INSULIN RECEPTOR AND ITS RELATIONSHIP WITH DIFFERENT FORMS OF INSULIN RESISTANCE

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Summary: Insulin plays an important role in maintaining the whole organism's homeostasis. The presence of insulin receptors in all vertebrates and invertebrates cells reflects the diversity of regulatory processes in which this hormone is involved. Furthermore, many different factors may influence the level of insulin receptor expression. These factors include e.g. the sole insulin or stage of development. Mutations in the receptor may lead to the development of insulin resistance. These mutations differ in the level of severity and are frequently associated with diabetes mellitus, hypertension, cardiovascular disorders, heart failure, metabolic syndrome and infertility in women. More than 50 mutations in insulin receptor gene have already been characterized. These mutations are associated with rare forms of insulin resistance like leprechaunism, insulin resistance type A or Rabson-Mendenhall syndrome. Molecular analysis of insulin receptor gene may lead to a better understanding of molecular mechanisms underlying various types of insulin resistance and help to develop more efficient treatment.

Key words: insulin receptor, *INSR* gene, alternative splicing, insulin receptor isoforms, hybrid receptors, insulin resistance.

Abbreviations: **RMS** – Rabson-Mendenhall Syndrome; **INSR** – insulin receptor gene; **INSR** – insulin receptor gene's transcript or insulin receptor; **PCOS** – polycystic ovary syndrome; **SNP** – single nucleotide polymorphism; **EGFR** – epidermal growth factor; **C/EBP β** – CAAT/enhancer binding protein β ; **TBP** – TATA binding protein; **TAFs** – TATA associated factors; **GRE** – glucocorticoid response element; **CAT** – chloramphenicol acetyltransferase; **EMSA** – electrophoretic mobility shift assay; **IR-DBP** – insulin receptor DNA binding protein; **HMGI-Y** – high mobility group; **HT-FIR** – hepatocyte-specific transcription factor of the insulin receptor gene; **IGF-I**, **IGF-II** – insulin-like growth factor I, II; **ISE** – intronic sequence enhancer; **ESE** – exonic sequence enhancer; **ISS** – intronic sequence silencer; **ESS** – exonic sequence silencer; **5'**, **3'UTR** – 5', 3' untranslated region; **pre-mRNA** – precursor-messenger RNA; **BP** – branch point; **U1snRNP** – U-rich 1 small nuclear ribonucleoprotein particle; **SF2/ASF** – splicing factor 2/alternative splicing factor; **hnRNP-A1**, **F** – heterogenous nuclear ribonucleoprotein A1, F; **IRS** – insulin receptor substrate; **APS** – adaptor protein; **PI3K** – phosphatidylinositol 3-kinase; **PIP2** – phosphatidylinositol 4,5-bisphosphate; **PIP3** – phosphatidylinositol 3,4,5-triphosphate; **GLUT4** – glucose transporter 4; **Grb-2** – growth factor

receptor-binding protein 2; **ATP** – adenosinotrisphosphate; **LRR** –leucine-rich repeat; **CR** – cysteine rich; **FU** – furin-like; **Fn0**, **Fn1**, **Fn2** – fibronectin type III; **TM** – transmembrane domain; **JM** – juxtamembrane domain; **TK** – tyrosine kinase domain; **CT** – carboxyterminal tail; **ER** – endoplasmatic reticulum; **Grp94** – glucose-regulated protein; **kDa** – kilodalton; **HSP90** – heat shock protein 90; **E11⁺** – transcript of the insulin receptor gene containing alternative exon 11 or a protein encoded by this transcript; **E11⁻** – transcript of the insulin receptor gene without alternative exon 11 or a protein encoded by this transcript; **IgG** – immunoglobulin G; **Met** – methionine; **VLDL** – very low density lipoproteins; **CGL** – congenital generalized lipodystrophy; **FPL** – familial partial lipodystrophy; **AGPAT2** – 1-acylglycerol-3-phosphate-O-acyltransferase 2; **BSCL2** –Berardinelli-Seip Congenital Lipodystrophy type 2; **PPAR γ** – peroxisome proliferator-activated receptor gamma; **ZMPSTE24** – zinc metalloproteinase, STE24 *Saccharomyces cerevisiae* homologue

INTRODUCTION

Insulin signal transduction pathways play important roles in maintaining the cellular homeostasis through regulation of glucose metabolism, glycogen synthesis, lipids and proteins metabolism, ions and amino-acids transport, control of cell cycle, proliferation and cell differentiation in addition to gene transcription and nitrogen oxide synthesis.

The presence of insulin receptors in most vertebrate cells further reflects the diversity of regulatory processes in which insulin is involved. However, it was noted that several factors may influence the level of insulin receptor expression, including the sole insulin, glucocorticoids or stage of development. Furthermore, splicing of the insulin receptor transcript is also regulated in a developmentally dependent manner [42, 81].

Mutations in the insulin receptor are correlated with insulin resistance syndromes of differing severity and are frequently associated with type 2 diabetes mellitus, hypertension, cardiovascular disorders, heart failure, metabolic syndrome and even infertility in women [152].

Health consequences of the insulin resistance are best represented by polycystic ovary syndrome (PCOS) or predisposition to breast cancer, colorectal carcinoma or pancreatic cancer [1, 16, 28-30, 33, 41, 44, 47, 55, 56, 79, 89, 90, 96, 102, 103, 115, 116, 121, 124, 156, 163].

In which way does insulin resistance predispose women to polycystic ovary syndrome?

