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Type 2 diabetes mellitus (T2DM) is a chronic and progressive disease that is strongly associated with the all-cause and cardiovascular mortality. The present study aimed to analyze the abundance and functionality of intrinsically disordered regions in several biomarkers of insulin resistance, adiponectin, and endothelial dysfunction found in the T2DM patients. In fact, in comparison to controls, obese T2DM patients are known to have significantly higher levels of inter-cellular adhesion molecule (iCAM-1), vascular cell adhesion molecule (vCAM-1), and E-selectin, whereas their adiponectin levels are relatively low. Bioinformatics analysis revealed that these selected biomarkers (iCAM-1, vCAM-1, E-selectin, and adiponectin) are characterized by the noticeable levels of intrinsic disorder propensity and high binding promiscuity, which are important features expected for proteins serving as biomarkers. Within the limit of studied groups, there is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction.

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Running title: Disorder of T2DM biomarkers

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic and progressive disease that is strongly associated with the all-cause and cardiovascular mortality. The present study aimed to analyze the abundance and functionality of intrinsically disordered regions in several biomarkers of insulin resistance, adiponectin, and endothelial dysfunction found in the T2DM patients. In fact, in comparison to controls, obese T2DM patients are known to have significantly higher levels of inter-cellular adhesion molecule (iCAM-1), vascular cell adhesion molecule (vCAM-1), and E-selectin, whereas their adiponectin levels are relatively low. Bioinformatics analysis revealed that these selected biomarkers (iCAM-1, vCAM-1, E-selectin, and adiponectin) are characterized by the noticeable levels of intrinsic disorder propensity and high binding promiscuity, which are important features expected for proteins serving as biomarkers. Within the limit of studied groups, there is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction.

Keywords: Adiponectin; Insulin resistance; Intrinsically disordered protein; Type 2 Diabetes; iCAM-1; vCAM-1; E-selectin.

Introduction

The word-wide ubiquity of diabetes is disturbingly high and is growing. Here are just a few illustrative facts: if in the 2000 word there were 171 million people with diabetes, this number is expected to upsurge to 366 million by 2030 (Wild et al. 2004). In another study, the national levels of diabetes prevalence among adults (aged 20-79 years) of 216 countries in 2010 were evaluated and used to make a projection for 2030 (Shaw et al. 2010). This analysis revealed that in 2010, the world prevalence of diabetes was 6.4% and this affliction affected 285 million adult patients, whereas by 2030, the diabetes pervasiveness is expected to rise to 7.7% affecting 439 million adults (Shaw et al. 2010). It is also expected that by 2030, there will be 69% and 20% increase in the numbers of adults with diabetes in developing and developed countries, respectively (Shaw et al. 2010).

Among other health threats, type 2 diabetes mellitus (T2DM) is an important cardiovascular disease (CVD) risk factor that causes the reduction of the life expectancy (Leiter et al. 2011; Seshasai et al. 2011) and is accompanied by the hypertension (Chen et al. 2012). T2DM is often associated with obesity brought about by the increased adipose tissue mass originating from the increase in the number and size of adipocytes (DeFronzo 2010). In addition, T2DM is typically associated with the reduced levels of high density lipoprotein (HDL) cholesterol (HDL-C) and is characterized by the HDL functional impairment (Besler et al. 2012; Farbstein & Levy 2012).

Being an active endocrine organ, adipose tissue is known to secrete various adipocytokines or adipokines, which are biologically active substances involved in the local and systemic regulation of numerous metabolic and inflammatory processes (Greenberg & Obin 2006). When this endocrine function of the adipose tissue is deregulated, serious obesity-related metabolic

61 disorders start to develop including T2DM, insulin resistance, and atherosclerosis (Murdolo &
62 Smith 2006).
63 Among the variety of adipokines secreted by the adipose tissue is an important protein
64 adiponectin, which is known to have the insulin sensitizing and anti-inflammatory activities, is
65 able to improve systemic glucose tolerance, and has vasodilatory function protecting the
66 vasculature from atherosclerosis (Li et al. 2011). In the insulin-resistant humans and animals
67 (Zhao et al. 2014), as well as in obesity and in the patients with T2DM (Ndumele et al. 2006),
68 the circulating levels of adiponectin are decreased. Therefore, hypoadiponectinemia is
69 considered as an independent risk factor for the T2DM and CVD development (Misu et al.
70 2012).
71 Overall, there is a close association between the T2DM incidence, insulin resistance, the
72 abnormal levels of inflammatory markers (Ndumele et al. 2006), and the adiponectin circulating
73 levels. In fact, it is believed that the abnormal levels of inflammatory markers and the endothelial
74 cell dysfunction (Ansar et al. 2011; Gomez et al. 2008) associated with hyperlipidemia,
75 hyperinsulinemia, and pancreatic β-cell failure (Ansar et al. 2011) are typical for the T2DM
76 patients. Furthermore, endothelial dysfunction is characterized by the prothrombic properties,
77 pro-inflammatory state, and reduced vasodilation (Endemann & Schiffrin 2004; Gomez et al.
78 2007). One of the reasons for the association between the endothelial dysfunction and insulin
79 resistance is an essential role played by insulin in the vascular function regulation via stimulation
80 of the expression of vascular cell adhesion molecules, such as soluble vascular cell adhesion
81 molecule-1 (vCAM-1), soluble intercellular cell adhesion molecule-1 (iCAM-1), and E-selectin
82 on endothelium (Cersosimo & DeFronzo 2006).

