolism and signaling, without affecting aldehyde detoxification in the prevention of inflammation associated with the development of diabetic retinopathy. When the relationship between increased aldose reductase activity and abnormal endothelium-dependent relaxation was examined in experimental animals with alloxan-induced diabetes, it was noted that basal and acetylcholine-stimulated levels of cyclic GMP and the relaxations in response to an endothelium-independent vasodilator, sodium nitroprusside, were not significantly different between diabetic and normal rabbits, indicating that nitric oxide release and action on the vascular smooth muscle were unchanged. The release of thromboxane A2 from diabetic vessels was found to be increased, whereas aldose reductase inhibitor, zopolrestat, normalized the elevated red blood cell sorbitol levels in diabetic rabbits, and restored the abnormal acetylcholine- and adenosine diphosphate-induced relaxations of the aorta. On the other hand, aldose reductase inhibitor had no effect on the levels of cyclic GMP or on the increased release of thromboxane A2 in diabetic aorta. These findings suggest that increased activity of the aldose reductase pathway in hyperglycemia is responsible for the abnormal endothelium-dependent relaxation in diabetic blood vessels [21]. In this context, it is interesting to note that aldose reductase and nitric oxide synthase (NOS) share NADPH as an obligate cofactor, suggesting that enhanced glucose flux by aldose reductase inhibited NO production by blunting NOS activity [22]. However, aldose reductase inhibitors prevented the inhibition of NO production, implying that aldose reductase inhibitors decrease glucose-mediated inhibition of NO production and thus, ameliorate endothelial function associated with diabetes.

Human umbilical vein endothelial cells (HUVeCs) cultured in the presence of a high concentration of glucose (27.8 mM for 48 h) increased neutrophil-endothelial cell adhesion and surface expression of intercellular adhesion molecule-1 (ICAM-1), P-selectin, and E-selectin on endothelial cells, which was significantly inhibited by epalrestat, an aldose reductase inhibitor, while NO synthase (NOS) inhibitors reduced the inhibitory effects of this compound. In contrast, phorbol 12-myristate 13-acetate (PMA), a PKC activator, showed similar effects as high glucose, and these effects were also inhibited by epalrestat, suggesting that aldose reductase inhibitors inhibit high glucose-mediated neutrophil-endothelial cell adhesion and expression of endothelial adhesion molecules not only through inhibition of a PKC-dependent pathway, but also through increased endothelial NO production [23]. These results are supported by the observation that exposure of cultured retinal endothelial cells to high glucose levels and osmotic stress similar to those in diabetic patients increased the formation of nitrotyrosine by increasing NOS activity and causing superoxide formation due to eNOS uncoupling and aldose reductase activation [24]. Thus, there is a close interaction between aldose reductase activity and eNOS generation and the development of diabetic retinopathy. These results also suggest that increase in eNOS generation is a compensatory phenomena in response to enhanced oxidative stress induced by hyperglycemia since inhibiting NOS or aldose reductase, scavenging superoxide or peroxynitrite, or supplementing the NOS substrate l-arginine or cofactor tetrahydrobiopterin blocked the formation of reactive oxygen.
species and prevented protein tyrosine nitration [24]. This is supported by the observation that increased flux of glucose through the polyol pathway is involved in the pathophysiology of secondary diabetic complications, and the first step of this pathway, which generates sorbitol from glucose, is catalyzed by aldose reductase. In vitro, the binding of substrates and inhibitors to aldose reductase is highly sensitive to the oxidation state of the enzyme due to the presence of a hyper-reactive cysteine residue (Cys-298) at the active site of the enzyme that can be readily modified by thiol-modifying reagents, nitric oxide (NO) donors and nitrosothiols. Studies revealed that exposure of erythrocytes to NO donors inhibited aldose reductase activity and aldose reductase-mediated accumulation of sorbitol, suggesting that NO regulates the cellular activity of aldose reductase and, in turn, the flux of glucose via the polyol pathway. This inhibition of aldose reductase by exogenous or endogenous NO is related to reversible S-glutathiolation of the aldose reductase protein [25]. Since, hyperglycemia is associated with a decrease in NO generation [26,27], the loss of NO-mediated repression of aldose reductase is a significant factor in the activation of the polyol pathway and the development of diabetic retinopathy and other diabetic complications. These results also suggest that NO is a physiological regulator of aldose reductase. Furthermore, it was reported that NOS inhibitor, N(G)-nitro-L-arginine methyl ester (l-NAME), increased sorbitol accumulation, whereas treatment with l-arginine (a precursor of NO) or nitroglycerine patches prevented sorbitol accumulation. When incubated ex vivo with high glucose, sorbitol accumulation was increased by l-NAME and prevented by L-arginine in wild type, but not eNOS-deficient, mice. Exposure to NO donors also inhibited aldose reductase and prevented sorbitol accumulation in rat aortic vascular smooth muscle cells (VSMC) in culture [25,28]. These observations suggest that NO regulates the vascular synthesis of polyols by S-thiolating aldose reductase and, therefore, increasing NO synthesis or bioavailability may be useful in preventing diabetes-induced changes in the polyol pathway and secondary complications due to diabetes mellitus.

