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The Role of Single Nucleotide Polymorphisms of Untranslated Regions (Utrs) in Insulin Resistance Pathogenesis in Patients with Type 2 Diabetes

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1. Introduction

Insulin resistance (IR) is defined as a condition, in which regular amount of insulin is insufficient to develop physiological respond of the cell. For this reason there is constantly great need for increased level of this hormone within insulin resistant body. Very important factors leading to insulin resistance development are environmental components such as inappropriate diet and sedentary life style. A great important role in insulin resistance pathogenesis plays genetic background, as IR develops more frequently in families with positive history of metabolic disorders. There are severe anomalies in expression level of genes playing role in regulation of insulin action detected in patients with impaired insulin sensitivity. Regulation of gene expression can be exerted at either transcriptional level or post-transcriptional level. The former is related to the primary gene sequence located in the promoter region and it is responsible for controlling whether a gene is transcribed or not. The later utilizes regions of transcript not being translated, located at 5' and 3' end of the mRNA named the Untranslated Regions (UTRs). The main roles of UTRs are transcript stability control, initiation or inhibition of translation and sub-cellular localization in the cytoplasm. Regulation by UTRs is mediated in several ways, mainly by interaction of regulatory motifs in UTRs with numerous proteins as well as regulation by microRNA.

1.1 IR: Insulin resistance

The main tissues affected by IR are adipose tissue, skeletal muscle and liver (Hernandez-Morante et al., 2008; Karlsson & Zierath, 2007). Insulin resistance also affects lymphocytes and other peripheral blood leucocytes (Maratou et al, 2007; Piątkiewicz et al. 2007). The first diagnosed symptom of insulin resistance development is the decrease in glucose utilization by skeletal muscles (Patti, 2004), what is mediated by decrease in glycogen synthase (GYS) activity. Furthermore the expression rate and the phosphorylation state of numerous kinases (mainly PI-3K) of insulin pathway is decreased. The phosphorylation of serine residues of IRS-1 and IRS-2 is increased (Boura-Halfon & Zick, 2002). Impaired activity of GYS leads to insulin resistance in liver. Next, the number and metabolism of mitochondria decline (Morino, 2006). At this stage, the glucose utilization becomes impaired in adipose tissue. In parallel, the lipids

metabolism deregulation, with increased FFA and TG levels, further impair insulin sensitivity in adipose tissue. Insulin resistance is characterized by dysfunction in GLUT4 translocation and glucose uptake in all cells, where insulin is essential. Despite long and intense studies, the origin and pathomechanism of insulin resistance remain unknown. It is believed that both environmental and genetic factors play role in its pathogenesis. IR causes increase in insulin production and secretion as a compensatory mechanism. The prolong demand for insulin results in decreased in pancreatic β-cells efficiency and insulin secretion. This is a theoretical pathomechanism of type 2 diabetes (T2DM) development.

1.1.1 Insulin resistance, mutations and genes expression

The body of literature reports that at insulin resistant state, severe anomalies in gene expressions encoded proteins involved in insulin pathway are diagnosed. In subjects with insulin resistance the decreased levels of *INSR* gene expression have been reported (Stentz & Kitabchi, 2007). Mutations in *INSR* gene are very rare and their presences result in a severe insulin resistance attendant by *acanthosis nigricans* (Hansen & Shafrir, 2002). Most of described so far changes in *INSR* gene sequence were localized mainly in coding region (Højlund et al., 2006; Kusari et al., 1991). Depending on the place of nucleotide variance the effects might influence diverse INSR function. If the change is placed in α chain, the decrease in affinity to insulin might be observed. On the other hand, nucleotide changes localized in β chain influence the activity of tyrosine kinase and thus the rate of tyrosine domain phosphorylation. Only for few nucleotide changes the relationship with insulin resistance has been confirmed.

The downstream kinases such as IRS proteins, PI-3K, Akt have been also shown to be decreased in insulin resistant patients. (Hansen & Shafrir, 2002). Numerous studies provide data for impairments in *IRS-1, IRS-2* or *PIK3R1* gene expression in all tissues affected by insulin resistance (Boura-Halfon & Zick, 2002; Stentz & Kitabchi, 2007; Andreelli et al., 2000). There are some reports suggesting association between SNPs in *PIK3R1* gene with resistance to insulin, such as Met326Ile (Barroso et al, 2003), however other reports negate this findings (Almind et al., 2002).

The last part of insulin pathway is GLUT4 activation and translocation into cell membrane what is essential for insulin-dependent glucose uptake. There are few polymorphisms in *SLC2A4* gene, mostly located in coding region of *SLC2A4* gene, that have been correlated with insulin resistance (Kusari et al, 1991). However, their presence is relatively rare, so it is unlikely they are responsible for insulin resistance by itself, taking into account the prevalence of this disorder. The mRNA analyses have revealed dysfunction in *SLC2A4* gene expression as well as GLUT4 activation and translocation to the cell membrane in adipose tissue (Shephard & Khan, 1999) and in skeletal muscles (Garvey et al, 1998) after insulin stimulation in insulin resistant subjects. The *SLC2A4* gene expression as well as expression rate of genes encoding others GLUT members, are also decreased in lymphocytes, what is correlated with dysfunction of these cells and very severe and long lasting infections in insulin resistant patients (Maratou et al, 2007; Piątkiewicz et al. 2007).

1.1.2 Insulin resistance and active kinases dephosphorylation – Protein Tyrosine Phosphatases (PTPs)

The initial animal models experiments performed during last decade allowed for accurate assessment of the PTPs role in insulin action and glucose metabolism. Further evaluation of PTPs action in humans correlated their activity with obesity, metabolism dysfunction and

impairment in insulin action (LeRoith et al, 1996). Several PTPs have been implicated in insulin signal regulation. The most important in insulin signaling is Protein Tyrosine Phosphatase 1B (PTP1B) encoded by *PTPN1* gene. The PTP1B has been for the first time associated with insulin resistance after improving insulin sensitivity in mice injected with antibodies against PTP1B (Ahmad et al, 1995). Further experiments performed on cell lines confirmed the role of PTP1B in insulin sensitivity regulation (Venable et al., 2000). Increased PTP1B level as well as increased *PTPN1* gene expression rate have been diagnosed in skeletal muscles of obese subject comparing to lean, correlated with decrease in glucose uptake by muscle cells in those patients (Ahmad et al., 1997). On the other hand analyses performed in adipose tissue revealed significantly higher PTP1B level in obese subjects while decreased activity (Cheung et al., 1999). The explanation of this phenomenon might be fact, that PTP1B is activated by INSR. Furthermore, *PTPN1* gene expression is induced by inflammation.

Another phosphatase that has been correlated with insulin resistance is Leukocyte Antigen-Related Phosphatase (LAR). Main data suggesting role of this phosphatase in insulin resistance pathogenesis came from knockout animal model studies that demonstrated severe insulin resistance in animals with decreased glucose uptake rate by skeletal muscles and decreased PI-3K activity (Zabolotny et al., 1999). Studies in humans showed similar pattern, that is significantly higher abundant of mRNA and protein in skeletal muscles and adipose tissue in obese subject, that positively correlated with obesity and insulin resistant state (Worm et al., 1995).

1.1.3 Insulin resistance and obesity

The average human body contains from 10 to 15 kg of adipose tissue that performs diverse functions ranging from energy storage to endocrine secretion. The excess accumulation of adipose tissue impairs insulin sensitivity by (1) excessive secretion of FFA into blood stream and its oxidation, (2) secretion of numerous cytokines that modulate insulin sensitivity, (3) chronic inflammatory state induction (George, 1996).