Further support of the importance of adiponectin in regulation of the endothelial function is given by Lisowska *et al*., who found that in the angina pectoris patients undergoing coronary artery bypass grafting (CABG) the concentrations of adiponectin and cell adhesion molecule CD146 before the surgery were significantly lower than those in the control group (Lisowska et al. 2014). However, three months after the CABG, the adiponectin and CD146 levels were significant increased, correlating with the concentrations of thrombomodulin, which is a natural antithrombin glycoprotein, and the well-established endothelial dysfunction marker, Von Willebrand factor (Lisowska et al. 2014). These findings suggested that adiponectin and CD146 can serve as markers of endothelial cell dysfunction (Lisowska et al. 2014; Saito et al. 2007), and that the cardiovascular system can be protected by the high adiponectin levels. However, no association between the adiponectin levels and the development of coronary artery disease was found in some other studies (Lindsay et al. 2005). To address some of these controversies, our study aimed to detect an association between insulin resistance, adiponectin, and endothelial dysfunction biomarkers and their intrinsic disorder status.

Recent studies revealed that many proteins with biologically important functions do not possess unique 3D-structures (Dunker et al. 2001; Dunker et al. 2008a; Dunker et al. 2008b; Dyson & Wright 2005; Tompa 2002; Uversky 2002a; Uversky 2002b; Uversky 2010; Uversky & Dunker 2010; Uversky et al. 2000; Wright & Dyson 1999). These intrinsically disordered proteins (IDPs) and hybrid proteins containing both ordered and intrinsically disordered domains/regions (Dunker et al. 2013) are very common in nature (Dunker et al. 2000; Tokuriki et al. 2009; Uversky 2010; Ward et al. 2004; Xue et al. 2012a; Xue et al. 2010b), with IDPs constituting significant fractions of all known proteomes (Dunker et al. 2000; Oldfield et al. 2005; Uversky 2010; Ward et al. 2004; Xue et al. 2012b), and therefore being considered now as an important

extension of the protein kingdom (Dunker et al. 2008a; Dyson 2011; Tompa 2012; Turoverov et al. 2010; Uversky 2002a; Uversky 2003; Uversky 2013; Wright & Dyson 1999). Despite their lack of unique 3D-structures, these proteins are involved in crucial biological processes (such as signaling, regulation, and recognition) (Daughdrill et al. 2005; Dunker et al. 2002a; Dunker et al. 2002b; Dunker et al. 2005; Dunker et al. 1998; Dunker et al. 2001; Dyson & Wright 2005; Tompa 2002; Tompa 2005; Tompa & Csermely 2004; Tompa et al. 2005; Uversky 2002a; Uversky 2002b; Uversky 2003; Uversky 2010; Uversky et al. 2000; Uversky et al. 2005; Vucetic et al. 2007; Wright & Dyson 1999; Xie et al. 2007a; Xie et al. 2007b), and their functions complement the functional repertoire of the ordered proteins (Vucetic et al. 2007; Xie et al. 2007a; Xie et al. 2007b). Furthermore, being general regulators and controllers, many IDPs are intimately associated with the variety of human diseases (Uversky et al. 2014; Uversky et al. 2008). Since earlier study revealed that proteins related to CVD are enriched in intrinsic disorder (Cheng et al. 2006), and since T2DM is an important CVD risk factor, we analyzed the intrinsic disorder propensity and the presence of disorder-based functional sites in the biomarkers utilized in this study (human adiponectin, iCAM-1, vCAM-1, and E-selectin) by a series of bioinformatics tools.

Materials and Methods

Computational Analyses of the Amino Acid Sequences of Biomarkers

Amino acid sequences of four human proteins utilized as biomarkers, adiponectin (UniProt ID: Q15848), inter-cellular adhesion molecule (iCAM-1, UniProt ID: P05362), vascular cell adhesion molecule (vCAM-1, UniProt ID: P19320), and E-selectin (UniProt ID: P16581) were downloaded from UniProt (http://www.uniprot.org/uniprot/). The intrinsic disorder propensities of these

biomarkers were evaluated by several per-residues disorder predictors, such as PONDR® VLXT (Romero et al. 2001), PONDR® VSL2 (Peng et al. 2005), PONDR® VL3 (Peng et al. 2006b), and PONDR® FIT (Xue et al. 2010a), which were chosen based on their different sensitivities to the various protein intrinsic disorder-related features. PONDR® VSL2B is one of the more accurate stand-alone disorder predictors (Fan & Kurgan 2014; Peng et al. 2005; Peng & Kurgan 2012), PONDR® VLXT is known to have high sensitivity to local sequence peculiarities and can be used for identifying disorder-based interaction sites (Romero et al. 2001), whereas a metapredictor PONDR-FIT (Xue et al. 2010a) is moderately more accurate than each of its component predictors, PONDR® VLXT (Romero et al. 2001), PONDR® VSL2 (Peng et al. 2005), PONDR® VL3 (Peng et al. 2006b), FoldIndex (Prilusky et al. 2005), and IUPred (Dosztanyi et al. 2005).