Previous studies also suggested a link between aldose reductase and VEGF. It was noted that enhanced aldose reductase activity triggers retinal oxidative stress and VEGF protein expression, events that could be prevented by aldose reductase inhibitor [29]. Impaired eNOS activity seen in diabetes causes vasospasm that results in hypoxia. The resultant hypoxia augments VEGF synthesis that could induce increase in vascular permeability. Impaired basal NO production facilitates leukocyte adhesion to the endothelium, which would result in the breakdown of the blood retinal barrier (BRB) leading to capillary non-perfusion. Alternatively, impaired eNOS activity may directly increase microvascular permeability [14].

Figure 2 Scheme showing the role of various factors involved in the pathogenesis of diabetic retinopathy and their interaction(s).

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Based on the results of the previous studies and the Phylogram constructed using Neighbor-Joining Algorithm using bioinformatics approach of the present study, it is suggested that hyperglycemia induces increase in the activity of the enzyme aldose reductase that, in turn, triggers a series of events leading to enhanced expression of iNOS, VEGF, PIGF (placental growth factor) and free radicals and activation of PKC. These molecular cause endothelial cell migration and replication (early steps in angiogenesis), enhance retinal vascular permeability that ultimately lead to the onset of diabetic retinopathy. AGE–RAGE interaction also elicits angiogenesis through the transcriptional activation of the VEGF gene via NF-κB [30] that can be prevented by aldose reductase inhibitors. This suggests that interaction between aldose reductase and VEGF is crucial in the pathogenesis of diabetic retinopathy. VEGF, in turn, works in concert with angiopoietins, a set of growth factors that modulate physiological angiogenesis and pathological neovascularization, particularly in association with VEGF [31]. Furthermore, adhesion of leukocytes to the retinal vasculature is one of the earliest events in the experimental diabetes and results in blood retinal breakdown, endothelial cell damage and capillary non-reperfusion. ICAM-1 and other adhesion molecules are up-regulated during diabetic retinopathy and VEGF drive the up-regulation of retinal ICAM-1, mostly via NO and NF-κB dependent pathways.

Conclusion

It is evident from the preceding discussion that diabetic retinopathy is a complex process in which several cytokines, growth factors, and free radicals play a significant role. In general, it is likely that hyperglycemia causes an increase in the activity of the enzyme aldose reductase that, in turn, triggers a series of events leading to enhanced expression of iNOS, VEGF, PIGF, and free radicals. However, clinical trials of the aldose reductase inhibitors were disappointing, whereas VEGF antagonists showed limited beneficial actions suggesting that changes in the concentrations and activities of other proteins and enzymes such as endothelial nitric oxide synthase and various growth factors also play a significant role in the pathogenesis of diabetic retinopathy. Consistent with this observation, the present bioinformatics study suggests a close association exists between aldose reductase, VEGF, NOS, PIGF, AGE–RAGE, angiopoietins, and cytokines in the pathogenesis of diabetic retinopathy (Fig. 2) and hence, a multi-pronged approach is needed to tackle this condition. Hence, development of drugs that inhibit aldose reductase and simultaneously enhance eNOS activity will be useful in the prevention and treatment of diabetic retinopathy and other complications of diabetes mellitus.

Competing interests

The author(s) declared that they have no competing interests.

Acknowledgements

A.A.R., Thota participated in the design of the study, interpretation of the results and prepared manuscript in bioinformatics aspects. G.R.S., U.N.D., A.A. participated in the design of study, interpretation of the results and prepared manuscript. S.B.C., S.R.C., S.P.A., T.P., D.C. and S.K. participated in the study, performed bioinformatics aspects and participated in the preparation of manuscript. All authors read and approved the final manuscript.

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Alzheimer’s disease care and management: Role of information technology

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Abstract
Alzheimer’s disease (AD) an ailment that is supposed to affect people in old age. There are evidences that it might affect others also. The number of elders is increasing as the average life expectancy is increasing. AD afflicts its patients with the dementia and AD might increase in malignance over time. People with cognitive disabilities can be overwhelmed through cognitive prosthetics. With the help of Information technology we can enhance the quality of life. Significant achievements are possible with an interdisciplinary approach that includes genomic, genetic, technological and therapeutic measures. The combination and coordination of Bioinformatics facilitates generation of various diagnostic tools for the people who are suffering from Alzheimer’s disease. These tools help the care providers also. In this article, we emphasize the literature regarding the use of technology and its methodologies to improve the quality of care for the people with Alzheimer’s disease.