Almost 30 year ago, patients with extreme insulin resistance were shown to have enlarged and polycystic ovaries combined with hyperandrogenism of ovarian origin [62]. Recently, it has been stated that most common forms of insulin resistance are associated with enlarged ovaries [6, 97, 98, 108]. While metformin therapy can increase ovulation rate in patients with common forms of polycystic ovary syndrome (PCOS), recently the main focus in PCOS treatment has been the use of insulin receptor sensitizing drugs [29, 30, 41, 47, 90, 115, 156]. As insulin is

a potential ovarian growth factor and stimulator of the androgen production, hyperinsulinemia is frequently observed in PCOS and is a common factor in extreme forms of insulin resistance syndromes. In contrast, gonadotropins stimulate normal ovarian growth during puberty as well as estrogens and progesterone production. However, in the case of insulin resistance syndromes or in common forms of PCOS, the unique role of insulin as a stimulatory ovarian growth factor has not been clarified. While gonadotropins stimulate ovarian growth and steroidogenesis during puberty, hyperinsulinemia has been shown to stimulate pathological ovarian growth and androgenesis during the whole life [99]. Moreover, insulin stimulates the ovary independently from gonadotropins [6, 22, 62, 97, 98, 108, 109]. Furthermore, in infants and small children suffering from leprechaunism, ovarian enlargement may be extreme in comparison to healthy infants with circulating gonadotropins, where it may be expected that the volume of ovaries is less than 1 cm³ [2, 7, 9, 59, 63, 118, 157].

While ovarian function has been partially restored (reduction in androgens secretion and recovery of menstruation) in older patients with lipodystrophy who were treated with leptin and in patients with insulin receptor auto-antibodies; no change in ovarian size was observed [6,97,98]. Hyperandrogenism in women with PCOS is often associated with a general increase of ovarian steroidogenesis, involving P450c17 α cytochrome activity which is a crucial enzyme involved in ovarian androgen biosynthesis. Hyperinsulinism either directly stimulates ovarian steroidogenesis through an increase in P450c17 α activity or indirectly by stimulation of gonadotropins secretion [28, 102, 103]. Interestingly, in younger patients ovarian growth predominates the androgen increase, while in older patients both effects (ovarian growth and ovarian androgen over-secretion) are usually observed [98].

Insulin resistance and neoplasia

Metabolic syndrome refers to the relationship between insulin resistance, obesity and the risk of chronic disorders including cancer. While many of these associations remain unsolved, there is evidence to suggest that night lipolysis which is secondary to sympathetic stimulation, may not only lead to insulin resistance, but it may also be responsible for hyperinsulinism due to the stimulation of insulin secretion and a decrease in insulin clearance by the liver. Disorders generated in this way – an increase in night-time free fatty acids and insulin concentration – may synergistically lead to a higher risk of some types of cancer [55].

Obesity and insulin resistance are risk factors for the development of breast cancer and are often associated with late stages of the disease and with poor prognosis. Angiogenesis has been shown to be relevant to the development of breast cancer as it is likely that an increase in the production of some adipocytokines including VEGF, HGF, leptin, TNF α , HBEGF and IL-6, in

conjunction with a decrease in adiponectin which is known to suppress angiogenesis, enhances angiogenesis in obese individuals [116]. Obesity is also an independent risk factor for the development and progression of pancreatic carcinoma and *in vivo* studies revealed that the decreased levels of adiponectin combined with insulin resistance may directly lead to changes in a tumor's microenvironment, enhancing growth and metastasis of pancreatic carcinoma [163].

More than 50 mutations in insulin receptor gene, *INSR*, have been described. Such mutations are associated with rare forms of insulin resistance including leprechaunism, insulin resistance type A and Rabson-Mendenhall syndrome. Analysis of DNA and RNA from patients diagnosed with one of these syndromes has provided insights into the receptor's structure and function in the activation of downstream genes. These mutations were divided into 4 groups. Group 1 includes mutations that lead to a down-regulation of insulin receptor gene, while mutations that result in intracellular insulin receptor transport disorders and its maturation process (such as signal peptide cleavage or glycosylation) belong to group 2. Group 3 consists of mutations that are associated with insulin receptor binding defects and group 4 includes mutations that are characterized by disruptions in the catalytic activity of the tyrosine kinase domain.

The aim of the present work is to summarize the current knowledge on insulin receptor and its relationship with different forms of insulin resistance.

INSULIN RECEPTOR GENE (*INSR*)

The insulin receptor gene, *INSR*, (Gene ID: 3643) located on short arm of chromosome 19 (19p13.3), is 180 kbp (kilobase pairs) long and consists of 22 exons (Fig. 1).

Most of the first exon encodes for 27 amino acid long signal peptide which directs the protein to plasma membrane. The first 11 exons of *INSR* gene encode the receptor's α subunit (this part of the gene spreads 90 kbp), while another 11 exons encode the β subunit and spreads approximately 30 kbp [27, 57, 131, 153]. Knowing the exon-intron *INSR* gene's structure enabled establishment of the functional and structural regions of the encoded protein. First exon encodes for signal peptide, the second one encodes for insulin binding region. Exon 3 encodes for cysteine-rich region and exons 4-10 encode for a major part of the receptor's α subunit. Exon 11 stands for alternatively spliced miniexon, while exons 12-14 encode for extracellular part of β subunit. Exon 15 encodes for transmembrane domain and exon 16 encodes for juxtamembrane domain. Exons 17-21 with a little part of exon 22 encode for tyrosine kinase domain and the last one exon 22 encodes for a carboxyterminal part of the protein. In addition, the region spanning

exons 2-5 has been shown to be homologous to a segment of the human epidermal growth factor (EGFR) gene [131].

Over 20 years ago the first several single nucleotide polymorphism (SNP) were found in *INSR* and included a range of silent mutations and missense mutations I421T (ATC-ACC) and Q465K (CAG-AAG) [131]. Subsequently, over 1820 SNP polymorphisms have been identified over the entire human *INSR* locus including the promoter region as well as 5' and 3' untranslated regions (UTRs) (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp&cmd=search&term=>; 17.06.2009).

Interestingly, four of these polymorphisms have been clinically associated with different forms of insulin resistance. In 1988, Kadowaki *et al.* identified two different *INSR* alleles from a patient with leprechaunism. The maternal allele contained a missense mutation K460E (AAG-GAG) in exon 6, while a nonsense mutation D672X was identified in the paternal allele. As a result of these mutations, the protein encoded by this transcript lacked the transmembrane domain and the entire β subunit [60].

The presence of the polymorphism N462S (AAT-AGT) also in exon 6 was identified in a patient diagnosed with insulin resistance and the skin disorder, Acanthosis nigricans [60].