The D^2P^2 internet database,(Oates et al. 2013) which is a community resource for the pre-computed disorder predictions on a large library of proteins from completely sequenced genomes (http://d2p2.pro/) was used to provide more information on the presence of functional disordered regions in the query proteins. As the measure of disorder predisposition, D^2P^2 database uses outputs of PONDR® VLXT (Romero et al. 2001), IUPred (Dosztanyi et al. 2005), PONDR® VSL2B (Obradovic et al. 2005; Peng et al. 2006a), PrDOS (Ishida & Kinoshita 2007), ESpritz (Walsh et al. 2012), and PV2 (Oates et al. 2013). This database is further enhanced by the information on the curated sites of various posttranslational modifications and on the location of predicted disorder-based potential binding sites.

Finally, the interactivity of biomarkers used in this study was further evaluated by STRING (Search Tool for the Retrieval of Interacting Genes) database, which is the online resource that

provides both experimental and predicted interaction information for query proteins (Szklarczyk et al. 2011).

Results

As it follows from literature data and our own results (data not shown) in comparison with controls, T2DM patients typically show significantly higher levels of iCAM-1, vCAM-1, and E-selectin, whereas their adiponectin levels are significantly lower than those of the matched controls. To check the intrinsic disorder status of the biomarkers used in our study, we evaluated the disorder propensities of human adiponectin, iCAM-1, vCAM-1, and E-selectin by several per-residues disorder predictors. Results of this analysis are summarized in Figure 1, which clearly shows that the N-terminal half of adiponectin is predicted to be highly disordered, and that all other biomarkers contain very substantial amounts of disorder.

Next, the disorder predispositions of these proteins were analyzed by the D^2P^2 database (Oates et al. 2013). Figure 2 represents the results of this analysis and confirms that human adiponectin, iCAM-1, vCAM-1, and E-selectin all possess functional disordered regions containing potential phosphorylation and protein-protein interaction sites. These observations are in agreement with the well-known fact that phosphorylation sites (Iakoucheva et al. 2004) and sites of various posttranslational modifications (PTMs) are preferentially located within the intrinsically disordered regions (Pejaver et al. 2014).

Finally, Figure 3 represents the outputs of the STRING online computational platform (Szklarczyk et al. 2011) that illustrate the interactability of human adiponectin, iCAM-1, vCAM-1, and E-selectin and shows that these biomarkers are characterized by high binding promiscuity.

Discussion

The hyperglycemia caused in T2DM by the insulin resistance can eventually caused the development of a multitude of the micro- and macrovascular complications. As a result, in comparison with healthy controls, the T2DM patients experience two to four times higher risk of the development of coronary artery disease (Haffner et al. 1998), peripheral vascular disease (Newman et al. 1993), and cerebrovascular disease (Wannamethee et al. 1999). Furthermore, CVD-related mortality among the patients with T2DM may be up to four times higher than that seeing in the background population (Almdal et al. 2004). It is known that insulin resistance and its manifestations precede T2DM and its cardiovascular complications and therefore can be used to predict these maladies (Yki-Ja¨rvinen 2001). Furthermore, there is an association between the insulin resistance characterizing the obese subjects and the endothelial dysfunction (Al Suwaidi et al. 2001; Steinberg et al. 1996). Also, the obese individuals are characterized by the down regulation of an important circulating adipose tissue-derived hormone, adiponectin (Kim et al. 2010). Experimental studies show that adiponectin may protect against the development of insulin resistance, atherosclerosis, and inflammation (Shibata et al. 2005).

Therefore, it is likely that the obese T2DM patients have insulin resistance and are characterized by the reduction of the adiponectin levels and alterations in the levels of the endothelial function biomarkers. This hypothesis is in agreement with the work by Meigs *et al*., who reported that the endothelial dysfunction can be used to predict T2DM among women (Meigs et al. 2004). Also, Thorand *et al*. supported the role of the endothelial dysfunction in the T2DM pathogenesis (Thorand et al. 2006). The levels of soluble E-selectin were independently found to be associated with diabetes (Laaksonen et al. 2004; Song et al. 2007). In addition, the Women's Health

Initiative Observational Study proved that the E-selectin levels could be considered as a predictor of diabetes among the U.S.A. women (Ingelsson et al. 2008). The decreased adiponectin levels among the obese T2DM patients could be due to the decreased adiponectin production by the enlarged adipocytes in the states of increased adiposity, since adiponectin is predominantly secreted by the pre-adipocytes (Hajer et al. 2008). One of the mechanisms for the negative correlation between the adiposity and adiponectin levels might be the increased secretion of TNF-alpha (TNF-α) from the accumulated visceral fat which potentially inhibits adiponectin secretion (Fernandez-Veledo et al. 2009; Matsuzawa 2010; Rui et al. 2001).

A study conducted by Vaverkova *et al*. independently found a positive association of soluble vCAM-1 with adiponectin (Vaverkova et al. 2013). In another study of the high risk dyslipidemic patients, these authors found altered levels of these biomarkers in the patients with vascular disease or dyslipidemia (Vaverkova et al. 2008). The existence of the positive association between the soluble vCAM-1 and adiponectin was also recently described in T2DM patients with diabetic nephropathy and was associated with the endothelial dysfunction measured by the flow-mediated dilatation (Ran et al. 2010).