Key words: information technology; bioinformatics; alzheimer’s disease; electronic medical records

Background:
Alzheimer’s disease (AD) was first discovered in 1907 by German neurologist Alois Alzheimer. [1] Alzheimer’s disease becomes one of the major hurdles for further survival of elders thereby a many number of people may suffer from AD in the next few decades. Molecular genetics reached human genetics about 1976, when the first human genes were cloned [2] Transgenic methods, ‘knock-outs’ and ‘knock-ins’ began in about 1986, and in about 1996, database searching became a fruitful way to do genomic research [3] The term ‘genome’ refers to an organism’s complete set of genes and chromosomes. The term ‘genomics’ describes the scientific discipline of mapping, sequencing, and analysing genomes. [4]

The fact that most diseases do not follow a simple inheritance patterns has led to a significant challenge in the genetic dissection of the complex traits of diseases such as hypertension, Alzheimer’s disease, schizophrenia and diabetes. Alzheimer’s (AD) afflicts its patients with a dementia that increases in malignance over time: the older an AD patient is, the worse the dementia is. Dementia is a result of the loss of neurons in the brain that assist in engagement of intellectual activities. The loss of neurons specifically affects the hippocampus, which is a central region for memory operation, and the cerebral cortex. The cerebral cortex is also involved in memory functions, but also works to accomplish reasoning and language functions. A big difference between a normal brain and a brain afflicted with AD is the presence of protein clusters inside and between neurons. The clusters inside are known as neurofibrillary tangles, which consist of a protein named tau. Another type of protein known as beta-amyloid is the protein that exists between neurons. While the presence of tau seems to be proportionate to the degree of dementia experienced by the patient, indicating a possible connection to the cause of AD, it is not unique to AD the way beta-amyloid plaques are in their unique concentrations. These beta-amyloid proteins that cluster between neurons and are accompanied by the immune system’s microglia, reactive inflammatory cells that are thought to remove already damaged neurons and/or the amyloid plaques themselves. [1] They originate from the beta-amyloid precursor protein (bAPP) when a bAPP fragment that is 99 amino acids is cut by gamma-secretase, creating a beta-amyloid peptide, while the amyloid plaques themselves are present in most old people, the high concentration of them in the hippocampus and cerebral cortex in AD patients suggests a role in the neuronal degenerative process.

Memory loss is the most common and well known symptom for Alzheimer’s disease. Other symptoms include loss of cognitive abilities, judgment, thinking and disorientation to place and time. Identification loss, depression, confusion, anxiety, fear, frustration, paranoia are also symptoms for Alzheimer’s disease. The above symptoms may have different effects on different people. Currently medicines that are available for Alzheimer’s disease slow down its progression or help control the
symptoms such as anxiety or sleeplessness. However, there is no available cure for Alzheimer's disease. While the curative approach is certainly crucial to combating the effects of AD, one avenue we might consider looking down is a focus on supplemental measures.

The development of new technologies that could help AD patients cope with loss of mental function might be appropriate, given the nature of the ailment. Developments in information technology could offer assistance to AD patients in a way that could supplement the loss of biological function with mechanical functions. For example, a computer could be used to keep records of family members to help remind the patient about his or her past. While a desktop PC seems somewhat impractical for this, a computer small enough to fit into someone's eyeglasses, coupled with voice and image recognition technology, could provide AD patients with the kinds of information they need to continue to function. This, along with drugs to at least slow the process, could provide a treatment that could restore a quality of life to the patient in a way that is currently unavailable.

A substantial number of the IMI patients reflected regional hypometabolism similar to AD, suggesting that IMI is likely an early stage in progressive dementia. A large percentage of IMI patients converted clinically to AD within three years of initial study, though they observed impaired memory functioning well before a clinical diagnosis of AD could be made. In addition to potential clinical utility, IMI and PET represent an opportunity to study dementia in relation to brain chemistry at a time when brain pathology is in the process of development.

Genomics of Alzheimer's disease

Genome analysis may be divided into structural and functional genomics. Structural genomics is an initial phase of genome analysis, and has a clear end point which is the construction of high-resolution genetic, physical, and transcript maps of an organism (its complete DNA sequence). This genotypic approach focuses on understanding how genotypic variation gives rise to phenotypic variation, relying on physical and genetic maps and easily-typed DNA sequence polymorphisms. The expression approach (functional genomics) relies on the large collection of partially sequenced cDNA clones. The benefits of the information arising from the accumulation of human gene sequences includes developing systematic ways of finding genes of interest, and their functions; hence 'functional genomics'. The genes cloned and their corresponding DNA sequences provide the tools for comprehensive characterization of the expression patterns of this entire set of genes, and for systematic experimental investigations of the functional properties of their products. Thus, functional genomics, which represents a new phase of genome analysis, makes use of the structural genomics information. The investigation is primarily a systematic approach to elucidate the genome and its functions.

Pathologically, Alzheimer's disease (AD) is associated with generalized degeneration of the cerebral cortical and hippocampal neurons. Cholinergic neurons in the basal forebrain which project to cortex and hippocampus appear to be particularly vulnerable, and to an extent, so are serotonergic and noradrenergic afferents to cortical regions. The extracellular deposition of peptide fragments (amyloid-beta) from the larger membrane precursor protein (APP) is typical in affected brain tissue. Intracellular accumulations of tau-proteins (tangles) are present in many cortical and cortico-limbic regions. [5]

Genetic studies have led to the identification of three genes in which mutations can cause AD: the $\beta$-amyloid precursor protein gene located on chromosome 21, presenilin 1 (PS1) located on chromosome 14 and presenilin 2 (PS2) located on chromosome 1. [6, 7, 8] In addition, the E4 allele of the apolipoprotein E (ApoE) gene is a risk factor for AD. While mutations associated with APP are extremely rare, the 50 or so mutations associated with PS1 may explain up to half of all cases of early-onset AD. A study which investigated the association of two candidate genes (PS1 and $\alpha$-antichymotrypsin (ACT)) with the risk of sporadic Alzheimer's disease on chromosome 14 reported that the frequency of the ACT*A allele was significantly higher in AD patients than in controls and the stratification of the ACT data by PS1 genotypes showed that the risk associated with the ACT*A allele was confined to PS1*1 carriers only. [9]