Abundant FFA level and the metabolite of its oxidation (Acylo-CoA) impair insulin action mainly by IRS-1 and IRS-2 serine/threoine residues phosphorylation, what is mediated through NF-κB pathway (Ragheb et al., 2009). Furthermore, NF-κB pathway activation is associated with increased rate of proinflammatory cytokines production.

Adipose tissue is known as an active endocrine organ producing and secreting into blood stream various cytokines like lepin, adiponectin, RBP4, resistin. Thanks to adipocytokines, adipose tissue connects with the central nervous system (CNS) and regulates energy balance. Some cytokines are implicated in insulin sensitivity regulation (George, 1996). Dysregulation in adipocytokines production, what has place in obesity, causes impairment in phosphorylation rate of numerous important kinases involved in insulin signal transduction (Cohen et al., 2002; Greenspan & Baxter, 1994). Adipose tissue in obese subjects is highly infiltrated by macrophages that change their phenotype into pro-inflammatory cells, secreting pro-inflammatory cytokines like IL -1, 6, 10 (Interleukin 1, 6, 10), TNF-α (Tumor Necrosis Factor-α), MCP-1 (Monocyte Chemotactic Protein-1). Mentioned cytokines are well known factors leading to JNK kinase activation, serine residues phophorylation and insulin sensitivity impairment (Müssig et al., 2005).

Macrophages are not the only cells causing inflammatory state. Some reports provide information that adipocytes hypertrophy is associated with preadipocytes differentiation deregulation and changing their phenotypes into pro-inflammatory cells secreting, similar

to macrophages, various cytokines. These cells become typical pro-inflammatory cells with markers expression characteristic for immune cells like CD68 (Gustafson et al., 2009).

1.2 UTRs: Localization, structure and function in post-transcriptional regulation of gene expression

The UTRs are localized at both ends of transcript (mRNA), but are not transcribed to proteins (do not encode proteins). The 5'UTR is localized upstream the start codon AUG (Met), on the other hand 3'UTR is placed downstream the stop codons UAA, UAG, UGA. The average size of 5'UTR in humans is 210,2 nt (nucleotides) with maximal size 2803 nt and minimal size equal 18 nt. The average size for 3'UTR is 1027 nt with maximal size 8555 nt and minimal size equal 21 nt (Mignone et al., 2002). The characteristic feature of those regions is GC content with higher amount of GC in 5'UTR about 60%, on the other hand in 3'UTR GC contents is about 45% (Pesole et al., 1999). Very interesting feature has been seen by Pesole (Pesole et al., 1999), who observed that the higher amount of GC the shorter 3'UTR.

UTRs make numerous conformational structures and tridimensional loops that interact with proteins and other functional and regulatory compounds like ribosomes or microRNA. Within the UTRs a specific functional motifs can be observed, that play important role in function and transcription control (Carmody & Wente, 2009). The most important 5'UTR motif is 5'7-methylguanin (5'7mG), which is added to the transcript just after the transcription initiation, before the whole transcript synthesis is completed (Mignone et al., 2002). Within 5'UTR other motifs can be distinguished like region IRES (Internal Ribosomal Entry Site), which stands for the 5'UTR region that interact with ribosome during translation initiation, numerous hairpins that interact with various proteins controlling transcripts stabilization and ORF (Open Reading Frames). Furthermore others motifs like IRE (Iron Respons Element) that is composed of about 30 nucleotides and has a stem-loop structure (Pickering & Willis, 2005) can be found. The most important motifs localized in 3'UTR are CPE (Cytoplasmic Polyadenylation Element), MRE (microRNA Regulatory Element) and poli(A) tail (Mignone et al., 2002; Carmody & Wente, 2009; Conne et al., 2000).

UTRs play their role thanks to many motifs, conformational loops and hairpins that interact with numerous proteins and others factors like microRNA or ribosomes. The main role of 5'UTR is controlling of translation initialization as well as transcript stabilization, on the other hand 3'UTR is mostly implicated in regulation of transcript stabilization and its localization in cytoplasm (Carmody & Wente, 2009; Pickering & Willis, 2005). Furthermore 3'UTR is the place of microRNA action via MRE. Depending on the cell type various mechanisms and regulatory motifs might regulate transcript stability. For example, IRE element regulates iron homeostasis. On the other hand regulatory proteins, growth factors as well as proto-oncogenes posses long 5'UTRs, what inhibits translation initiation and as a consequence, protein synthesis (Pickering & Willis, 2005).

1.2.1 Regulation of translation initiation

The main regulatory element of translation regulation is the regulation of translation initiation. This process requires interaction of ribosomal 40 S subunit with 5'7-methylguanin (5'7mG). The resulting complex (so called 43 S) further interacts with translation initiation factors like eIF2, eIF4F, eIF4G, eIF3. Next, eIF1A and eIF3 facilitate binding the 43 S subunit with eIF2-GTP-Met-tRNA, what begins the mRNA scanning process and searching for the initiation (start) codon (AUG) in 5'→3' direction (Carmody & Wente, 2009; Meijer &

Thomas, 2002). Once the start codon has been achieved, the eIF5 facilitates the 40 S and 60 S subunits joining resulting in 80 S ribosomal subunit. The 80 S subunit initiates the protein synthesis and elongation. In the regulation of efficiency of translation initiation and 5'UTR scanning the secondary structures of 5'UTR play a great role. However, the effect on translation initiation has been shown for structures posses the bounding energy higher $\Delta R <$ -50 kcal/mol (Svitkin et al., 2001). In the initiation translation process the most important is 5'7mG and its interaction with 40 S ribosomal subunit as well as with translation initiation factors. The mRNA binding with 40 S subunit is facilitated also by the IRES motif and this process dominates in situation when the translation initiation by 5'7mG is impaired by stress, apoptosis or suppressed by cell-cycle stage (Pickering & Willis, 2005; Meijer & Thomas, 2002). This mechanism is common for mRNA encoding growth factors or transcription factors. IRES is localized close to AUG codon and the IRES-mediated translation regulation depends on the secondary and tertiary structure (Peng et al., 1996) as well as on the complementarity to 18 S rRNA (Chappell et al., 2000). Many genes posses the IRES in 5'UTR e.g. genes involved in apoptosis like *c-myc* (Stoneley et al., 2000), *Apaf-1* (Coldwell et al., 2000), *XIAP* (Holcik & Korneluk, 2000). The *VEGF* gene contains two IRESs in 5'UTR (Meiron et al., 2001).

Very important role in translation initiation plays the nucleotide sequence flanking start codon with following sequence: GCC**R**CC**AUGG**. The **R** stands for purine, usually adenine. The purine in -3 position and **G** in +4 position is the rule of start codon determination and is strong consensus sequence present in animals and plants (Svitkin et al., 2001). The presence of AUG in 5'UTR and false determination of the main ORF decrease the translation initiation process via assignment of upstream open reading frames (uORF). The uORF results in translation initiation and synthesis of false proteins (Mignone et al., 2002; Svitkin et al., 2001). The fate of 40 S subunit that recognized the wrong ORF depends on the size of uORF. The 40 S subunit might dissociate and restart scanning, however, if the uORF is greater than 30 codons, the rescanning is not possible (Peng et al., 1996).

The mechanisms mediated translation initiation vary depending mostly on environmental condition and, in situation, when one mechanism is inhibited, the second is active. For example when the cap-dependent mechanism of translation initiation is inhibited by hypoxia or apoptosis, the translation is initiated by IRES-dependent mechanism. The same changeable mechanisms might be seen for translation regulation via uORF. It is believed that this switch hypothesis is an adaptive mechanism of gene expression regulation in various cellular conditions (Meijer & Thomas, 2002).