The association between the insulin resistance and the endothelial dysfunction can be explained by the decreased dihydropterin reductase activity caused by the insulin resistance with the subsequent depletion of an essential cofactor of the catalytic activity of nitric oxide synthase (NOS), the tetrahydrobiopterin (BH4) (Shinozaki et al. 2001). This BH4 depletion might lead to the increased levels of the oxidative stress and endothelial dysfunction (Shinozaki et al. 2001). In agreement with the observations that insulin can serve as a vasodilator and stimulate endothelial nitric oxide (NO) production (Scherrer et al. 1994; Steinberg et al. 1994), several studies have demonstrated that the T2DM patients might be characterized by the abnormal NO-mediated

vasodilation (Williams et al. 1996). In the same way as insulin resistance may contribute to the endothelial dysfunction, the defects in the NO-mediated vasodilation may contribute to the insulin resistance (Baron et al. 1995).

Another possible mechanism of the endothelial dysfunction induced by insulin resistance is based on the impaired ability of insulin to inhibit very low density lipoprotein (VLDL) production in the liver of the T2DN patients (Malmstrom et al. 1997). An increase in serum triglycerides is accompanied by generation of small dense low density lipoprotein (LDL) particles that also contributes to the endothelial dysfunction in patients with type 2 diabetes (Makimattila et al. 1999).

The association between the insulin resistance and the hypoadiponectinemia can be due to the effects of high blood levels of glucose and fatty acids. High blood levels of fatty acids are the direct cause of the insulin resistance. According to Dresner *et al*. (Dresner et al. 1999) and Griffin *et al*. (Griffin et al. 1999), an increase in the delivery of fatty acids to the muscles or a decrease in the intracellular metabolism of fatty acids might lead to the accumulation of the intracellular fatty acid metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. These metabolytes activate a serine/threonine kinase cascade leading to the phosphorylation of serine and threonine sites of the insulin receptor substrate 1 (IRS1) and the insulin receptor substrate 2 (IRS2). This, in turn, reduces the ability of these IRS1 and IRS2 to activate phosphatidylinositol 3 kinase (PI 3 kinase) and eventually leads to the reduced activity of the glucose transporter 4 (GLUT4) (Dresner et al. 1999; Griffin et al. 1999). As a consequence, the glucose uptake in the skeletal muscle cells is reduced because the diminished glucose transport activity of the insulin receptors. The decrease in the levels of plasma adiponectin can cause the decreased glucose uptake, increased gluconeogenesis, and decreased fatty acid oxidation in the skeletal muscles

and the liver. The decrease in the oxidation of fatty acids defines the increase in the levels of free fatty acids, followed by the increase in the insulin resistance, and finally leading to the decrease in the glucose uptake (Dresner et al. 1999; Griffin et al. 1999). The decrease in the glucose uptake and the increase in the gluconeogenesis ultimately result in the increase in the levels of plasma glucose leading to T2DM (Sheng & Yang 2008).

Four biomarkers used in our study clearly belong to the class of multitasking proteins which often rely on intrinsic disorder for their multifunctionality. For example, adiponectin is a unique and abundant protein hormone that serves as an adipokine responsible for the modulation of numerous metabolic processes, such as glucose regulation and fatty acid catabolism (Lau et al. 2011; Takeda et al. 2012). At least four major biological functions were ascribed to adiponectin, regulation of metabolism, vascular protection, anti-inflammatory response, and cardioprotection/anti-ischemic function (Goldstein et al. 2009; Lau et al. 2011). In these functions, adiponectin regulates metabolism by participating in the increase in the insulin sensitivity, glucose utilization, and fatty acid oxidation. The vascular protective function is based on the adiponectin's roles in the enhancement of the NO production and the angiogenesis stimulation, whereas its anti-inflammatory role relies on the decrease in both neutrophil adhesion and macrophage activation (Goldstein et al. 2009). Human adiponectin has 244 residues and includes a signal peptide (residues 1-18) that targets adiponectin for extracellular section and is cleaved in the mature protein, a non-conserved N-terminal region (residues 19-41) followed by the collagen-like domain (residues 42-107), and a C-terminal globular domain (residues 108-244). Structurally, this C-terminal globular domain of human adiponectin is similar to TNF-α, despite dissimilar amino acid sequences (Lau et al. 2011). In agreement with the results of our computational analysis, biological activities of the full-length adiponectin are known to be

controlled by numerous post-translational modifications (*e.g.*, hydroxylation and glycosylation). The adipocyte-secreted adiponectin exists in three forms, trimers (~90 kDa; the basic unit), low-molecular-weight hexamers (~180 kDa), and high-molecular-weight isoforms consisting of 12-mers to 18-mers (which can exceed 400 kDa) (Lau et al. 2011). Again, in agreement with our bioinformatics analysis, monomeric adiponectin is thermodynamically unstable and has not been observed under native conditions, whereas its proteolytic cleavage product containing globular C-terminal domain has been postulated to exist *in vivo*.(Lau et al. 2011) Curiously, the Arg112Cys and Ile164Thr mutations that prevent adiponectin from trimer formation and result in the impaired secretion of this protein from the cell were shown to be clinically associated with hypoadiponectinemia (Waki et al. 2003).