The two-site haplotype data for PS1 and ACT indicated that the A1 haplotype, carrying the ACT*A and PS1 alleles, was more frequent in Alzheimer's disease patients, and these results may also suggest that there is a possible synergistic effect of these two loci on the risk of AD. In contrast to early-onset AD, there is to date only one genetic factor indisputably linked with late-onset forms of this disorder; the E4 allele of apolipoprotein E. [10] Differences in ApoE genotyping appear to explain differences in patients' responses to drug therapy. With tacrine, a better response was seen in patients with the ApoE E2 or ApoE E3 allele than in those carrying the ApoE E4. The ApoE E4 allele has an inverse relationship with residual brain choline acetyltransferase (the acetyl-choline synthesizing enzyme) activity, and it appears that patients with this genotype may not have sufficient acetylcholine to benefit from a drug which acts as an inhibitor of acetylcholinesterase. However, patients with the ApoE E4 genotype appear to have a better response than other AD patients to treatment with another drug, Servier's S12024 (morpholinyl-2 methoxyl-8 tetrahydro-1, 2, 3, 4 quinoline) which is currently in phase II clinical trials. In fact, this drug had no detectable effect in patients with the other ApoE genotypes. S12024 does not appear to affect the cholinergic system, but rather to facilitate brain noradrenergic and vasopressinergic activity, and increases vasopressin synthesis and release in a dose-dependent manner. There may be a balance between cholinomimetic and vasopressinergic pathways, according to ApoE E4 allele presence or absence. [11] With relevance
to design of clinical trials, the important observation may be that alleles that appear to be conclusively associated with a therapeutically relevant phenotype can be used to select a subgroup of patients for clinical trials.

A polymorphic site need not be part of the target for the drug; it only needs to be associated with a response to the treatment. In responsive patients, the selective treatment could be more effective, and associated with fewer or less severe side effects. Furthermore, pre-emptive genotyping aimed at drug-associated genes could mean that fewer drug candidates would fail to reach the market place because of poor toxicity/efficacy profiles in the general population. For example, genotyping of early-onset AD is likely to include the two PS1 and PS2 genes involved in this disease. Predictive and diagnostic tests for PS1 mutations and diagnostic tests for ApoE alleles are already commercially available and other tests are being developed. Thus, genetic testing for AD exists for clinical use, and is likely to be used more often to stratify patients in Alzheimer's disease research, both in trials of preventive products and in tests of new pharmacological treatments. Therefore, predictive and diagnostic genetic testing for these highly penetrant mutations such as PS1 or PS2 may be appropriate for adults from families with a clear autosomal dominant pattern or inheritance, particularly those with a family history of early onset of symptoms. Testing is an option that could be discussed and that could reasonably be accepted or declined by the patients. However, the application of the ApoE test raises concerns, because although the E4 allele is associated with an increased risk of AD, its predictive value for individuals is quite limited. The small increase in diagnostic confidence provided by ApoE genotyping does not justify the burden of testing: such testing may have value in AD research, but its widespread clinical use is premature until practical benefits outweigh its costs.

Information technology-Alzheimer's disease:
Information technology role on Alzheimer's disease has already begun. In 2003, Intel entered into a consortium with the Alzheimer's association, granting $1 million in Information technology research to be directed towards AD patients. Technology such as sensor networks is being used to study the habits of Alzheimer's disease patient behavior in hopes of finding ways to learn more about AD and to make it more livable. This is an example of how Information technology can work for AD patients. Scientist Hans Moravec has suggested that someday, entire human brains and the consciousnesses they hold will be able to be downloaded into a computer. This would certainly avoid the problem of neuronal deterioration, but it's possible that by the time, we have the technology to move minds into machines, we will know enough about AD to make it a livable or curable illness.

The early stage of AD primarily causes memory problems. At this stage a person can live independently and only requires assistance in remembering certain tasks. Cognitive prosthetics are helpful in aiding a person to remember such tasks. A pager was used to help a person remember the tasks during the early stage of AD. The Pager was able to record 80 letter alphanumeric message and then display the message at scheduled time. The person was able to perform the tasks independently within a week by using the pager.

The functional memory of the person had improved after using the pager for six months.

The majority of people with AD usually fit into the category of mild to moderate disease progression. At this stage it is common for patients with AD to move-in with a caregiver. During these stages, persons with AD are prone to forgetting their way home or wandering in the streets. The use of technology for people with AD provides hope that they can live on their own for longer period. Global Positioning System (GPS) is the technology that may prove to be very helpful in these stages. GPS is a tracking device that can be used to identify the location of the patients. A patient can wear a GPS unit and a caregiver can be notified when the patient wanders out of the designated area. Currently GPS devices used to locate Alzheimer’s patients are being used with personal locater devices.