1.2.2 Transcript stability

The mRNA stability is mostly regulated via 3'UTR, especially by elements rich in AU repeats – ARE (AU – Rich Elements) (Griffin et al., 2004). AREs are classified into 3 groups depending on AUUUA repetitive units, regulation mechanisms and degradation efficiency. However, the result of its action is fast deadenylation and mRNA degradation (Peng et al., 1996). mRNA degradation is also regulated by numerous endonuclease enzymes, that hydrolyze the poli(A) tail of transcript with following fast degradation of the whole mRNA (Mignone et al., 2002). In the transcript stability regulation important role play hnRNPs (ribonucleoproteins) that stabilize the mRNA and is responsible for its localization in the cells. The recognition site for hnRNPs is located in the 3'UTR (Mignone et al., 2002).

The 5'UTR also plays role in the regulation of transcript stability by process named Nonsense-Mediated mRNA Decay (NMD) (Nicholson et al., 2010). The mechanism of regulation is connected with the proper identification of uORF as the false reading frame and its degradation. It is also responsible for accurate identification of stop codon and the translation termination in a proper position. In physiological conditions the premature translation termination codons (PTCs) are produced in variety of organisms. To prevent from the production of protein lacking C-terminal domains, those transcripts are recognized and subsequently degraded by NMD (Nicholson et al., 2010). The NMD mechanisms are also responsible for controlling the abundance of physiological full length transcripts (Mendell et al., 2004; Rehwinkel et al., 2005).

1.2.3 Regulation of transcript localization in cytoplasm

Subcelluar transcripts localization depends mainly on the type of protein encoded by gene and its demand in the cell. The mRNAs in cytoplasm are connected with ribonucleoproteins (Mignone et al., 2002). Three main mechanisms regulating subcellular localization of mRNA in the cell are known. The prevalent mechanism relies on the active transcript transport into the particular compartments of the cell by cytoskeleton elements and specific proteins interacting with mRNA (in particular with the 3'UTR). The second mechanism is connected with interaction of various proteins with motifs located in 3'UTR thus influencing the transcript localization. The third mechanism relies on the diffusion of mRNA (Mignone et al., 2002).

1.2.4 Post-transcriptional regulation via microRNA

MicroRNAs are short, single stranded classes of RNAs of 19-25 nucleotides (nt) in length. MiRNAs are produced from longer precursors containing hairpin structure (pre-miRNAs) that are generated from pri-miRNAs by nuclear RNase III Drosha. Pre-miRNAs are then transported into the cytoplasm and processed by Dicer RNase III complex to produce about 22 nt mature miRNAs (Kim, 2005). Mature miRNAs appear in the cell as complexes with proteins known as miRNP (miRNA containing ribonucleoprotins complex), or mirgonaute or miRISC (miRNA containing RNA induced silencing complex) (Kim, 2005). Generally one strand of miRNA is cleaved whilst one strand stands for active strand of mature miRNA. In animals including humans miRNAs act by imperfect pairing to the MRE (MiRNA Regulatory Element) in 3'UTR of target transcripts. Because of the mismatch between miRNA and target site in mRNA, one miRNA might target numerous different mRNAs, on the other hand one transcript might be regulated by various miRNAs (Jackson & Standart, 2007). There are several mechanisms of miRNA-dependent gene expression regulation, mainly through translation repression or mRNA decay (Jackson & Standart, 2007; Shuang & Fang, 2009; Zhao & Liu, 2009).

Translation repression might occur at initiation stage or after initiation stage. Three distinct mechanisms mediate translation repression by miRNA. First mechanism relies on blocking translation initiation by repression the assembly of ribosome that is the 60 S subunit to form complete 80 S translation active form (Thermann & Hentze, 2007). Second mechanism targets the translation initiation by repression the translation complex formation, mainly by blocking the eIF4E assembly to 5'7mG (Shuang & Fang, 2009). Third mechanism by which miRNA modulated translation initiation is connected with PolyA Binding Proteins (PABPs) action, or rather with the PolyA tail deadenylation (Wakiyama et al., 2007). There are

various post-initiation mechanisms that influence translation initiation mediated by miRNA. It has been shown that microRNA represses the IRES depended translation initiation, inhibits the LIN4 protein synthesis or causes timely ribosome drop-off and early translation termination (Shuang & Fang, 2009).
Numerous studies provide data reported mRNA degradation as a main aspect of gene expression repression mediated by miRNAs. MiRNA acts, in contrary to siRNA not by endonucleolytic cleavage, but rather by deadenylation and decapping of target mRNA and its subsequent degradation (Wu et al., 2005). The process of mRNA decay by miRNA has place in a cytoplasms foci named P-bodies (Processing bodies) that contain miRNAs, target mRNAs and enzymes required for mRNA decay (Jackson & Standart, 2007).

1.2.5 UTRs data bases

In order to classify the knowledge and to comprehensively understand the mechanisms of post-transcriptional regulation of gene expression mediated by UTRs, various data bases were created. Data bases provide information about functions and regulation mechanisms on the basis of primary and secondary structure of regulatory motifs. All data assembled in UTRs data bases have been determined by experimental studies and published (Mignone et al., 2005; Huang et al., 2006). Data bases contain information about sequence and structure of regulatory motifs like IRES, IRE, MRE, ARE, indicating the region of transcript that the particular motif appears. Furthermore, data bases provide information about regulatory factors interacting with particular motif (transcription factor, regulatory protein, miRNA). All data bases stand for useful tool for therapeutic possibility anticipating and searching. Examples of the most common UTRs data bases with short characterization are presented in table 1.

Name	Web site	Description
ERPIN (*Easy RNA Profile IdentificatioN*)	http://tagc.univ.mrs.fr/erpin/	Identification of a wide range of secondary structure, orientation and regulatory motifs in mRNA sequence.
MicroInspector	http://bioinfo.uni-plovdiv.bg/microinspector/	Tools for miRNA target site prediction as well as for searching miRNAs of analyzed mRNA.
miRanda	http://www.microrna.org/microrna/home.do	
miRBase	http://www.mirbase.org/	
RegRNA	http://regrna/mbc.nctu.edu.tw/	Identification of regulatory motifs and elements in mRNA sequence with functional effects.
RNA Analyzer	http://rnaanalyzer.bioapps.biozentrum.uni-wuerzburg.de/	
UTRdb, UTRsite	http://utrdb.ba.itb.cnr.it/tool/utrscan	Identification regulatory motifs in 3′ and 5′UTRs important in post-transcriptional regulation.

Table 1. Characterization of main UTRs data bases.

1.3 Single nucleotide polymorphisms in UTRs and insulin resistance

5′ and 3′ UTRs are highly rich in polymorphisms like Alu elements or long polymorphisms LINE. In UTRs are present also mini and microSTRs as well as SNPs in a high abundance. The heterogeneity regions of UTRs for human are approximately 36% for 3′UTR and 12% for 5′UTR (Mignone et al., 2002). Single Nucleotide Polymorphism (SNP) is the replacement, deletion or insertion of a single base in genome sequence and is the most common change in human genome. The effects of nucleotide replacement vary, depend on the place, where the nucleotides have been changed. In most cases, SNPs do not have the phenotypic effects, and stand for the genomic heterogeneity within or between distinct populations. On the other hand SNPs in coding region of the genome (cSNPs – coding SNPs) might result in amino acids replacement and finally changes in protein structure and function. SNPs located in introns might influence the splicing process with effect on ensuing transcript. The great influence on mRNA and proteins synthesis possess SNPs located in regulatory regions like promoter region of the gene or in UTRs. Changes in those regions are associated with deregulation in gene expression at transcriptional and post-transcriptional levels (Doss et al., 2008).