Intercellular cell adhesion molecule-1 (iCAM-1), also known as CD54 (cluster of differentiation 54), is an immunoglobulin-like glycoprotein expressed on the surface of several cell types including endothelial cells and cells involved in the immune response (Pietruczuk et al. 2004), where it serves as a ligand for the leukocyte adhesion protein LFA-1 (integrin α-L/β-2). Besides its major role in stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration, iCAM-1 serves as a site for the cellular entry of human rhinovirus (Abraham & Colonno 1984; Bella et al. 1998) and is involved in a signal transduction (Etienne-Manneville et al. 1999) and spermatogenesis (Xiao et al. 2013). Human iCAM-1 has a signal peptide (residues 1-27) and is known to exist in the membrane-bound and soluble forms, with the transmembrane form possessing a large N-terminal extracellular domain (residues 28-480), a single-span transmembrane region (residues 481-503), and a small C-terminal cytoplasmic domain (residues 504-532). Human iCAM-1 is a heavily glycosylated and phosphorylated protein, with the extracellular domain being composed of multiple loops stabilized by seven disulfide bonds

(Cys48-Cys92, Cys52-Cys96, Cys135-186, Cys237-290, Cys332-Cys371, Cys403-Cys419, and Cys431-Cys457). Curiously, many of these loops correspond to the regions predicted to be disordered in our study.

Vascular cell adhesion molecule-1 (vCAM-1), also known as cluster of differentiation 106 (CD106), is an important cell adhesion protein mediating the adhesion of the components of the immune system (such as lymphocytes, monocytes, eosinophils, and basophils) to vascular endothelium (Petruzzelli et al. 1999; Wu 2007). It also functions in leukocyte-endothelial cell signal transduction, it may play a role in the development of atherosclerosis, and rheumatoid arthritis, and may be used by tumor cells to escape T-cell immunity (Wu 2007). Organization of vCAM-1 is very similar to that of iCAM-1, and human CD106 has a signal peptide (residues 1-24), a large N-terminal extracellular domain (residues 25-698), a single-span transmembrane region (residues 699-720), and a short C-terminal cytoplasmic domain (residues 721-739). The protein is glycosylated at multiple sites and has several disulfide bond-stabilized loops (Cys47-Cys95, Cys52-Cys99, Cys137-195, Cys246-291, Cys335-Cys383, and Cys534-Cys579). Similar to iCAM-1, many of these loops are predicted to contain significant amounts of disorder.

Finally, E-selectin, also known as cluster of differentiation 62 (CD62) antigen-like family member E (CD62E), endothelial-leukocyte adhesion molecule 1 (ELAM-1), or leukocyte-endothelial cell adhesion molecule 2 (LECAM2), is a cell adhesion protein expressed only by the cytokine-activated endothelial cells (Robbins et al. 1999). E-selectin plays a role in immunoadhesion by mediating the adhesion of blood neutrophils in cytokine-activated endothelium through interaction with PSGL1/SELPLG (Hession et al. 1990). Since E-selectin is a typical adhesin, its topological structure, being similar to those of iCAM-1 and vCAM-1, includes a signal peptide (residues 1-21), a large N-terminal extracellular domain (residues 22-

556) that contains C-type lectin domain (residues 22-139), an EGF (epidermal-growth-factor)-like domain (residues 140-175), six Sushi domain (SCR repeat) units (residues 178-249, 240-301, 303-364, 366-427, 429-490, and 491-549), a single-span transmembrane region (residues 557-578), and a short C-terminal cytoplasmic tail (residues 579-610). There are several glycosylation and phosphorylation sites in human E-selecting, and this protein is heavily cross-linked by 16 disulfide bridges (Cys40-Cys138, Cys111-Cys130, Cys143-154, Cys148-163, Cys164-Cys174, Cys210-Cys237, Cys242-Cys286, Cys272-Cys299, Cys304-349, Cys335-362, Cys367-Cys412, Cys398-Cys425, Cys430-Cys475, Cys461-Cys488, Cys493-534, and Cys520-Cys547). Since E-selectin is predicted to be noticeably more disordered than the iCAM-1 and vCAM-1 (the content of the PONDR® VSL2-predicted disordered residues in E-selectin is 42.0%, whereas iCAM-1 and vCAM-1 contain 36.1% and 35.3% disordered residues, respectively), it is likely that the larger number of disulfide bonds and their more complex pattern are needed to keep the structure of this protein stable.

Conclusions

There is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction, and proteins used as biomarkers to emphasize these connections are predicted to contain substantial amount if intrinsic disorder. The elevated disorder content in these important proteins might explain their exceptional multifunctionality.

Acknowledgements

O.H.A. wrote the manuscript and analyzed data. F.A.M. analyzed data and contributed to discussion. E.H.A. contributed to discussion and reviewed/edited manuscript. V.N.U. analyzed data, contributed to discussion, and wrote the manuscript.

Disclosure

None declared.

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Figure legends

Figure 1. Evaluating the intrinsic disorder propensities of the human adiponectin (UniProt ID: Q15848; **A**), iCAM-1 (UniProt ID: P05362; **B**), vCAM-1 (UniProt ID: P19320; **C**), and E-selectin (UniProt ID: P16581; **D**) by the family of PONDR predictors. A disorder threshold is indicated as a thin line (at score = 0.5) in all plots to show a boundary between disorder (>0.5) and order (<0.5).