In 1990, during the examination of family members, Moller *et al.* identified a polymorphism in exon 19 of the *INSR* gene. While the father suffered from hyperinsulinism (together with other abnormalities) and the mother was unaffected, their three daughters were diagnosed with insulin resistance type A (OMIM ID: 610549). Molecular studies revealed that the father and offspring carried an abnormal *INSR* allele that contained a dominant mutation A1134T (GCA-ACA) which disrupts the tyrosine kinase domains. Transfection analysis of CHO cells expressing mutant insulin receptors revealed that the mutation caused a decrease in the autophosphorylation of the receptor protein after binding its ligand, whereas the process of receptor maturation remained normal, as well as its ability to bind insulin [92].

Four years later, the heterozygous SNP polymorphism (CGG-CAG) in exon 20 of the *INSR* gene was found in 22 unrelated women, which suffered from insulin resistance, acanthosis nigricans and polycystic ovary syndrome (OMIM ID: 610549). The result of this SNP polymorphism was a R1174Q substitution in tyrosine kinase domain in the intracellular part of the receptor's β subunit. As the mutation was present in patient's affected sister, and absent in her mother, presumably two affected parents' aunts were also carriers of this mutation, thus SNP polymorphism CGG-CAG (R1174Q) is considered to be the main cause of the dominant inherited insulin resistance [93].

INSULIN RECEPTOR GENE PROMOTER REGION'S CHARACTERISTICS

The *INSR* promoter region was first described in 1987 by Araki *et al.* [3] while subsequent characterization was undertaken by Seino *et al.* [131] (Fig. 2). Spanning approximately 1800 bp upstream of the translation start codon bioinformatics analysis revealed that the promoter region is extremely GC rich. Interestingly, the promoter region lacks a TATA-box, which is present in most of genes transcribed by RNA polymerase II, as well as a CAAT-box. These characteristics are similar to the regulatory regions of housekeeping genes, which are expressed at steady levels including the human 3-phosphoglycerate kinase, human epidermal growth factor receptor, human hypoxanthine phosphoribosyltransferase, human adenosine deaminase, as well as hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase and murine hypoxanthine phosphoribosyltransferase [3]. In addition, a transcription enhancer element located 410-480 bp upstream start codon has been identified. Furthermore, several additional enhancer elements have been found within the promoter region that may bind C/EBP β protein (CAAT/enhancer binding protein β). Other DNA binding proteins elements were also found.

Additional features of the *INSR* promoter include seven potential Sp1 binding sites (GGGCGG or CCGCCC), two pairs of inverted CCGGGCCCG and CCCGGGCCGC repeats, which can be bound by transcription factors playing a role in the regulation of transcription initiation, three CCCGGGCGCAG and two CCGCCC direct repeats [3].

As the transcription factor, Sp1, enhances RNA polymerase II-directed transcription, it has been suggested that *INSR* transcript levels is modulated by Sp1 [3].

Further studies have discovered that the four GC-rich regions located at position -593 to -618 of the promoter, including Sp1 binding sites are the main elements regulating *INSR* expression in CHO and COS cells [4, 54]. Interestingly, the Sp1 activation domain, located in the amino terminus, possesses two glutamine-rich domains A and B which are able to interact with RNA polymerase II transcription factor TFIID, consisting of TATA binding protein (TBP) and several TBP associated factors – TAFs. It has also been shown that TAFII-110 directly interacts with Sp1 [54].

Furthermore, it was noted that there are IRNF-I and IRNF-II (Insulin Receptor Nuclear Factor) binding sites located at positions -530 to -550 and -500 to -520 of the *INSR* gene promoter region. It was shown that mutations which alter these binding sites strongly down-regulate insulin receptor gene expression. This shows that both factors are necessary for its proper expression pattern [76]. Interestingly, adenoviral E1a protein may also bind to Sp1 factor binding sites within the promoter region and thus regulate *INSR* gene expression [66].

FIGURE 2. The promoter region of the human insulin receptor gene. Due to the presence of several transcription initiation sites, nucleotide numbering is relative to the first nucleotide of the translation start codon +1 ATG (encoding for methionine, Met). The *Altu* sequence is underlined. 7 Sp1 transcription factor binding sites are also shown [3]. Start 1, start 2, start 3 – transcription initiation sites according to [131], start 4 – transcription initiation site according to [3]. Black arrows stand for transcription direction, grey for direct repeats and broken arrows stand for inverted repeats. HMGI-Y, Sp1 and C/EBP β transcription factors binding sites contained in E3 and C2 fragment are also shown [37] (according to [3, 37, 131, 150], modified)

Using primer extension and nuclease S1 mapping/ nuclease S1 protection assays, three transcription initiation sites at positions 276, 282 and 283 bp upstream the ATG codon have been identified, with the most abundant transcripts starting at positions 276 and 282 [32]. Similar features such as *inter alia* lack of TATA-box and a presence of several transcription initiation sites, are a characteristic feature of promoters regions of many housekeeping genes [58, 67, 139].

It was observed that *INSR* gene is up-regulated by glucocorticoids [84]. While it has been suggested that these hormones may lead to the induction of *INSR* gene transcription, analysis of the 5' promoter region failed to reveal the presence of the glucocorticoid response elements (GREs) [64]. In turn, Rubin *et al.* identified *Alu* sequence in the 5' end of the *INSR* promoter, which is thought to play a role in protein binding and may thus potentially regulate insulin receptor gene expression [119]. Several potential regulatory promoter regions have also been identified which are important for cell-specific receptor protein expression [12, 14, 145]. DNase I footprinting analysis revealed that two AT-rich regions, which were named as C2 and E3 fragment located at positions: -671 to -874 and -1661 to -1818, respectively, are essential for *INSR* gene expression during differentiation of myoblasts into myocytes in BC3H-1 muscle cells [13]. Interestingly, a chloramphenicol acetyltransferase reporter gene assay revealed that these sequences acted rather as promoters than transcription enhancer elements. Accordingly Brunetti *et al.* suggested that the proteins binding to these regions play a crucial role in the spatial expression of *INSR* [13]. Further experiments revealed that these regions (C2 and E3) are bound by high mobility group proteins (HMGI-Ys), in this way activating *INSR* gene transcription [15]. Electrophoretic mobility shift assay (EMSA) and Western blot experiments using nuclear proteins isolated from human lymphocyte IM-9 cell line enabled identification of the protein, which was named IR-DBP (Insulin Receptor DNA Binding Protein) and was highly similar to HMGI-Y proteins. While IR-DBP protein is a distant member of HMG protein family, it is able to bind and bend the minor groove of double-stranded DNA helix, leading to the changes in local DNA conformation. Through interactions with numerous transcription factors, IR-DBP may activate gene transcription [20, 112]. It was observed that the HMGI-Y inhibition in cells up-regulating *INSR*, led to its down-regulation. In turn, HMGI-Y over-expression in cells down-regulating the *INSR*, led to its up-regulation [15]. Taken together, it has been suggested that HMGI-Y positively regulates the expression of *INSR*. Accordingly, mutations affected HMGI-Y may contribute to abnormal function of the insulin receptor and subsequently to the disorders in insulin signal transduction pathway.