The body of literature associate SNPs located in UTRs with gene expression regulation (Mendell et al., 2005; Chen et al., 2006). The proper nucleotide sequence in motifs described above ensures accurate function of these regions and gene expression regulation (Mendell & Dietz, 2001). Nucleotide changes in the functional transcripts regions influence the mRNA synthesis, splicing, transcripts stabilization and decay. Other mechanisms that SNPs might influence post-transcriptional gene expression regulation are the translation initiation or uORF generation. Nucleotide changes cause conformation changes in UTRs, especially in 5′UTR what notably influence the efficiency of translation initiation, the 5′UTR scanning and start codon searching. Furthermore changes in motifs that interact with 40 S ribosomal subunit, proteins, transcription factors or miRNA abolish the binding sites for these factors thus impair regulatory mechanisms.

So far, majority of investigators have focused mainly on genetic variants located in coding region. In recent decade polymorphisms in functional region have been emphasized. The growing interest dues to the fact that most diseases have been associated with abnormalities in gene expression rate as the main cause, thus the regulatory mechanisms have been widely investigated. Initially, investigators focused on genetic variants in regulatory regions as the cause of abnormities in gene expression during carcinogenesis. Next, more diseases have been correlated with changes in those regions (Conne et al., 2000; Pickering & Willis, 2005; Chen et al., 2006; Sethupathy & Collins, 2008; Halvorsen et al., 2010).

1.3.1 SNP in 5' and 3'UTRs and insulin resistance

The correlations between insulin resistance and genetic variants in UTRs have been reported previously by many investigators (Xia et al., 1999; Chen et al., 2006; Nelsøe et al, 2006). The associations linking genetic variations in UTRs of such genes as *PPP1R3A*, *PTPN1*, *KCNJ9*, *RETN*, *SOCS1, INSR,PIK3R1* or *FASL* with insulin resistance have been shown previously. In *PPP1R3A* gene the polymorphism has been connected with ARE and the nucleotide change (5bp deletion) impaired gene expression rate by increased mRNA degradation (Xia et al., 1999). Dinucleotide GT microSTR polymorphism in *FASL* gene has been correlated with insulin resistance in type 2 diabetes by affecting the mRNA stability (Nelsøe et al, 2006). The SNP in *PTPN1* gene, where change relyed on additional guanine insertion in 3′UTR, increased transcript stability, thus protein abundance and its activity. Increased PTP1B activity on the

other hand impaired phophorylation rate of INSR and IRSs and insulin sensitivity (Di Paola et al., 2002). The A allele of SNP located in 3'UTR of resistin gene showed decreased risk of type 2 diabetes and hypertension comparing to G allele (Tan et al., 2003). In independent study the same SNP showed association with obesity and insulin-related phenotype (Duman et al., 2007). Wolford et al. have shown the association of SNPs including the SNPs located in 3'UTR of *KCNJ9* gene with insulin resistance and type 2 diabetes in Pima Indians, however, the associations was not strong. It is believed that investigated SNPs are in linkage disequilibrium with others functional SNPs (Wolford et al., 2001). Zhang et al. demonstrated allele dependent gene expression rate of *GFPT2* gene. The SNP located in 3'UTR of this gene positively correlated with mRNA abundance. The lower mRNA amount has been diagnosed in C allele carries, on the other hand significantly higher amount of mRNA has been diagnosed in T allele carriers (Zhang et al., 2004). Furthermore the T allele correlated with higher risk of type 2 diabetes development and its complications. Further examples of polymorphism in UTRs implicated in insulin resistance provided Villuendas et al. that revealed association between ACAA insertion/deletion polymorphism in *IGFIIR* gene with type 2 diabetes. The ACAA deletion variant played protective role. What's more authors suggested that this polymorphism might be a good surrogate insulin resistance marker (Villuendas et al., 2006). Bennet et al. in their work performed computational analysis in order to assess the influence of SNPs in insulin resistance syndrome development. Using bioinformatics and experimental approaches they revealed the association of nineteen SNPs located in 3'UTR with IR and T2DM. Nine SNPs were located in UTRs of genes implicated in insulin function and regulation pathway, nine SNPs were placed in UTRs of genes regulating cytokines synthesis and inflammation processes and one SNP in 3'UTR of gene classified into regulation of glucose metabolism and glucose transport (Bennet et al., 2001).

1.3.2 SNPs in microRNA genes/ microRNA target sites and insulin resistance

Gene expression regulation via microRNAs is crucial for maintaining body homeostasis. Dysregulation of this process might be a reason for various metabolic diseases (Sethupathy & Collins, 2008). Numerous factors affect miRNA translation regulation such as mutations in the proteins involved in miRNA processing and maturation (*trans factors*) as well as mutations in miRNA target sites (*cis factors*). Many SNPs located in target sites for miRNA action are correlated with miRNA mediated metabolic disorders like Tourette's Syndrome, Spastic Paraplegia, Hypertension, Parcinson Disease and various types of cancers (Sethupathy & Collins, 2008). MiRNA dysfunction is also correlated with obesity, insulin resistance and type 2 diabetes pathogenesis (Ferland-McCollough et al., 2010; Poy et al., 2007). Genome wide association studies (GWA) as well as meta-analysis studies demonstrated several SNPs correlated with higher risk of type 2 diabetes (Salonen et al., 2007; Zeggini et al., 2008; Dupuis et al., 2010). Among all SNPs with the highest significant association for type 2 diabetes, Glinsky selected twelve SNPs that possessed homology sequence to 8 microRNAs (Glinsky, 2008). Furthermore ten of twelve SNPs exhibited sequence homology to microRNAs targeting mRNA of *KPNA1* gene. According to authors SNPs that demonstrated sequence homology to following miRNAs: let-7, miR-548, miR-519, miR-520, miR-181, miR-541 act as phenocode of type 2 diabetes risk. These data suggested that SNPs in non coding but functional genome regions play important role in common human systemic diseases.

In our previous study we have shown difference in genotype distribution of two SNPs located in 3'UTR of *INSR* gene and *PIK3R1* gene and its correlation with insulin resistant

phenotype (Malodobra et al., 2011). It has been shown that G/G genotypes of two SNPs (rs3756668 of *PIK3R1* gene and rs3745551 of *INSR* gene) correlated with BMI, insulin resistant ratios (HOMA-IR and QUICKI) and increased risk of IR .
INSR (Insulin Receptor) is a transmembrane glycoprotein formed by four chains: 2 α and 2 β subunits. The α subunit is responsible for ligand (insulin) binding, whilst the β subunit possesses the activity of tyrosine kinase that autophosphorylate tyrosine residues of β subunit and further kinases (IRS proteins) (Hubbard et al., 1994).
PIK3R1 gene encodes the p85α regulatory subunit of phosphatydylinositol 3-kinase that plays important role in insulin signal transduction, GLUT4 activation and substantially glucose uptake by the cell. Both kinases are extremely important for insulin action (Boura-Halfon & Zick, 2009).
The present work is devoted to evaluation of the influence of two investigated SNPs (rs3756668 of *PIK3R1* gene and rs3745551 of *INSR* gene) on gene expression rate and insulin resistance and type 2 diabetes risk. In presented study the relationships between genotypes and the mRNA levels of interest genes were elucidated.