Figure 2. Evaluation of the functional intrinsic disorder propensity of the human adiponectin (UniProt ID: Q15848; **A**), iCAM-1 (UniProt ID: P05362; **B**), vCAM-1 (UniProt ID: P19320; **C**), and E-selectin (UniProt ID: P16581; **D**) by the D^2P^2 platform (http://d2p2.pro/) (Oates et al. 2013). In this plot, top nine colored bars represent location of disordered regions predicted by different computational tools (Espritz-D, Espritz-N, Espritz-X, IUPred-L, IUPred-S, PV2, PrDOS, PONDR® VSL2b, and PONDR® VLXT, see keys for the corresponding color codes). Dark red bar shows the location of the functional domain found by the Pfam platform, which is a database of protein families that includes their annotations and multiple sequence alignments generated using hidden Markov models (Bateman et al. 2004; Finn et al. 2006; Finn et al. 2008). Green-and-white bar in the middle of the plot shows the predicted disorder agreement between these nine predictors, with green parts corresponding to disordered regions by consensus. Red, yellow and purple circles at the bottom of the plot show the locations of phosphorylation, acetylation and ubiquitination sites, respectively.

Figure 3. Analysis of the interactivity of the human adiponectin (UniProt ID: Q15848; **A**), iCAM-1 (UniProt ID: P05362; **B**), vCAM-1 (UniProt ID: P19320; **C**), and E-selectin (UniProt

ID: P16581; **D**) by STRING (Szklarczyk et al. 2011). STRING produces the network of predicted associations for a particular group of proteins. The network nodes are proteins, whereas the edges represent the predicted or known functional associations. An edge may be drawn with up to 7 differently colored lines that represent the existence of the seven types of evidence used in predicting the associations. A red line indicates the presence of fusion evidence; a green line - neighborhood evidence; a blue line – co-occurrence evidence; a purple line - experimental evidence; a yellow line – text mining evidence; a light blue line - database evidence; a black line – co-expression evidence (Szklarczyk et al. 2011).

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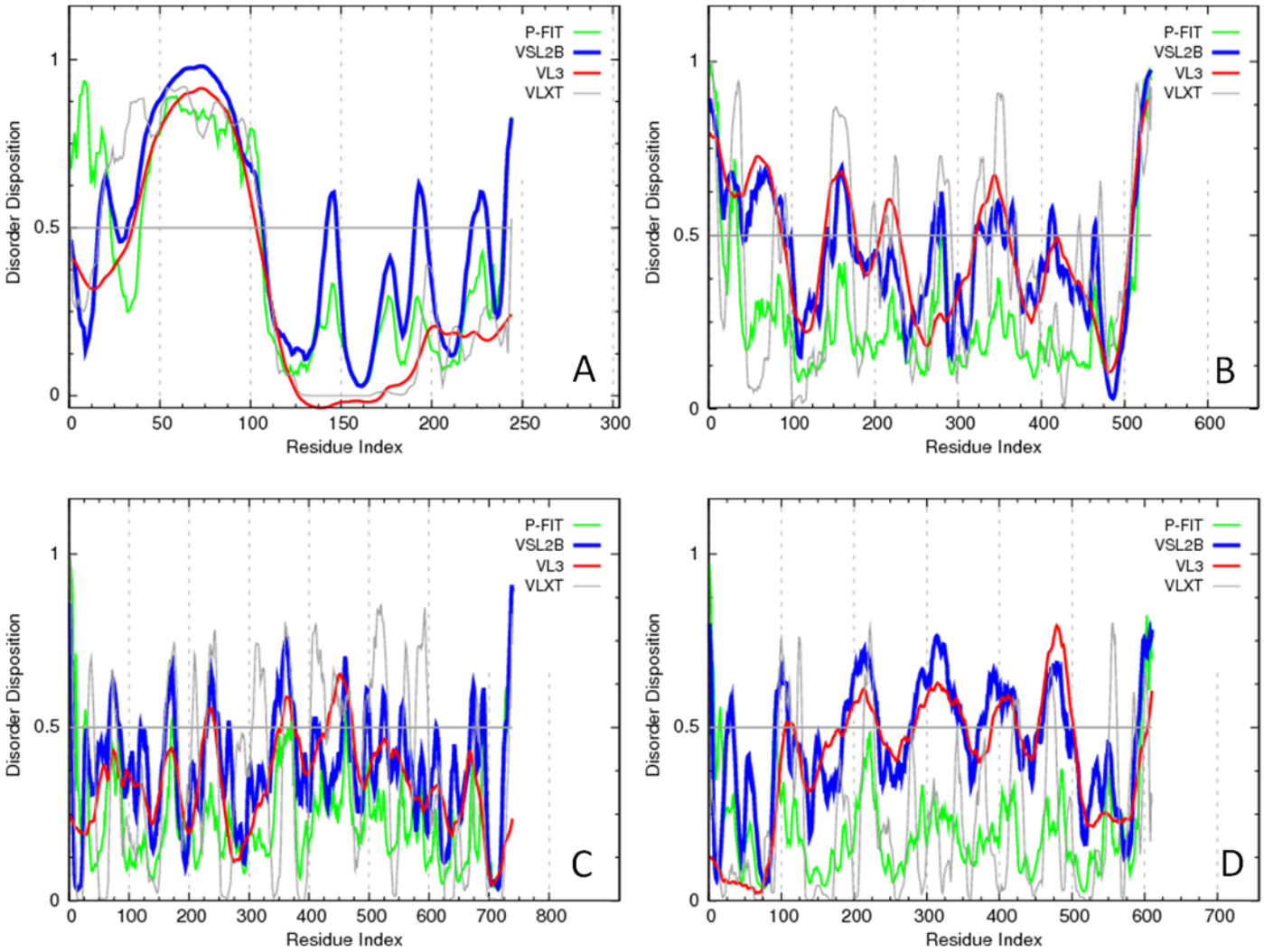
Figure 1

Figure 1. Evaluating the intrinsic disorder propensities of the human adiponectin (UniProt ID: Q15848; **A**), iCAM-1 (UniProt ID: P05362; **B**), vCAM-1 (UniProt ID: P19320; **C**), and E-selectin (UniProt ID: P16581; **D**) by the family of PONDR predictors. A disorder threshold is indicated as a thin line (at score = 0.5) in all plots to show a boundary between disorder (>0.5) and order (<0.5).