During the moderate to late stages of AD, behavioral disturbances need for more care. At this stage, notes can be programmed to inform caregiver of all the conditions of the patient. Sensors could be placed in the person’s bed to monitor weight loss. A combination of sensors placed in chairs and infrared tags detected by cameras could inform the caregiver, if the AD patient has fallen down or is sitting in the chair.

Information technology can be used to help the caregivers with the responsibilities of monitoring the AD patients as well as informing the caregivers about AD and answering their questions. For example, a telephone-based intervention has been used to provide help and support to caregivers. The Project is known as REACH (Resource for Enhancing Caregiver Health) used an interactive voice response (IVR) to provide support and answers to caregivers. It is necessary for the caregiver to receive the support they need in order to perform their job easily. The World Wide Web can easily solve the problem by providing knowledge and support to the caregivers. They can get the validity of online information from the online service called Alzonline.net. [12]

Healthcare providers and caregivers are responsible for constantly monitoring the patient’s condition. It is not possible for many caregivers to constantly monitor the patient’s health because of lack of medical knowledge or equipment. [13] An important part of care giving consists of taking the patients to the physician, this can add further stress to the care giver and affect the accuracy of physician’s judgment about the patient’s condition. A healthcare provider might have a better idea regarding a patient’s condition if they could monitor the patient in a natural setting. A telehealth system could be used in order
for the healthcare provider to monitor the patients in their home setting.

Telehealth technology can be useful for rural caregivers. They need not drive long distances. Telehealth application can be used to monitor patients at home and eliminate unnecessary travel. A number of telehealth applications have been used to monitor the patients. Information technology can play a very important role to improve the condition of the people with Alzheimer’s disease. It can provide a caregiver with information and support. On the other hand it can engage the patients in many different activities to reduce the caregivers stress. A combination of telemedicine, telecommunication projects and technologies for daily living can help us with the aging of our elders the medical conditions they will face.

Electronic Medical Records
The main purpose of clinical care and research is to discern patterns and modify treatment according to changing parameters, be they weight, blood pressure, plasma glucose or serum lipids. Records on paper can be scanned and browsed easily but the data may not be recorded in a uniform fashion, papers may be lost, misplaced or become complex. The relevant paper records cannot easily be sorted and they cannot be accessed across different locations. [14] Electronic medical records have advantages over paper records, viz: complete and comprehensive flexibility in storage and retrieval of data, which can be used for publication, presentation and research. Computerized guidelines can provide evidence-based recommendations by allowing access to references, showing errors and sending reminders. [15] Besides, interactive telemedicine support is possible. Information laws of the country can be expected to consider EMR as a legal document. They may be electronically signed and must be permanent. An identifier must be attached to any further modifications.

Bioinformatics-Biological Databases
Bioinformatics is the science of using and developing computational tools and algorithms to help in solve different biological problems. These problems include similarity searches of unknown DNA/protein sequences and the prediction of protein structure and function. Bioinformatics can be useful in finding the causative genes for Alzheimer’s disease. The genome information can be obtained from existing biological databanks to analyze their structure and function. Therefore Biological databases plays important role in bioinformatics. There are around 500 public and commercial biological databases. These databases usually contain genomics and proteomics data, but databases are also used in taxonomy. The data are nucleotide sequences of genes or amino acid sequences of proteins. Furthermore information about function, structure, localization on chromosome, clinical effects of mutations as well as similarities of biological effects can be found. Biological databases have become an important tool in assisting scientists to understand and explain a host of biological phenomena from the structure of biomolecules and their interaction, to the whole metabolism of organisms and to understanding the evolution of species. This knowledge helps facilitate the fight against diseases, assists in the development of medications and in discovering basic relationships amongst species in the history of life. The biological knowledge of databases is usually (locally) distributed amongst many different specialized databases. This makes it difficult to ensure the consistency of information, which sometimes leads to low data quality. We can extract the genes that are causing AD from gene cards using bioinformatics.

Computer Applications for Clinical Questions
Although prospective learning will always be important for health care professionals, it is not possible to prospectively obtain all of the knowledge required to appropriately treat all patients. Therefore, clinicians must be able to access knowledge at the point of care that answers clinical questions and provides diagnostic and therapeutic decision support. A number of available computer applications address this need. Three of these are highlighted here. Many Internet sites can provide valuable information to clinicians, and the applications can be accessed on the Internet using the websites like www.UpToDate.com, www.infoapoems.com, etc.

Conclusion:
Information technology plays a vital role in identification of the genes that are causing AD, disease management, progression and online data collection using electronic medical records for future research. The use of technology has the potential to help the patients to be more independent and reduce stress on the caregiver. The use of Information technology is might be worth pursuing if technology advances faster than a treatment or cure.

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Short paper

Alzheimer's disease and Type 2 diabetes mellitus: the cholinesterase connection?

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Abstract

Alzheimer's disease and type 2 diabetes mellitus tend to occur together. We sought to identify protein(s) common to both conditions that could suggest a possible unifying pathogenic role. Using human neuronal butyrylcholinesterase (AAH08396.1) as the reference protein we used BLAST Tool for protein to protein comparison in humans. We found three groups of sequences among a series of 12, with an E-value between 0-12, common to both Alzheimer's disease and diabetes: butyrylcholinesterase precursor K allele (NP_000046.1), acetylcholinesterase isoform E4-E6 precursor (NP_000656.1), and apoptosis-related acetylcholinesterase (IB41|A).