2. Material and methods

The experimental protocols were approved by ethical review boards at Wroclaw Medical University, No. KB – 556/2008, November 30, 2008.

2.1 Peripheral lymphocytes and adipose tissue collection

Visceral adipose tissue biopsies were taken during abdominal surgery after receiving written agreement. Samples were immediately preserved in RNA*Later* (Ambion), incubated in 4°C for 24 h and then stored at -70°C until analysis. Lymphocytes were isolated from the whole blood taken on anticoagulant by centrifugation on Gradisol G (AquaLab). The 5 ml of the whole blood were placed on 2 ml of Gradisol and centrifuged 2000 rpm for 30 min in 4°C. The lymphocytes ring was collected and washed twice with PBS, the red blood cells were removed by lysis buffer (NH_4Cl, $KHCO_3$, EDTA-Na_2). Then lymphocytes were suspended in PBS, counted and portioned for $3x10^6$ cells per one tube, next centrifuged at max speed for 2 min in 4°C. PBS was discarded and lymphocytes pellet was frozen in -75°C till analysis.
Adipose tissue biopsies were collected from 15 patients with T2DM (6 men and 9 women) and from 24 controls (11 men and 13 women) in similar age (56±8 years for patients and 49±10 years for healthy subjects). Adipose tissue donors were inpatients of First Department and Clinic of General, Gastroenterological and Endocrinological Surgery, Wroclaw Medical University and of Provincial Specialist Hospital, Kamieńskiego in Wroclaw. The aims of abdominal surgeries were mainly cholecystectomy, surgery of abdominal hernia or gastric surgery. Lymphocytes were collected from 34 type 2 diabetic patients (21 men and 13 women) and from equal number of control subjects (17 men and 17 women) in similar age (mean age of diabetic patients was 58±7 years, controls 52±8 years). T2DM patients were inpatients of Department of Angiology, Hypertension and Diabetology, Wroclaw Medical University. Control subjects were selected based on fast glucose level below 106 mg/dl, lack of diabetes in family history, additionally for women no gestational diabetes in the past. Diabetic patients were divided into two subgroups depending on the insulin sensitivity: *IS* – insulin sensitive and *IR* – insulin resistant.

2.2 BMI and insulin resistance ratios

BMI was assessed dividing weight in kilograms by square of height in meters [kg/m^2]. Overweight was assigned with BMI > 25 kg/m^2, obesity with BMI > 30 kg/m^2.
Insulin resistance rate was estimated by insulin resistance ratios, calculated using following formulas:

1. HOMA-IR – [(glucose [mmol/l] * insulin [μU/ml])/22.5],
2. QUICKI – [(log glucose [mg/dl] + log insulin [μU/ml])].

Insulin resistance state was diagnosed with HOMA-IR > 2.5 and QUICKI < 0.321 (Ruano et al., 2006).

2.3 Bioinformatics analysis of investigated SNPs

The bioinformatical analyses of investigated SNPs were done with the use of bioinformatics tools available on-line: http://utrdb.ba.itb.cnr.it/tool/utrscan. The analyses were done to assess the localization of investigated SNPs in functional motifs of *INSR* and *PIK3R1* genes 3'UTRs.

2.4 RNA isolation and gene expression level

RNA from peripheral lymphocytes was isolated with the use of mirVana™ miRNA Isolation Kit (Ambion) according to manufactures protocol dedicated for total RNA isolation. RNA from visceral adipose tissues was isolated using TriPure Isolation Reagent (Roche) according to manufactured protocol. The tissues were homogenized using 2,0 mm Zirconia Beads (BioSpec Products, Inc). After homogenization tissues were centrifuged at max speed for 5 min in 4°C in order to collect the fat depot at the top of tube. The fat was discarded and the homogenate was extracted with 200 μl of chloroform, briefly vortexed and centrifuged for 15 min at max speed in 4°C. The aqueous phase was collected and RNA was precipitated with 500 μl of isopropanol, centrifuged for 10 min at max speed and washed with 1 ml of 70% ethanol in DEPC-treated water. RNA pellet was suspended in RNase-Free water and stored in -75°C.
Reverse transcription was performed with the use of High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufactured protocol. The *PIK3R1* and *INSR* genes expression levels were analyzed in real-time PCR with the use of TaqMan Gene Expression Assay (Applied Biosystems) and Real-Time PCR Universal MasterMix (Applied Biosystems). The quantitative analysis was done as relative gene expression level normalized to two housekeeping genes: *β-actin* and *GUS-β* utilizing delta-delta (ΔΔCt) mathematical model (Pfaffl, 2001).

2.5 Statistical analysis

Statistical analysis was done using STATISTICA8 software. Statistical significance was considered with $p < 0.05$. The association of investigated SNPs with clinical parameters and *PIK3R1* and *INSR* gene expression levels were done with use of one way variance analysis ANOVA. Correlation between gene expression and biochemical parameters were assessed by Pearson's coefficient of correlation.

3. Results

3.1 Anthropometrical and biochemical characterization of analyzed groups

67.5% of all diabetic patients were insulin resistant (*IR*). 32.5% diabetic patients displayed proper insulin sensitivity (*IS*). *IR* patients were characterized by increased BMI value

(*p=0.0203*) and fasting insulin level (*p=0.0000*) as well as insulin resistance ratios (*p=2.1E-06* and *p=1.6E-13* for HOMA-IR and QUICKI, respectively) in comparison to *IS* patients. Glucose level did not show statistical difference between groups of patients with slight increase in *IR* group (*p=0.0538*). Furthermore, *IR* patients manifested higher hypertension and increased TG level. Moreover insulin resistance rate correlated positively with BMI value (HOMA-IR: R=0.44, *p=0.000*; QUICKI R=-0.53. *p=0.000*). Type 2 diabetic patients displayed higher TG (p=0.0316) and lower HDL (0.0006) level.

In previous study the correlation between genotype and insulin resistant phenotype has been presented (Malodobra et al., 2011). Furthermore we have noticed higher frequency of G/G genotypes of both SNPs in *INSR* and *PIK3R1* genes higher risk of this disorders (OR = 1.83 (0.21-0.84) of rs3756668; OR = 2.27 (0.13-0.69) of rs3745551 and OR = 3.14 (0.11-0.28) of G/G_G/G haplotype). Increasing the number of investigated subjects we have revealed the association of rs3756668 G/G_rs3745551 G/G haplotype with insulin resistant phenotype. Carriers of both G/G genotypes were more insulin resistant and were characterized with higher BMI, glucose and insulin level. Results are presented in figure 1.