It was also observed that 278 bp long region located in the 5' end of the first intron of *INSR* is essential for expression in HIRIN3 adipocytes [85].

In 2001 Youshizato *et al.* identified a *cis*- regulatory element (-592 to -577) and *trans*-acting factor which influenced the expression of *INSR* in *hepatocarcinoma* HepG2 cells and in rat hepatocytes. Subsequent analysis revealed

that characterized the 35 kDa *trans*-acting nuclear protein, HT-FIR factor (Hepatocyte-specific Transcription Factor of the Insulin Receptor gene) could bind a 5'-TCCCTCCC-3' sequence located within this promoter region (position -588 to -581).

Although the insulin receptor gene promoter activity is associated with a region located approximately 579 bp upstream the translation initiation codon, it is suggested that the whole promoter region is necessary for its maximal activity [14].

While basal promoter activity is observed in all cells, up-regulation of *INSR* was observed in skeletal muscles, the liver, adipose tissue or the brain. This may suggest the existence of tissue-specific factors regulating *INSR* expression, whereas differences in the level of gene expression in different tissues may result from the presence or absence of tissue-specific transcription factors. It is known that promoters of many genes which are tissue-specifically expressed are frequently under the control tissue specific and general transcription factors [88].

In 2003, Foti *et al.* [37] uncovered that the regulation of *INSR* is dependent on the formation of a nucleoprotein complex (called enhanceosome) which in HepG2 cells is composed of HMGI-Y and general transcription factors. Foti *et al.* also showed that HMGI-Y played an essential role in this complex and was necessary for the assembly of an active transcription initiation complex [37]. Additional *in vitro* and *in vivo* experiments also revealed that the interactions between HMGI-Y, Sp1 and C/EBP β significantly increased the insulin receptor gene promoter region activity and activated RNA polymerase II-directed transcription [37]. Consequently, any defects of transcription factors described above may influence the insulin receptor gene expression and thereby a lack of response on insulin.

INSULIN RECEPTOR TRANSCRIPT'S CHARACTERISTICS

INSR was first identified in 1971 by Freycht *et al.* [39] and the cDNA sequence relevant to the *INSR* gene transcript sequence was established independently by Ullrich *et al.* [153] and Ebina *et al.* [27] in 1985.

Surprisingly, the research groups obtained sequences that differed in length by 36 nucleotides. Bioinformatic analysis of these sequences revealed that the insulin receptor transcript undergoes alternative splicing, in which exon 11 (encoding the carboxyterminal end of the α subunit) is occasionally skipped. This splicing event (exon exclusion/exon skipping) results in the formation of a shortened transcript called the 'short variant' (or second variant, E11⁻, A) as opposed to the unspliced transcript or the 'long variant' (or the first one, E11⁺, B) that contains exon 11 (36 bp long, corresponding to the 717-728 amino acid residues). These two isoforms encode for proteins of 1370 and 1382 amino acids

respectively [27, 153] (Fig. 1). Alternative splicing is a tissue-specific process and has been shown to alter the receptor ability to bind insulin. Experiments using transfected cell lines has revealed that there are differences in the internalization of different forms of the receptor induced by insulin binding [48]. There are also studies which suggest that the short form is expressed in leukocytes, and the longer form dominates in the liver, adipocytes and skeletal muscles, while in the placenta the level of both forms remains similar. Interestingly, the insulin binding affinity of the short variant is twice as much as the long variant E11⁺ [91]. Some of the studies noted that the shorter protein form is characterized by an increased rate of internalization process after ligand binding, whereas the longer protein with lower insulin binding affinity has higher catalytic activity in comparison to its shorter isoform [48]. It was also found that the isoform without the protein part encoded by exon 11 may bind insulin as well as IGF-II with high affinity, while the other isoform can bind only insulin [91].

While the biological significance of the two alternative spliced transcripts is yet to be established, it may be possible that disorders with alternative *INSR* transcript assembly leading to changes in both forms ratio in different tissues may lead to the development of various severe diseases in human [65, 70, 95, 104, 106, 126], though, the differences in both insulin receptor mRNA expression level has not been observed yet in patients suffering from various forms of insulin resistance in comparison to healthy ones [130].

The overall length of *INSR* transcript differs depending not only on the presence of several transcription initiation sites, but also a number of polyadenylation sites. Transcripts ranging from 5400 to 9400 bp have been observed, the most abundant are 6,900 bp and 9,400 bp long [150]. Interestingly, though, the small protein size, transcript length is significant and depends on the considerably 3'UTR length (4801 bp) what is similar to transcripts isolated from other organisms such as e.g. rat [150]. It is possible, that this region may play some regulatory functions. It has also been suggested that some of the post-transcriptional mechanisms may also be implied in the control of *INSR* mRNA as well as a sole protein expression. Alternative splicing mechanism remains under control of insulin as well as other factors. Disorders of this process may lead to higher insulin sensitivity, what suggests that the regulation of insulin receptor mRNA alternative assembly is crucial for insulin receptor sensitivity and the cell response [71]. In the alternative splicing sites selection there are numerous proteins and additional factors involved, including RNA secondary structure [8, 32, 142, 143], exon length and the "strength" of alternative splicing sites [101]. While changes in these sites selection have been frequently observed in genes during cell differentiation [86], hormonal regulation is not common. For instance, glucocorticoids have been shown to modulate insulin receptor mRNA alternative splicing in HepG2 cells, while insulin has been shown to modulate the same mechanism in *hepatocarcinoma* FaO cells [70, 132]. Studies conducted by Kadowaki *et al.* identified sites important for alternative *INSR* transcript splicing.