Butyrylcholinesterase and acetylcholinesterase related proteins were found common to both Alzheimer's disease and diabetes; they may play an etiological role via influencing insulin resistance and lipid metabolism.

Background

Alzheimer's disease and type 2 diabetes mellitus occur with increasing frequency as age advances. Besides, the development of one increases the risk of the other [1]. Epidemiological studies have shown an association of diabetes mellitus and Alzheimer's disease. A population-based historical cohort study estimated that the risk of Alzheimer's disease increased with adult onset diabetes mellitus [2]. A longitudinal study of 1,262 elderly subjects without dementia at baseline, adjusted relative risk of Alzheimer's disease among persons with diabetes was 1.3 [95% CI: 0.8, 1.9], [3]. In a more recent community-based study among 1301 dementia-free persons aged 75 and above, diabetes mellitus was associated with subsequent development of Alzheimer's disease [4]. Similarly patients with Alzheimer's disease were more vulnerable to developing impaired fasting glucose and type 2 diabetes mellitus [5]. A variety of mechanisms has been postulated in the risk of Alzheimer's disease and type 2 diabetes mellitus: metabolic abnormalities of insulin resistance (dyslipidemia, hypertension), hyperglycemia per se or insulin, by disturbing synaptic plasticity, learning and memory [6].

The enzyme butyrylcholinesterase (EC 3.1.1.1.8) does not have a well-defined physiological function, although it may modulate the phenotypic expression of dyslipidemia and insulin resistance. It is affected by dietary factors, obesity, and dyslipidemia [5,7,8].
While acetylcholinesterase in the brain is chiefly localized to neurons, butyrylcholinesterase is primarily associated with glial cells and endothelial cells [9].

Butyrylcholinesterase was studied in relation to both type 2 diabetes mellitus and Alzheimer's disease in different ethnic groups [10-13].

Despite originating from the endoderm, the pancreas is highly innervated and shares molecular similarities with brain at the level of transcriptome and proteome [14]. Localized and progressive amyloidosis is characteristic of both type 2 diabetes and Alzheimer's disease. Neurofibrillary tangles, the manifestations of pancreatic islet neurodegeneration have not been as extensively studied in diabetes mellitus as in Alzheimer's disease. Cytotoxic effects of fibril in both conditions involve an interaction of cell membranes with mis-folded insoluble peptides [15]. The two may therefore be mediated by common regulatory elements.

Considering that butyrylcholinesterase was associated with both type 2 diabetes mellitus and with Alzheimer's disease, and that leads existed for the α role of butyrylcholinesterase in their pathogeneses, we chose it as the reference protein. In addition, genetic variants of the enzyme exist, which may play a role in biological fitness of individuals [16]. Identification of such sequences would provide leads for further understanding etiological, therapeutic or prognostic aspects [17-20].

Data was retrieved from National Centre for Biotechnology Information (NCBI). We took the amino acid sequence of human neuronal butyrylcholinesterase (AAH08396.1) as the reference protein and copied it to Word programme in FASTA format. We used BLAST tool in NCBI, and performed protein to protein comparison in humans from the available databases. Taking E-value between 0–12, we obtained 12 sequences that were common to both Alzheimer's disease and diabetes mellitus (Table 1). The E-value refers to the probability due to chance, that there is another alignment with a similarity greater than the given similarity score. In general, An E-e< sub>5 of an alignment means that that alignment is highly unique, and not due to error, whereas an E ≥ e<sub>-6 means that the alignment might be strong, but more research is needed to verify. The lower the E value, the more significant the score.

The following sequences were obtained (Table 1):

Essentially the sequences may be grouped into butyrylcholinesterase precursor K allele (NP_000046.1), acetylcholinesterase isofrom E4-E6 precursor (NP_000656.1), and apoptosis-related acetylcholinesterase (1B41|A).

Dementia and diabetes increase with age. This is related to the occurrence of atherosclerosis, advanced glycation end product, oxidative stress and the deposit of amyloid. [1,5]. Neurofibrillary tangles are deposited in brain of Alzheimer's disease, along with senile plaques, which also contain extracellular deposits of human amyloid beta peptide. Amyloid deposits, consisting of elongated unbranched fibrils that bind to Congo Red dye, consist of among others, acetylcholinesterase and apolipoprotein E [21]. Amyloid fibrils are also seen in pancreatic beta cells in type 2 diabetes mellitus [22]. Association of butyrylcholinesterase with Alzheimer's disease showed conflicting results: some showed positive associations of BChE polymorphism acting in synergy with APOE 4 [10], others no significant differences while yet others protection among women. The rationale for looking at the association is that BChE gene codes for butyrylcholinesterase, which is found in amyloid plaque [23]. BChE acts in concert with apolipoprotein E which are both astrocyte pro-