2

Figure 2

Figure 2. Evaluation of the functional intrinsic disorder propensity of the human adiponectin (UniProt ID: Q15848; **A**), iCAM-1 (UniProt ID: P05362; **B**), vCAM-1 (UniProt ID: P19320; **C**), and E-selectin (UniProt ID: P16581; **D**) by the D^2P^2 platform (http://d2p2.pro/) (Oates et al. 2013). In this plot, top nine colored bars represent location of disordered regions predicted by different computational tools (Espritz-D, Espritz-N, Espritz-X, IUPred-L, IUPred-S, PV2, PrDOS, PONDR® VSL2b, and PONDR® VLXT, see keys for the corresponding color codes). Dark red bar shows the location of the functional domain found by the Pfam platform, which is a database of protein families that includes their annotations and multiple sequence alignments generated using hidden Markov models (Bateman et al. 2004; Finn et al. 2006; Finn et al. 2008). Green-and-white bar in the middle of the plot shows the predicted disorder agreement between these nine predictors, with green parts corresponding to disordered regions by consensus. Red, yellow and purple circles at the bottom of the plot show the locations of phosphorylation, acetylation and ubiquitination sites, respectively.

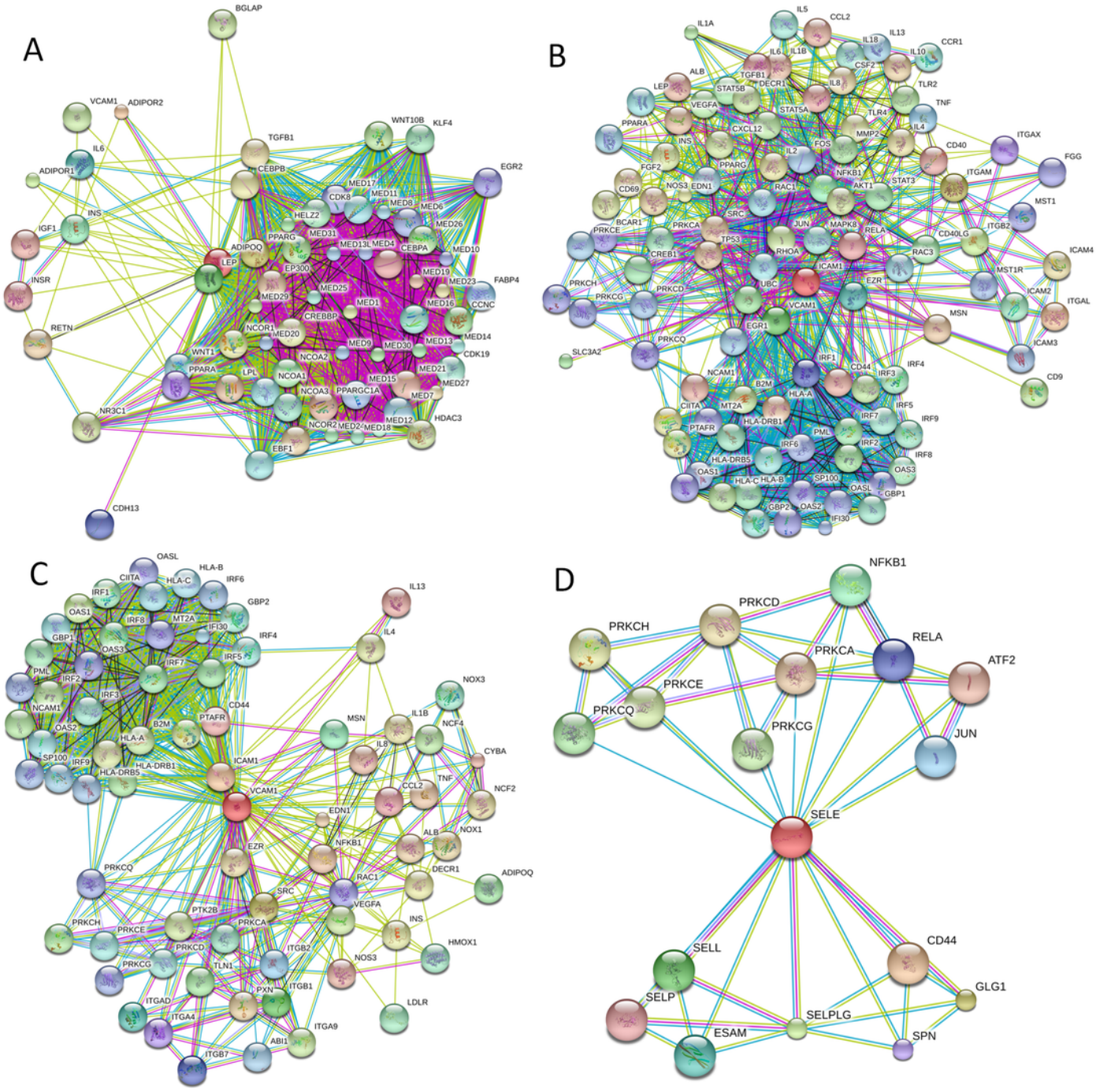

A
B
C
D
Espritz-D
Espritz-X
Espritz-N
IUPred-L
IUPred-S
PV2
PrDOS
VSL2b
VLXT
Predicted Domains
Predicted DisorderAgreement
MoRF Regions
PTM Sites
Key:
Predicted SCOP Structure
Weaker Support
Pfam Conserved Domain
Predicted Disorder
Predicted MoRFs
Curated PTM Site
Disorder:
Superfamilies:
[1] TNF-like
[1] Immunoglobulin
[1] C-type lectin-like
[2] Complement control module/SCR domain
Pfams:
[2] C1q domain
[3] PB016127 (Pfam-B)
[4] PB003601 (Pfam-B)
[5] Collagen triple helix repeat (20 copies)
[6] PB002564 (Pfam-B)
[7] PB005698 (Pfam-B)
[2] PB019874 (Pfam-B)
[3] Intercellular adhesion molecule (ICAM), N-terminal domain
[4] Immunoglobulin domain
[2] Immunoglobulin C2-set domain
[3] Immunoglobulin I-set domain
[4] Immunoglobulin domain
[3] Lectin C-type domain
[4] Sushi domain (SCR repeat)
[5] EGF-like domain

3

Figure 3