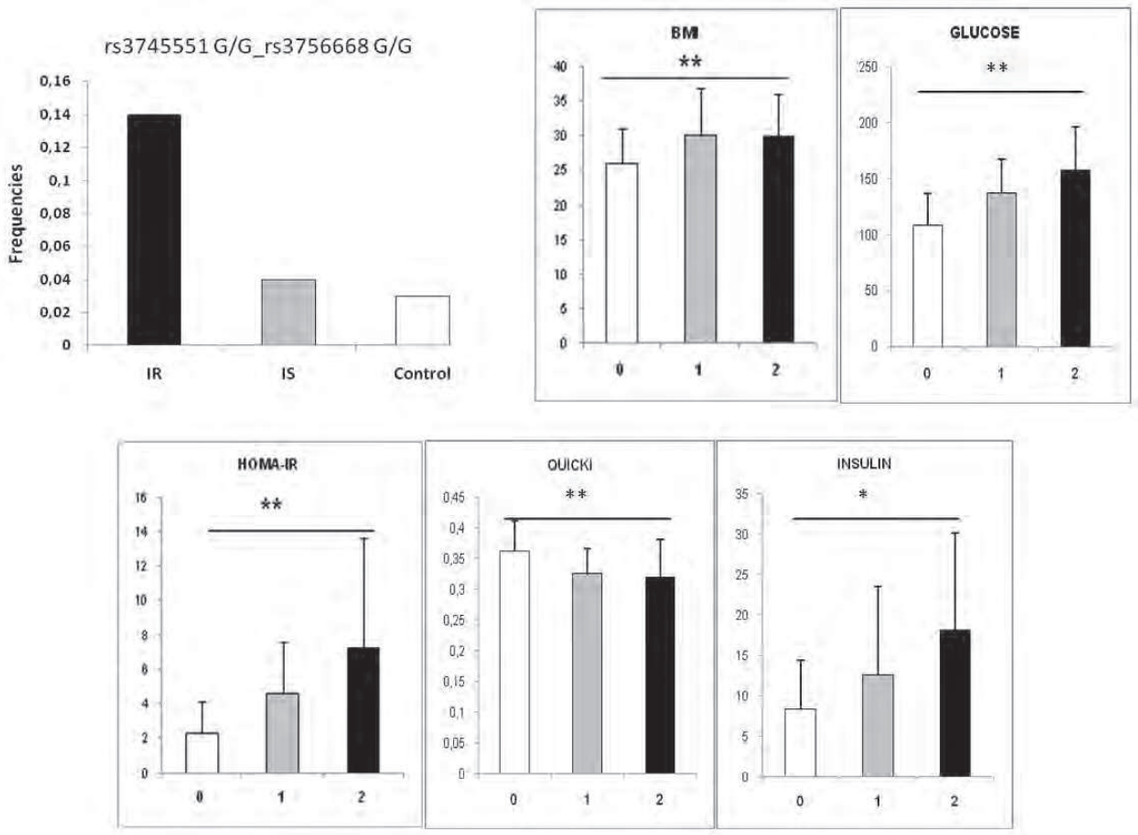


Fig. 1. The relationship between clinical features and number of G/G genotype (**0** - lack, **1** – one G/G genotype either rs3745551 or rs3756668, **2** – carriers of both genotypes; ** < 0.001, * <0.05).

3.2 Bioinformatics analysis of investigated SNPs

The bioinformatics analysis was done in order to evaluate the localization of investigated SNPs in regulatory motifs of *INSR* and *PIK3R1* genes UTRs as well as to determine how the

nucleotide change influence the regulatory elements structure and potentially their function. The analysis has been done for all isoforms of *PIK3R1* and *INSR* genes. The analysis revealed that all investigated SNPs were located in uORF of 3'UTR of examined genes. However, uORF in 3'UTR did not posses functional importance. What's more, the range of regulatory motifs reading frame did not change with the nucleotide substitution. The results of bioinformatics analysis are presented in table 2.

Gene name	rs ID	Type of change	Region	Position in mRNA	Regulatory Element	The wild type reading frame	The mutation type reading frame
INSR (NM_000208.2)	rs1052371 rs3745551	C>T A>G	3′ UTR 3′ UTR	8739 7034	uORF[1] uORF	8735÷9004 7030÷7174	8735÷9004 7030÷7174
INSR (NM_001079817.1)	rs1052371 rs3745551	C>T A>G	3′ UTR 3′ UTR	8696 7001	uORF	8669÷8968 ----------	8669÷8968 ----------
PIK3R1 (NM_181523.1)	rs3756668	A>G	3′ UTR	4876	uORF	4853÷5038	4853÷5038
PIK3R1 (NM_181504.2)	rs3756668	A>G	3′ UTR	4086	uORF	4063÷4248	4063÷4248
PIK3R1 (NM_181524.1)	rs3756668	A>G	3′ UTR	4246	uORF	4223÷4408	4223÷4408

Table 2. The bioinformatics analysis of investigated SNPs.

3.3 *INSR* and *PIK3R1* genes expression rate measurements

The *INSR* and *PIK3R1* genes expression levels were measured in peripheral lymphocytes and visceral adipose tissues in both groups. There was no significant difference in *INSR* and *PIK3R1* genes expression levels performed in lymphocytes between analyzed groups. Similar results were obtained normalized to both housekeeping genes (data not shown). On the other hand there were a significant differences in investigated genes expression levels measured in visceral adipose tissues. Both, the *IR* and *IS* diabetic patients displayed significantly lower mRNA abundance of both analyzed genes comparing to healthy subjects. The differences in genes expression level were statistically significant when normalized to *β-actin* ((p=0.004 and p=0.0159 for *PIK3R1*gene and *INSR* gene respectively). The relative analyses of genes expression rate normalized to *GUS-β* were close to be significant.

3.4 Genotype association with the *INSR* and *PIK3R1* genes expression levels

In order to analyze whether the genotype of investigated SNPs located in 3'UTRs of *INSR* and *PIK3R1* gene might influence gene expression rate the genotyping results (described previously, Malodobra et al., 2011), and gene expression analyses results were analyzed

using ANOVA. There was no significant relationship between genotype of analyzed SNPs and gene expression rates in peripheral lymphocytes (data not shown). There was no relationship between genotypes of SNP located in 3'UTR of *PIK3R1* gene and *PIK3R1* gene expression in adipose tissue. Despite the fact, that *PIK3R1* gene showed decreased expression level in adipose tissue in diabetic patients and the rs3756668 of *PIK3R1* showed higher frequency in G/G genotype in those patients, the decreased *PIK3R1* gene expression did not correlate with genotype.

On the other hand there was statistically significant relationship between rs3745551 located in 3'UTR of *INSR* gene and *INSR* gene expression rate. The G/G genotype carriers displayed considerably lower mRNA abundance in visceral adipose tissue comparing to homozygotes A/A and heterozygotes A/G. However the relationship was seen for healthy subjects only. In type 2 diabetic patients, both in *IR* and *IS* the *INSR* gene expression level was low and with no substantially differences between genotype carriers. The relationship between gene expression rate of investigated genes in adipose tissue and genotype of analyzed SNPs is presented in figure 2.