### Table 1: Sequences common to Alzheimer's Disease and Diabetes mellitus

<table>
<thead>
<tr>
<th>SeqA Name</th>
<th>Len(aa)</th>
<th>SeqB Name</th>
<th>Len(aa)</th>
<th>E-Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAH08396.1</td>
<td>64</td>
<td>CHLE_HUMAN</td>
<td>602</td>
<td>4e-34</td>
</tr>
<tr>
<td>2 AAH08396.1</td>
<td>64</td>
<td>NP_000046.1</td>
<td>602</td>
<td>4e-34</td>
</tr>
<tr>
<td>3 AAH08396.1</td>
<td>64</td>
<td>NP_000656.1</td>
<td>614</td>
<td>1e-16</td>
</tr>
<tr>
<td>4 AAH08396.1</td>
<td>64</td>
<td>BAD97163.1</td>
<td>614</td>
<td>1e-16</td>
</tr>
<tr>
<td>5 AAH08396.1</td>
<td>64</td>
<td>IF8UJA</td>
<td>583</td>
<td>2e-16</td>
</tr>
<tr>
<td>6 AAH08396.1</td>
<td>64</td>
<td>AAO32948.1</td>
<td>526</td>
<td>3e-16</td>
</tr>
<tr>
<td>7 AAH08396.1</td>
<td>64</td>
<td>AAH94752.1</td>
<td>640</td>
<td>2e-15</td>
</tr>
<tr>
<td>8 AAH08396.1</td>
<td>64</td>
<td>AAKZ10033.1</td>
<td>94</td>
<td>6e-13</td>
</tr>
<tr>
<td>9 AAH08396.1</td>
<td>64</td>
<td>IPOQJA</td>
<td>529</td>
<td>3e-05</td>
</tr>
<tr>
<td>10 AAH08396.1</td>
<td>64</td>
<td>IVZJH</td>
<td>40</td>
<td>0.001</td>
</tr>
<tr>
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<td>64</td>
<td>AAIU3801.1</td>
<td>617</td>
<td>0.14</td>
</tr>
<tr>
<td>12 AAH08396.1</td>
<td>64</td>
<td>1B41</td>
<td>A</td>
<td>539</td>
</tr>
</tbody>
</table>

* The E-value refers to the probability due to chance, that there is another alignment with a similarity greater than the given similarity score.
proteins that interact with lipoproteins after being released into the circulation by the liver.

The variable association of BchE and variants with Alzheimer's disease [10,23-25] was attributed to ethnic differences in frequency of BchE variants, perhaps due to hidden population admixture, or of variant BchE being in linkage disequilibrium with an as yet unidentified AD susceptibility gene in some populations [12].

Studies have linked BchE with the pathogenesis of Alzheimer's disease and diabetes mellitus. BchE may modify the risk of Alzheimer's disease either alone, or in synergy with apoE-epsilon 4 [13]. Its active involvement is also suggested by plaque area of demented brains having higher BchE, and by its localization in neurofibrillary tangles, a pathological hallmark of Alzheimer's disease. A recent study showed that BchE attenuates amyloid formation by an interaction of C terminus with soluble species of beta amyloid in a polar environment [26]. It may prolong nucleation phase and reduce propagation phase of fibril formation and suppress amyloid fibril formation enhanced by purified AchE-S [26]. Similarly selective inhibition of BchE in aged rats improved cognitive navigation [9]. In cultured neuroblastoma cells, selective BchE inhibition reduced beta amyloid precursor protein and beta amyloid peptide levels. It is supportive in the hydrolysis of acetylcholinesterase and can partly compensate for the action of AchE. While AchE levels are reduced early in Alzheimer's disease, BchE levels rise with disease; selective BchE inhibition may be useful to ameliorate cholinergic defect. Therefore BchE may have a role in coregulating local concentrations of acetylcholinesterase in Alzheimer's disease.

BchE may be involved in the pathogenesis of type 2 diabetes either by way of amyloid fibrils or by modifying other risk factors of insulin resistance. Amyloid fibrils in pancreatic islets produce excessive superoxide radicals, lipid peroxidation and nitric oxide inactivation, contributing to apoptosis of beta cells [15]. K variant of BchE have propensity for beta sheet formation, which may be related to amyloidogenesis. In addition, a locus on chromosome 3q27, which is close to the chromosomal locus of BchE was linked to type 2 diabetes mellitus in a French population [27].

In addition, increased BchE may predispose aging cells to oxidative stress [16].

Conventional association studies with evaluation of limited phenotypes are laborious, and do not always give unambiguous answers proportionate to the effort incurred [15]. With the availability of nucleotide and amino acid sequences, we performed a bioinformatics approach to study butyrylcholinesterase-related proteins in Alzheimer's disease and in type 2 diabetes mellitus.

Comparative genomic study across species provides a more broad-based picture of proteins in terms of evolution; earlier we showed that butyrylcholinesterase (EC 3.1.1.1.8) and its variants were spread in mammals consistent with their evolutionary space, and that homologous sequences were expressed in other life forms, including plants and bacteria [11,28].

Butyrylcholinesterase (NP_000046.1, Table 1) whose gene is localized to a single autosomal location at 3q26 has extensive homology to other cholinesterases of various species. More than 30 genetic variants of BchE have been reported, and the number is increasing. The occurrence of such preadaptative pharmacogenetic variants was attributed to either balanced polymorphisms or to neutral mutations [29].