Exon 11 is very short (36 bp) and is flanked by long introns (~2,2 kb and ~7,5 kb). It was shown that intron 10 of the insulin receptor gene contains all the elements necessary for the proper splicing site selection and alternative exon 11 exclusion. There are two branch point (BP) sites in this intron which are crucial for splicing site selection. 300 bp upstream exon 11 there is an *Alu* sequence. 43 bp upstream the branch point in intron 10, there is an intronic splicing enhancer sequence (ISE) as well as intronic splicing silencing sequence (ISS) located at its 3' end responsible for exon skipping. ISE sequence located at 5' end of intron 10 contains a GA-rich sequence 48 bp long, crucial for exon 11 inclusion in the mature mRNA molecule [72].

The splicing site selection model during the insulin receptor transcript maturation was proposed in 1998 [72]. According to this model, the GA-rich sequence located on the 5' end of the intron 10 otherwise known as the intronic splicing enhancer sequence ISE, favours exon 11 inclusion in the mature transcript by direct interaction with 3' splicing site. It is possible that due to its location (more than 2 kb upstream branch point, BP), this region may interact with an adjacent 5' splice site (UAG:GUCAGGAC) considerably different from a consensus sequence (CAG:GUAAGUAU) [72]. Thus, the role of this enhancer may rely on the strengthening the interaction of U1snRNP (U-rich 1 small nuclear ribonucleoprotein particle) – one of the component of the spliceosome, with 5' splicing site [74]. However, the effect on the alternative splicing of the downstream exon, remains unknown. Furthermore, it has been suggested that the SF2/ASF protein is able to bind to GA-rich sequences which are similar to those present on the 5' end of intron 10. Accordingly, SF2/ASF protein may promote exon 11 retention in the *INSR* mature transcript, in similar way to splicing of the rat clathrin light B chain [21]. SF2/ASF protein may also preferentially use of proximal 5' or 3' splicing site. In turn, hnRNP-A1 splicing factor (which binds to UAGGGA or UAGGGU sequence) acts antagonistically, promoting distal splicing site selection. Thus, both factors act in an antagonistic way [19]. At the 5' end of intron 10 there is an additional CTTAGGGACC sequence, containing hnRNP-A1 binding site (underlined). It is already known that this nucleoprotein together with hnRNP-F is involved in the regulation of *INSR* transcript alternative splicing [148]. At the 3' end of intron 10 there are two regions located, which also play a role in *INSR* transcript alternative splicing. One of them, located upstream the branch point, promotes exon 11 exclusion from mRNA and is called intronic splicing sequence silencer (ISS). Furthermore, bioinformatic analysis has showed that this region may form a secondary stem-loop structure that plays a role in alternative splicing of mRNA. The second regulatory region, important for alternative splicing of *INSR* transcript is located in exon 11 and seems to act independently in the modulation process of 3' splice site selection. It can't be excluded that both regions are components of a larger secondary structure around the 3' splice site [72].

The function of intronic and exonic splicing sequence enhancers (ISE, ESE) and silencers (ISS, ESS) in alternative splicing regulation, relies on relevant

trans-acting factor binding, which in turn, influence splicing site selection by spliceosome [11]. Secondary structure of the premature transcript regulates availability of splicing factors. Such double-stranded stem-loop RNA structures are recognized by *trans*-acting factors and the whole complex lead to changes in mutual spatial distribution of *cis*-acting elements in this way forming additional regulatory “possibilities” [18, 45, 53]. Further studies investigating the alternative splicing of the insulin receptor has led to the identification of ISE and ISS elements in intron 10, as well as to better understanding of its mechanism [133, 158]. In addition, SRp20 and SF2/ASF have been shown to modulate exonic enhancer elements (ESE) activity. Moreover, it was shown, that ESS elements in exon 11 and ISS elements at the 3' end of intron 10 need CUG-BP1 protein binding. CUG-BP1 protein overexpression promoted exon 11 exclusion in *INSR* transcript, while SRp20 overexpression led to the enhancement of exon 11 retention [133]. Sen *et al.* consequently suggested that both proteins would act antagonistically and their mutual balance is crucial for regulation of exon 11 inclusion or elimination from transcript [133]. Schematic representation of the proposed mechanism for the alternative splicing of the insulin receptor mRNA is depicted in Figure 3.

Although the function of SRp20 and SF2/ASF has not been completely resolved, it has been postulated that these proteins lead to the recruitment of U1 and U2 snRNPs or prevent from CUG-BP1 proteins binding. It is possible that the activity of these factors may also be regulated by each other. It is still proposed that exon 11 together with an upstream polypyrimidine tract and a sequence containing branch point BP, form a large secondary stem-loop type structure, which occurrence inhibits U2AF, SF1 and U2 snRNP binding and, in this way, blocks 3' splice site availability. The ability of a secondary RNA structure to inhibit splicing was shown in studies that characterized splicing of *SMN2* (Survival Motor Neuron 2) gene, one of genes associated with spinal muscles atrophy [140]. The overlapping SF2/ASF and CUG-BP1 protein binding sites in exon 11 are located in a secondary RNA structure, upstream of which there is a SRp20 protein binding site. It is assumed that SRp20 and SF2/ASF binding prevent the formation of a secondary RNA stem-loop type structure. In turn, CUG-BP1 binds to its binding site and to the silencer sequence and stabilizes a double-stranded stem-loop fragment that aid in exon 11 being spliced out [133]. In order to confirm this hypothesis, molecular studies are currently being conducted at the University of California.