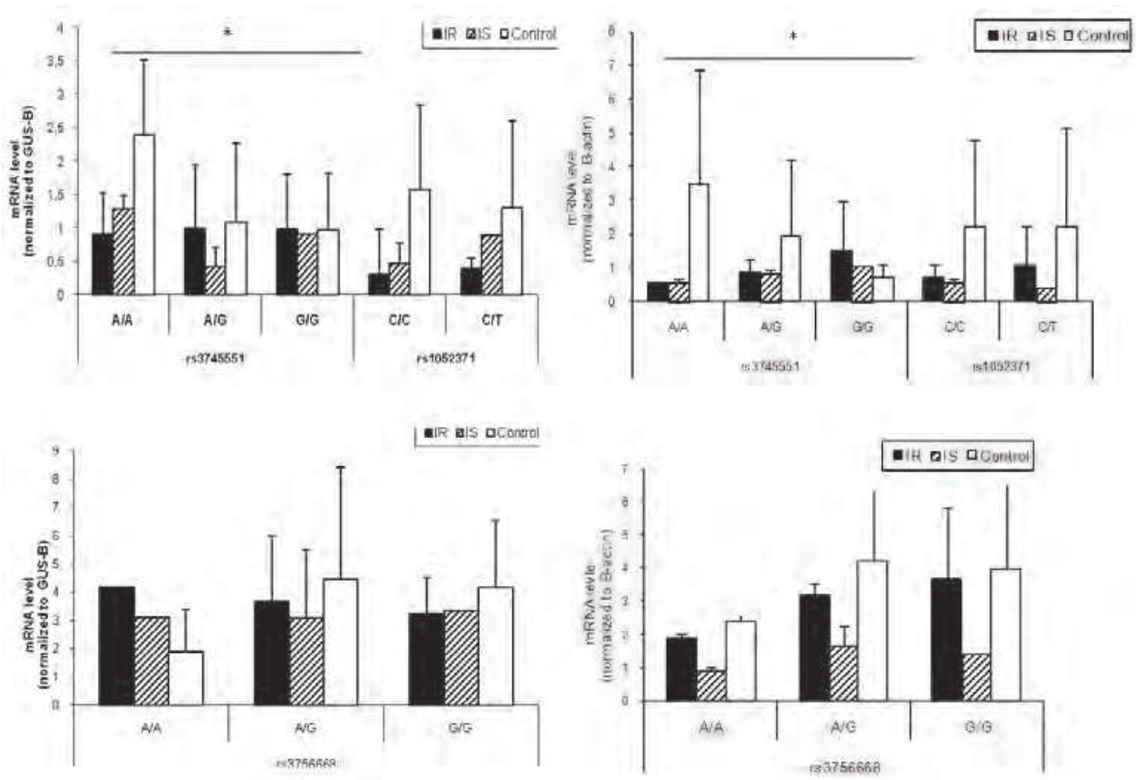


Fig. 2. Relationship between *INSR* and *PIK3R1* genes expression level and genotypes of investigated SNPs (* $p<0.05$).

3.5 Correlation between *INSR* and *PIK3R1* genes expression levels, biochemical parameters and insulin resistant phenotype

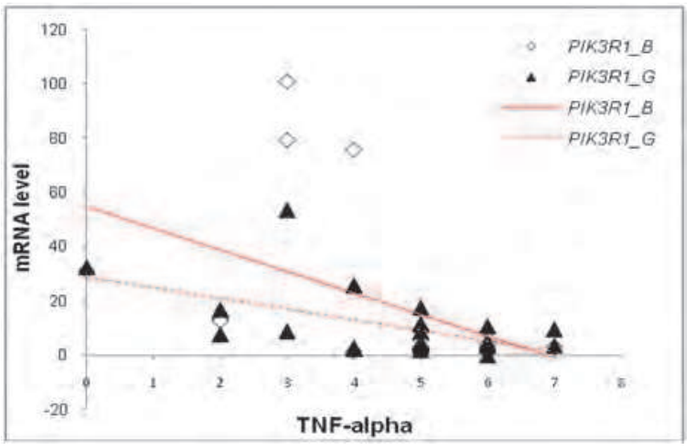
The *INSR* and *PIK3R1* genes expression rates measured in lymphocytes showed correlation with insulin resistance ratios. There was negative correlation between *INSR* and *PIK3R1* mRNA level and fasting insulin concentration and HOMA-IR as well as positive correlation

between mRNA level and QUICKI. In addition *INSR* gene expression rate showed negative correlation with TG and CHOL. Similar results were seen for gene expression normalization to both housekeeping genes. The correlation between investigated gene expression rate and clinical parameters is presented in table 3.

The *INSR* and *PIK3R1* genes expression rates measured in adipose tissue correlated neither with analyzed clinical feature nor with insulin resistance ratios (data not shown). However, *PIK3R1* gene expression rate showed negative correlation with TNF-α concentration (R = (-0.82), p=0.026 normalized to *β-actin* and R = (-0.70), p=0.08 normalized to *GUS-β*). The relationship between *PIK3R1* mRNA level and TNF-α concentration presents figure 3.

	Glucose [R(p)]	**Insulin [R(p)]**	**HOMA [R(p)]**	**QUICKI [R(p)]**	**TG [R(p)]**	**CHOL [R(p)]**	**HDL [R(p)]**	**LDL [R(p)]**	**BMI [R(p)]**
PIK3R 1_B	n.s.	-0.42 (0.02)	-0.44 (0.022)	0.46 (0.015)	n.s.	n.s.	n.s.	n.s.	n.s.
PIK3R 1_G	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
INSR_ B	-0.33 (0.02)	-0.48 (0.02)	-0.53 (0.01)	0.53 (0.01)	-0.31 (0.09)	-0.35 (0.06)	n.s.	n.s.	n.s.
INSR_ G	n.s.	-0.57 (0.006)	-0.59 (0.004)	0.66 (0.001)	-0.32 (0.06)	-0.54 (0.005)	n.s.	-0.37 (0.05)	n.s.

Table 3. The correlation between genes expression and clinical parameters (_B – normalized to *β-actin*; _G – normalized to *GUS-β*; n.s. – not significant).



PIK3R1_B R = (-0.82), p = 0.026
PIK3R1_G R = (-0.70), p = 0.08

Fig. 3. The correlation between *PIK3R1* mRNA and TNF-α (_B – normalized to *β-actin*; _G – normalized to *GUS-β*).

4. Discussion

The genetic predispositions are large components that trigger the IR and T2DM risk and pathogenesis. SNPs in functional region are in great interests of numerous investigators and are associated with variety of diseases pathogenesis. The relationships between IR and SNPs

in UTRs have been reported by many investigators (Xia et al., 1999; Chen et al., 2006; Nelsøe et al et al., 2006). Especially 3'UTR is considered as a "hot spot" of pathology and polymorphic sites located within 3'UTR are associated with increased risk of numerous diseases (Conne et al., 2000). Taking into consideration the fact that IR is characterized by deregulations in numerous genes expression rates encoding important for insulin signaling kinases, the SNPs located in UTRs of these genes were particularly under investigation. Numerous SNPs located in *INSR*, *PIK3R1*, *PTPN1* and *SLC2A4* genes were genotyped and correlated with insulin resistant phenotype (Malodobra et al., 2011). Overwhelmingly two out of seven genotyped SNPs showed the association with insulin resistant phenotype (BMI, HOMA-IR, QUICKI) as well as with increased risk of IR development (assessed based on OR (95%Cl)). The G/G genotypes of rs3756668 located in 3'UTR of *PIK3R1* gene and rs3745551 located in 3'UTR of *INSR* gene positively correlated with higher BMI value, fasting glucose and insulin level, as well as G/G carriers were more insulin resistant (based on HOMA-IR and QUICKI ratios). It is worth to mention that in logistic regression analysis we displayed additional interaction between G/G genotypes of those two SNPs with increased OR value (Malodobra et al., 2011).

In present work, the *PIK3R1* and *INSR* genes expression analyses in peripheral lymphocytes and adipose tissue were presented. The main aim of this paper was to evaluate the role of those two SNPs in gene expression regulation at post-transcriptional level and how the nucleotide changes influence mRNA level in the cell.

The present work contains as well the influence the G/G haplotype of rs3745551 (*INSR*) and rs3756668 (*PIK3R1*) on insulin and glucose metabolism and insulin resistant phenotype.

Previously described results (Malodobra et al., 2011) demonstrated that two out of seven genotyped SNPs showed the association with insulin resistant phenotype. Those two SNPs displayed as well increased risk of IR development. Thus in present work thanks to increasing the number of analyzed subjects we were able to evaluate the relationship between two SNPs haplotype (rs3756668 G/G and rs3745551 G/G) and insulin resistant phenotype. Very interesting correlation has been observed with progressively increased insulin resistance state (assessed by clinical parameters: fasting glucose and insulin concentrations, HOMA-IR and QUICKI ratios) depending on number of G/G genotypes. The higher insulin resistant state has been seen for carriers of both G/G genotype, moderate values of measured parameters have been seen for carriers one of at risk genotypes. The lowest values of measured parameters possessed subject not affected by at risk G/G genotype.