A recent in vitro study showed that BchE attenuates amyloid fibril formation [9]. The association of BchE K variant in both Alzheimer's disease [10] and type 2 diabetes mellitus suggests a possible pathogenic role in both diseases.

Acetylcholinesterase (NP_000656.1, Table 1) regulates cholinergic nerve and neuromuscular transmission; the gene encoding this protein is mapped to chromosome 7. Acetylcholinesterase belongs to the family of hydrolases along with butyrylcholinesterase, with which it has 53% sequence homology [17].

Acetylcholinesterase-like proteins were shown to mediate cytoarchitectural changes that support neurotogenesis [30]. It also has a role in human neocortical neuroplastic processes [31].

There is substantial structural and functional evidence linking acetylcholinesterase to Alzheimer's disease: a region near C-terminus is weakly homologous to the N-terminus of amyloid-beta peptide [32]. In a comparative study on neurotoxicity comparison, human amyloid-beta peptide fibrils complexed with acetylcholinesterase showed greater toxicity than amyloid beta peptide fibrils along [21]. Acetylcholinesterase may potentiate both amyloid deposition and the toxicity of such deposits.

A recent study showed that hydrogen peroxide may be a regulator of AchE [33]. The reaction is similar to that which occurs in vitiligous skin, resulting in severe oxidative stress [34]. Similarly, increased membrane AchE activity was attributed to high homocysteine levels, which are correlated with neurological problems [34]. Oxidative stress could result from hyperhomocystinemia, leading to neuronal apoptosis [35] and to insulin resistance [36].
Similar suppression of butyrylcholinesterase has been shown to occur with homocysteine, probably acting via free radicals [35].

*Apoptosis-related acetylcholinesterase* (AAO32948.1) is an alternatively-spliced variant expressed in blood cells, associated with apoptosis. It could be a potential marker and a regulator of apoptosis, being expressed in apoptotic cells. Similarly it could be modulate pro-inflammatory cytokines in macrophages in the periphery, and be differentially expressed by stress in the periphery and in the central nervous system [37].

Insulin signaling abnormalities could be the underlying mechanism affecting the outcome of Alzheimer's disease; insulin resistance and disordered degradation of amyloid seem to link diabetes mellitus with Alzheimer's disease [38]. Insulin dysregulation could act in a variety of ways including decreased cortical glucose utilization, oxidative stress formation of advanced glycated proteins, increased neurofibrillar formation and increased b-amyloid aggregation through inhibition of insulin-degrading enzyme [39]. Insulin resistance could therefore be a link between Alzheimer's disease and type 2 diabetes mellitus [40]. Butyrylcholinesterase may be indirectly involved in the pathogenesis of insulin resistance [41]. It has been hypothesized that peripheral insulin resistance can affect CNS insulin levels, cognition and amyloid beta levels [40]. Peripheral insulin resistance downregulate insulin uptake at the blood brain barrier and lead to CNS insulinoopenia. Since insulin promotes intracellular amyloid beta release and alters expression of insulin degrading enzyme, low brain insulin levels can lead to amyloid beta accumulation in neurons. Peripheral insulin resistance may also inhibit clearance of amyloid beta from the brain to the periphery, either by blocking its transport from the brain or by interference with clearance in peripheral sites. Thus there could be a combination of accumulation of amyloid beta, with decreased clearance, both due to insulin resistance [40].

Antidiabetic drugs could be potentially useful in treating Alzheimer's disease: PPAR gamma agonists, by improving insulin sensitivity, decreasing inflammation and improving cerebral energy metabolism; intranasal insulin, by restoring brain insulin levels in Alzheimer's disease [40].

Another intriguing aspect is the occurrence of variant butyrylcholinesterases with low enzyme activity in certain ethnic populations [16]. It would be instructive to study the prevalence and course of both insulin resistance and Alzheimer's disease in these individuals who may be considered to be on butyrylcholinesterase inhibitors. Such studies are particularly instructive, considering that other forms of treatment such as beta-secretase inhibitors may be associated with potential negative consequences [42]. Individuals with BchE deficiency have been known to survive without any apparent adverse physiological consequences.

In summary, esterase group of enzymes may be an underlying thread in the coexistence of Alzheimer's disease and diabetes mellitus.

At present however, one cannot impute a direct cause-effect relationship among the variables discussed here (Alzheimer's disease, type 2 diabetes mellitus, insulin resistance, butyrylcholinesterase), although there are epidemiological, biochemical, pathological and now computational biological leads pointing to an association. In line with emerging paradigms of *in vivo to in silico biology and back* [43], our results offer direction in the iterative processes that drive biology forward in comprehending biological phenomena.

**Competing interests**

The author(s) declare that they have no competing interests.

**Authors' contributions**

GRS participated in the design of the study, interpretation of the results and prepared the manuscript. TH, SB, SP and DC participated in the design of the study, performed the bioinformatics aspects and participated in the preparation of the manuscript. AAR participated in the design of the study, guided in the bioinformatics aspects, and participated in the interpretation of the results and in the preparation of the manuscript. All authors read and approved the final manuscript.

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