FIGURE 3. Proposed model for human insulin receptor transcripts alternative splicing regulation. In the alternative splicing sites selection numerous proteins and additional factors are involved, including RNA secondary structure, exon length and the “strength” of alternative splicing sites. Intron 10 of the *INSR* gene contains all the elements necessary for the proper splicing site selection and alternative exon 11 exclusion. There are two branch point (BP) sites in this intron which are crucial for splicing site selection. 43 bp upstream the branch point in intron 10, there is an intronic splicing enhancer sequence (ISE) and intronic splicing silencing sequence (ISS) located at its 3' end responsible for exon skipping. GA-rich ISE sequence located at 5' end of intron 10 favours exon 11 inclusion in the mature transcript by direct interaction with 3' splicing site. It is possible that due to its location, this region may interact with an adjacent 5' splice site. Its role may rely on the strengthening the interaction of U1snRNP (U-rich 1 small nuclear ribonucleoprotein particle) – one of the component of the spliceosome, with 5' splicing site. It has also been suggested that the SF2/ASF protein is able to bind to GA-rich sequences which are similar to those present on the 5' end of intron 10. Accordingly, SF2/ASF protein may promote exon 11 retention in the *INSR* mature transcript (upper panel). In turn, hnRNP-A1 splicing factor acts antagonistically, promoting distal splicing site selection (middle panel). Intronic splicing sequence silencer (ISS) may form a secondary stem-loop structure (lower panel) that plays a role in alternative splicing of mRNA. The second regulatory region is located in exon 11 and seems to act independently in the modulation process of 3' splice site selection. ESS elements in exon 11 and ISS elements at the 3' end of intron 10 bind CUG-BP1 protein binding and promote exon 11 exclusion. In turn, SRp20 and SF2/ASF favour exon 11 retention in *INSR* transcript by leading to the recruitment of U1 and U2 snRNPs or preventing from CUG-BP1 proteins binding (according to [72, 133], modified)

HUMAN INSULIN RECEPTOR'S STRUCTURE

The mature insulin receptor is a glycoprotein that functions as a heterotetramer consisting of two dimers of two subunits (each 1370 or 1382 amino-acid residues long) connected by 14 disulfide bridges. This receptor is synthesized inside the cell as a premature proreceptor precursor ($M_r \sim 180$ kDa) which undergoes proteolytic cleavage within a Arg-Lys-Arg-Arg sequence located at the connection of α and β subunits releasing, in this way, α - β monomers. The α - β connection is possible due to the existence of disulfide bonds formed between cysteine 647 of α subunit and cysteine 872 of β subunit. Two α - β monomers are subsequently connected by Cys-524 bonds between α subunits [73].

The α subunit ($M_r=135$ kDa) is an extracellular domain which can bind insulin, while the β subunit ($M_r=95$ kDa, consisting of 620 amino-acid residues) possesses a 194 amino-acid extracellular region a 23-amino-acid long transmembrane region and a 403-amino-acid long intracellular tyrosine kinase domain [27, 57, 131, 153]. In addition, the α subunit, as well as an extracellular region of the β subunit have several glycosylation sites [131].

Following the signal peptide, in aminoacid sequence, there is a L1 domain containing leucine-rich repeats (52-164 residues) which serves for an insulin binding site, a cysteine-rich region CR (179-340 residues) and a furin-like and a FU region (234-281 residues) containing furin-like repeats. The next region, the L2 domain (359-472 residues), is located upstream of three fibronectin type 3 Fn0, Fn1, Fn2 domains (Fig. 1). The aminoterminal end of the protein (encoded by exons 1-2) as well as cysteine-rich domains (encoded by exons 3-5) together with carboxyterminal end consisting of 704-719 residues are responsible for insulin binding with high affinity [73], and the presence of the 12-aminoacid long sequence encoded by alternative exon 11 modulates the ligand binding affinity. Several key residues of the receptor include positions 906 and 920 that are crucial for inter-domain contact. Other residues such as 921-922 and 924-925 stand for cytokine receptor motif that is followed by catalytic tyrosine kinase domain. Formed by the following residues: 1020-1021, 1025, 1043, 1045, 1092, 1094, 1098, 1151-1152, 1154, 1165 (Gly-X-Gly-X-X-Gly), the ATP-binding site is located in β subunit [125]. Two tyrosine residues (Tyr-965 and Tyr-972) located in the juxtamembrane JM domain of β subunit undergo autophosphorylation after insulin binding. Another phosphorylation site is located in the centre of the intracellular domain (within the following sequence: Tyr-1158-X-X-X-Tyr-1162-Tyr-1163), and two others are located on the carboxyterminal end of the protein (inside CT domain) (Tyr-1328 and Tyr-1334) [125]. Tyrosine 972 phosphorylation leads to the NPXpX motif formation, recognized by PTB domain of IRS-1 and Shc proteins. This NPXpX motif is also important for receptor internalization process [160]. In turn, it was shown that the autophosphorylation of the tyrosine residues present in a regulatory kinase domain is crucial for catalytic activity of the receptor

induced by insulin and insulin biological effect. In addition, the activated KRLB (Kinase Regulatory Loop Binding) domain is necessary for interactions between a unique region of IRS-2 protein spanning 591-768 residues and insulin receptor [125]. In turn, residues 1034-1037, 1115, 1117, 1157-1158, 1283 and 1288 form a non-catalytic PTP1B binding site, and amino acids at positions: 1151, 1180, 1182-1184, 1186-1187, 1196-1197, 1199, 1230 are a substrate protein binding site. The activating loop (A-loop) is located at positions 1164-1185 while residues at positions: 1171, 1173-1174, 1176-1177 are involved in a formation of interaction site with SH2 domains of the target proteins.

Insulin binding with α subunit is suggested to bring both subunits into close proximity. This enables the β subunit to bind ATP which triggers a phosphorylation cascade. Phosphorylation and activation of the IRS (insulin receptor substrate) proteins: IRS-1 and IRS-2, lead to binding and activation of other proteins containing SH2 (*Src* homology 2) domains such as Shc and adaptor APS proteins [149]. Activation of these proteins releases a subsequent cellular signaling cascade which leads to the accumulation of cellular effectors and insulin signal transduction process inside the cell.