In contradictory to results described by others investigators (Maratou et al., 2007; Piatkiewicz et al., 2007), we did not detect impairments in insulin signaling in patients with T2DM both in *IS* (insulin sensitive) and *IR* (insulin resistant) measured in lymphocytes. There was no difference in *INSR* and *PIK3R1* genes expression rates between investigated groups. In type 2 diabetic patients, both *IS* and *IR*, the mRNA levels of investigated genes were similar to those measured in healthy controls. Piatkiewicz et al. suggested that peripheral lymphocytes might stand for perfect cellular model for insulin signaling investigation in type 2 diabetes (Piatkiewicz et al., 2007). Obtained results did not confirm that statement, there was no evidence for defects in insulin signal transduction and GLUT4 activation (at mRNA level). However, presented results ought to be evaluated on protein level. On the other hand, expression rates of analyzed genes measured in lymphocytes negatively correlated with fasting glucose and insulin

concentrations as well as with HOMA-IR values, whilst positively with QUICKI ratio. These results suggest that peripheral lymphocytes are very sensitive for environmental changes in glucose and insulin level.

The pathomechanism of impairment in insulin sensitivity in adipose tissue is quite different than in other tissues (skeletal muscles, liver) (George, 1996). In adipose tissue the main dysfunction leading to insulin resistance is the adipogenesis deregulation favoring differentiation towards pro-inflammatory cells (Gustafson et al., 2009). In addition hypertrophy and hyperplasia of adypocytes further lead to insulin sensitivity impairment. The visceral adipose tissue is especially implicated in metabolic syndrome pathogenesis including IR (Preis et al., 2010), for that reason this type of adipose tissue has been collected for analysis. Adipose tissue is characterized by deregulation in expression as well as phosphorylation rate of numerous genes and kinases (Ahmad et al., 1995; Andreelli et al., 2000; Patti, 2004; Rasouli & Kern, 2008).

In presented study in type 2 diabetics adipose tissues, *IR* as well as *IS* patients, the mRNA levels of *INSR* and *PIK3R1* genes were significantly lower comparing to healthy controls. These data stand for the fact, that insulin signaling in adipocytes in type 2 diabetic patients is impaired by decrease in gene expression. Similar results have been observed and described by others authors, who also diagnosed lower mRNA as well as protein abundance of INSR and p85 α in T2DM patients (George, 1996; Hansen & Shafrir, 2002; Rasche et al., 2008).

It has been proved by many investigators that SNPs in UTRs might affect mRNA stability and translation initiation processes (Mendell et al., 2005; Chen et al., 2006). In order to assess whether investigated SNPs in *INSR* and *PIK3R1* genes might influence the mRNA abundance in the cells we analyzed genotyping and expression rates results. First of all, however, the bioinformatical tools were utilized in order to evaluate the exact localization of SNPs in regulatory elements of 3'UTRs and whether the nucleotide change might reorganize the frames of regulatory motifs in UTRs. The investigated SNPs were localized in uORFs of 3'UTR and the changes in nucleotide sequence did not influence the reading frames of those regulatory elements.

Despite the fact we performed evaluation how particular genotype of investigated SNPs affects the gene expression rate. We did not notice significant changes in *INSR* and *PIK3R1* genes expression rates measured in peripheral lymphocytes. There was no difference in mRNA abundance in relation to genotypes. Similar results were received for all groups and for genes expression analyses normalized to both housekeeping genes. Obtained results negate the possible role of investigated SNPs in genes expression regulation at post-transcriptional level.

Similar analysis has been done for *INSR* and *PIK3R1* genes expression measurements in adipose tissues and for genotypes of investigated SNPs (rs3745551 and rs1052371). One of the investigated SNP displayed relationship with mRNA abundance with difference statistically significant. The G/G carriers were characterized by the lowest mRNA level comparing to A/A and A/G carriers. It is worth to mention that G/G genotype of this SNP in previous study showed association with IR risk and with insulin resistant phenotype. However, the difference reached significance only in healthy subjects. In type 2 diabetic patients the mRNA level was very low and did not differ depending on genotype. Probably some others factors (genetic of environmental) strongly influenced *INSR* gene expression in those subjects thus, noticed in healthy controls relationship between mRNA level and

genotype, in groups exposed to those factors, has been abolished. Second polymorphism investigated in *INSR* gene (rs1052371) did not display relationship between mRNA abundant in relation to genotype.

The rs3756668 located in 3'UTR of *PIK3R1* gene in previous study, similar to rs3745551 of *INSR* gene, showed association with increased IR risk and with insulin resistant phenotype. However, when genotyping results were compared with *PIK3R1* gene expression rate, there was no relationship between genotypes of this SNP and mRNA level. Lack of correlation might be due to the low number of analyzed samples. Further experiments must be done in order to evaluate how this SNP influence increased risk to IR and T2DM.

Described in present study results provide the first evidence for association of SNPs in UTRs of *INSR* and *PIKR1* genes with genes expression rates. So far, all investigated in these genes SNPs were localized mainly in coding regions (Kusari et al., 1991; Baynes et al., 2000; Almind et al., 2002; Jamshidi et al., 2006; Højlund et al., 2006). Presented results stand for the first report where evaluation of SNPs in UTRs of *INSR* and *PIK3R1* genes with gene expression levels was performed in peripheral lymphocytes and adipose tissues.

5. Conclusion

Concluding genes expression measurements, presenting results negate the dysfunction in insulin signaling in peripheral lymphocytes, at least at mRNA level. On the other hand the expression rate of genes implicated in insulin action is decreased in adipose tissue of patients with T2DM. The rs3745551, that in previous study showed correlation with insulin resistance, in present work, displayed relationship with *ISNR* gene mRNA level in adipose tissue. The relationship however was seen for healthy controls only. The second SNP that in previous study showed association with insulin resistance, in present work we did not show the relationship between genotype and mRNA level. Further study must be done in order to evaluate how this SNP is implicated in increased risk to IR.

The present study also showed the effect of two SNPs haplotype influence on insulin resistant phenotype.

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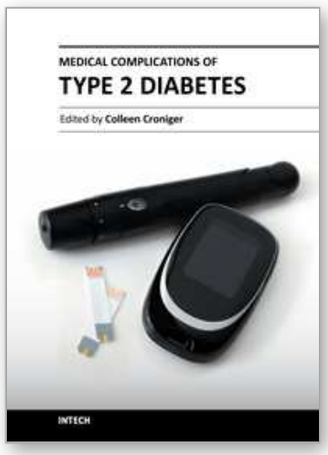

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Obesity and type 2 diabetes are increasing worldwide problems. In this book we reviewed insulin secretion in both healthy individuals and in patients with type 2 diabetes. Because of the risk associated with progression from insulin resistance to diabetes and cardiovascular complications increases along a continuum, we included several chapters on the damage of endothelial cells in type 2 diabetes and genetic influences on endothelial cell dysfunction. Cardiovascular complications occur at a much lower glucose levels, thus a review on the oral glucose tolerance test compared to other methods was included. The medical conditions associated with type 2 diabetes such as pancreatic cancer, sarcopenia and sleep disordered breathing with diabetes were also discussed. The book concludes with several chapters on the treatments for this disease offering us hope in prevention and successful alleviation of the co-morbidities associated with obesity and type 2 diabetes.

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