Figure 3.

Analysis of the interactivity of the human adiponectin (UniProt ID: Q15848; **A**), iCAM-1 (UniProt ID: P05362; **B**), vCAM-1 (UniProt ID: P19320; **C**), and E-selectin (UniProt ID: P16581; **D**) by STRING (Szklarczyk et al. 2011). STRING produces the network of predicted associations for a particular group of proteins. The network nodes are proteins, whereas the edges represent the predicted or known functional associations. An edge may be drawn with up to 7 differently colored lines that represent the existence of the seven types of evidence used in predicting the associations. A red line indicates the presence of fusion evidence; a green line - neighborhood evidence; a blue line – co-occurrence evidence; a purple line - experimental evidence; a yellow line – text mining evidence; a light blue line - database evidence; a black line – co-expression evidence (Szklarczyk et al. 2011).


A
BGLAP
VCAM1
ADIPOR2
WNT10B
KLF4
TGFB1
IL6
EGR2
ADIPOR1
CEBPB
INS
IGF1
ADIPOQ
LEP
INSR
FABP4
RETN
NR3C1
HDAC3
EBF1
CDH13
B
IL5
CCL2
IL1A
IL13
CCR1
IL18
IL10
IL6
IL1B
CSF2
ALB
TGFB1
LEP
IL8
TLR2
STAT5B
DECR1
VEGFA
TNF
STAT5A
TLR4
PPARA
CXCL12
IL4
MMP2
ITGAX
INS
FOS
CD40
IL2
FGG
FGF2
PPARG
NFKB1
ITGAM
CD69
NOS3
EDN1
RAC1
AKT1
STAT3
MST1
SRC
BCAR1
PRKCA
JUN
MAPK8
RELA
ITGB2
PRKCE
CD40LG
TP53
CREB1
RHOA
RAC3
ICAM4
ICAM1
MST1R
PRKCH
EZR
UBC
ICAM2
PRKCD
PRKCG
VCAM1
ITGAL
MSN
EGR1
PRKCQ
ICAM3
SLC3A2
IRF1
IRF4
CD44
IRF3
CD9
NCAM1
B2M
HLA-A
CIITA
MT2A
IRF5
HLA-DRB1
IRF7
IRF9
PTAFR
PML
IRF6
IRF2
IRF8
HLA-DRB5
OAS1
OAS3
HLA-C
HLA-B
SP100
OASL
GBP1
GBP2
OAS2
IFI30
C
OASL
HLA-B
CIITA
HLA-C
IRF6
IRF1
GBP2
IL13
OAS1
IRF8
MT2A
IFI30
IL4
GBP1
IRF4
OAS3
IRF5
IRF7
NOX3
PML
IRF2
IRF3
CD44
NCAM1
PTAFR
IL1B
MSN
OAS2
B2M
NCF4
HLA-A
IL8
CYBA
SP100
IRF9
ICAM1
HLA-DRB1
HLA-DRB5
TNF
VCAM1
CCL2
NCF2
EDN1
NOX1
ALB
EZR
NFKB1
PRKCQ
DECR1
ADIPOQ
RAC1
SRC
VEGFA
PTK2B
INS
PRKCH
PRKCA
HMOX1
PRKCE
PRKCD
ITGB2
NOS3
PRKCG
TLN1
ITGB1
PXN
LDLR
ITGAD
ITGA4
ITGA9
ABI1
ITGB7
D
NFKB1
PRKCD
PRKCH
RELA
PRKCA
ATF2
PRKCE
PRKCQ
PRKCG
JUN
SELE
SELL
CD44
GLG1
SELP
SELPLG
ESAM
SPN