Regulation of glucose transport into the cells as well as several other metabolic process are under the control of phosphatidylinositol 3-kinase. Its p85 regulatory subunit binds to the phosphorylated tyrosine residues of IRS-1 protein leading to its conformational change and activation of catalytic p110 subunit of IRS-1 protein. Subsequently, the whole enzyme transits to the cell plasma membrane. Phosphatidylinositol 3-kinase catalyzes phosphatidylinositol 4,5-bisphosphate phosphorylation to phosphatidylinositol 3,4,5-triphosphate which is involved in the protein B kinase (PKB) activation process, also named AKT kinase. Direct interaction of the PIP3 molecule with PH domain of the AKT kinase leads to its transfer to the plasma membrane, where it undergoes activation by phosphatidylinositol PDK-1 kinase. AKT kinase, in turn, is responsible for the phosphorylation of various cellular proteins involved in various metabolic pathways and pathways that regulate cellular growth and differentiation [1,33]. This enzyme, by inhibiting GSK-3 kinase, also enhances glycogen synthesis, and by regulating mTOR (mammalian target of rapamycin) kinase induces synthesis of various cellular proteins. AKT kinase is also responsible for "cell survival" by blocking several proapoptotic factors including Forkhead family transcription factors. The PI3K/AKT kinase signaling pathway also involves translocation of a membrane glucose transporter GLUT4, which transport glucose molecules to the cells' interior. Mitogenic effects of insulin action are caused, by not only AKT kinase pathway activation, but Ras/MAPK kinase as well [43, 77, 159].

Phosphorylated and activated Shc binds to the Grb2/SOCS complex (growth factor receptor-binding protein 2). Relocation of SOCS protein to plasma membrane enables its interaction with Raf kinase anchored to the plasma membrane and its activation. Subsequently, activated Ras, activates Raf kinase, and this leads to the activation of MAP and ERK kinases phosphorylation cascade [44,

69, 127, 155]. In the next step of signaling pathway, ERK kinases are translocated to the nucleus and activate transcription factors such as c-jun, c-fos and c-myc. Consequently, the response to insulin also involves the induction of genes' expression which are involved in the proliferation processes [46, 69, 146].

After insulin signal transduction inside the cell, insulin receptor undergo internalization process. Some of the receptors are directed back to the plasma membrane in clathrin-coated vesicles, while others are degraded in lysosomes [3, 122].

Tissue-specific alternative splicing of the insulin receptor transcript leads to the formation of two, fully functional forms of the receptor and the ratio of them differs depending on tissue.

Precursor receptor protein maturation occurs in the endoplasmic reticulum (ER). The INSR precursor is subsequently proteolytically cleaved in *trans* Golgi to form the mature INSR protein which is then transported to the cell's surface [122]. The growing polypeptide chain of the insulin receptor translocates through the Sec61 translocon to the endoplasmic reticulum lumen [5]. In the meantime, Glc3Man9GlcNAc2 oligosaccharide core is co-translationally added to the protein in N-glycosylation process by oligosaccharide transferase. This core is subsequently shortened by the activity of glucosylase I and II [31, 52, 120]. The next step in the insulin receptor maturation process, is thought to involve the two ER luminal proteins calnexin and calreticulin. Acting as a quality control system for improperly folded proteins, it has been suggested that calnexin and calreticulin aid in the addition of carbohydrate substrates and oligosaccharide shortening. Misfolded glycoproteins are directed by retrograde transport from the endoplasmic reticulum lumen back to the cytoplasm where they undergo ubiquitin-dependent proteolytic degradation by the 26S proteasome [5]. Cytoplasmic HSP90 as well as glucose-regulated protein Grp94 (Mr=94 kDa), a HSP90 homologue residing in the lumen of ER, are both involved in protein maturation. These protein ensures the proper maturation of other proteins and signal transducers translocation such as steroid hormones, growth factor receptors and others [17].

INSULIN RECEPTOR ISOFORMS AND INSR/IGF-1R HYBRID RECEPTORS

As mentioned above, there are two insulin receptor isoforms, which differ by lack of (short, E11⁻) or presence (long, E11⁺) of the carboxyterminal 12 amino acid long fragment located at the end of α subunit and encoded by alternatively spliced transcripts [27, 153].

It is believed that these two insulin receptor forms are immunologically and functionally distinct [83, 94, 123, 134-136, 154, 161, 162]. In 1988, Sakata *et al.* suggested that the carboxyterminal domain of α subunit of the E11⁺ receptor

isoform, relative to 705-731 amino-acids is the main auto-antigenic site [123]. In turn, Sesti *et al.* revealed that the IgG fraction, isolated from patients suffering from autoimmune hypoglycemia (autoantibodies against insulin receptor) inhibited insulin binding by receptors expressed in cells transfected with vectors and overexpressing E11⁻ form, but there was no effect on insulin binding in cells transfected with vectors and overexpressing E11⁺ form [134]. Two years later it was demonstrated that an antibody directed against the amino terminal region encoded by exon 11, was able to inhibit insulin binding by E11⁺, but not by E11⁻ form [135]. Furthermore, other studies have shown that the two insulin receptor isoforms have different ligand with the E11⁻ variant binding insulin twice as much as a protein encoded by transcript with exon 11 [94, 161, 162].

The differing ligand affinity may reflect changes in insulin acting as a metabolic or mitogenic factor [83]. Moreover, it seems that E11⁻ isoform is characterized by a higher rate of internalization and recycling in the cell [154, 161]. In some cases, experiments using transfected mammalian cells expressing separately two receptor isoforms revealed down-regulation of insulin-induced E11⁻ form [162]. Frasca *et al.* [38] found that the E11⁻ isoform may bind insulin-like growth factor II (IGF-2). Interestingly, IGF-2 binds to the E11⁻ receptor with quite high affinity (approximately 40% of value observed for insulin), but it is not able to bind to the E11⁺ receptor. Furthermore, IGF-2 binding by insulin receptor was shown to have a mitogenic rather than metabolic effect inside the cell [38].

As mentioned earlier, the two isoforms are differentially expressed in a cell-, tissue- and developmentally-specific manner [38, 91, 130, 135, 136]. While both isoforms are expressed in placenta, kidneys, adipose tissue and skeletal muscles, the short variant is expressed in hematopoietic and nervous cells and E11⁺ isoform dominates in liver [91, 130, 135, 136]. Furthermore the short form of the receptor is expressed mainly in fetal tissues such as kidneys, skeletal muscles, liver and fibroblasts [38].

Cellular differentiation also influences the expression of both transcripts. For instance, in colorectal carcinoma and breast cancer, E11⁻ is the dominant form of the insulin receptor and there is thus an increase in regulatory possibilities of the cell's growth by IGF-2 [38, 128].

In vivo [104] and *in vitro* [105] studies conducted by Norgren *et al.* revealed that alternative splicing of the insulin receptor transcript may be regulated hormonally and metabolically.

The short 12 amino-acid long sequence encoded by exon 11 has been shown to modulate the receptor's affinity for insulin, by potentially forming the relevant binding site with other regions of the receptor. It can't be excluded that the presence of this region may influence other function of this extremely important protein in the cell. Whereas the insulin receptor occurs in two alternative splicing forms E11⁺ and E11⁻, insulin-like growth factor 1 receptor IGF-1R occurs only as a "E11⁻" form [82, 87]. Both the INSR and IGF-I receptors belong to the subclass II of tyrosine kinases, which have a high sequence and function homology [27, 153].

FIGURE 4. Insulin receptor, IGF-1 receptor and hybrid receptor INSR/IGF-1R together with bound ligands and metabolic or mitogenic effects caused within the cell. Both INSR and IGF-1R receptor have low affinity to the homologous hormone. Insulin receptor binds mainly insulin leading to the metabolic effect. In turn, IGF-1R receptor binds IGF-1 and IGF-2 with higher affinity in comparison to the insulin and thus lead to the mitogenic rather than metabolic effect. The hybrid receptor formed by dimerization of two different subunits of the INSR and IGF-1R receptor can bind IGF-1 and IGF-2 with high affinity, while the affinity of such hybrids to insulin is lower than the insulin receptors. Thus binding insulin-like growth factors lead to mitogenic rather than metabolic effects within the cell (according to [23, 24, 137], modified)

In contrast to other tyrosine kinases which dimerize after binding to their ligands, both INSR and IGF-1R receptors are present in cell membrane as already formed dimers of β - α - α - β structure. Proreceptors dimerization takes place in endoplasmic reticulum, where disulfide bridges are formed, stabilizing the whole structure of the receptor, before proteolytic cleavage into α and β subunits [107].

Insulin plays a role in the regulation of glucose concentration, lipid metabolism in muscles, liver and adipose tissue, whereas IGF-1 stands for growth factor for most of the cells “Cross-reaction” studies revealed, that both receptors have low affinity to the homologous hormone (INSR to IGF-1 and IGF1-R to insulin), thus it is suggested that at physiological condition these receptors should response only in the presence of its own ligand. In certain conditions however, insulin may play a role as a growth regulator, and IGF-1 may lead to metabolic

effects similar to those evoked by insulin action. Furthermore, most of the disulfide bridges in the α subunits are also conserved. It has accordingly been suggested that a hybrid INSR/IGF-1R receptor may form and be composed of α/β insulin receptor heterodimers and α/β IGF-1R receptor heterodimers (Fig. 4) [10].

Surprisingly, it was shown that the hybrid receptor binds IGF-2 with similar affinity to IGF-2 receptors, while the affinity of such hybrids to insulin is lower than the insulin receptors [75, 144]. In addition, these hybrid receptors resemble the IGF-2 receptors rather than the insulin receptors when it comes to protein autophosphorylation, internalization and degradation [129]. Hybrid distribution differs depending on tissue; for instance a significant percentage of such receptors have been seen in human tissues such as placenta, skeletal muscles, adipose tissue, erythrocytes, leukocytes and fibroblasts [34]. While the factors regulating hybrid receptors formation *in vivo* have not been defined yet, it has been proposed that they may form by chance with the same efficiency and in ratio determined by molar concentration of the defined forms. As insulin and IGF-1 regulate expression of its own receptors' genes, changes resulting from insulin and/or IGF-1 receptors number induced by these hormones may also modify the number of hybrid receptors. In skeletal muscles of patients with insulinoma and hyperinsulinism, it was observed that the number of hybrid receptors was elevated, what markedly correlated with higher insulin level in blood and smaller number of insulin receptors [35]. Other studies showed an up-regulation of hybrid receptors in skeletal muscles in obese patients with high fasting insulin level and lower IGF-1 level in blood. The ratio of hybrid receptors correlated with a down-regulation of insulin receptors and an up-regulation of IGF-1 receptors [36]. These observations suggest that changes in insulin level contribute to insulin receptor down-regulation, IGF-1 receptors up-regulation and up-regulation of hybrid receptors. Mixed receptors can bind IGF-1 with a high affinity, thus they may be activated in physiological conditions by IGF-1 rather than by insulin leading to mitogenic rather than metabolic effects (Fig. 4).

INSULIN RECEPTOR DEFECT AS A CAUSE OF RARE SYNDROMES

Insulin resistance is one of the most frequently observed disorders associated with defects in hormonal regulation and is a main cause of hyperglycemia in patients with diabetes mellitus type 2. Furthermore, insulin resistance is commonly related to insulin action defects downstream the insulin receptor [100]. Surprisingly, insulin resistance connected with defects in the function of the insulin receptor function are rare (Table 1).

TABLE 1. Characteristic features of genetic disorders associated with severe forms of insulin resistance [2, 6, 7, 22, 26, 40, 49, 51, 59, 61, 62, 68, 78, 80, 97, 110, 111, 113, 114, 117, 138, 141, 147, 152].

SYNDROME	CLINICAL FEATURES	MOLECULAR MECHANISM
Insulin resistance type A	Affects younger women, ovarian hyperandrogenism, virilisation, hirsutism, menstrual cycle and fertility disorders, acanthosis nigricans, severe insulin resistance, diabetes mellitus	Insulin receptor gene mutations or other target-cell defects in its action
Insulin resistance type B	Affects older women, may be associated with other autoimmune disorders	Autoimmune mechanism – the presence of antibodies against insulin receptor
Rabson-Mendenhall Syndrome	Insulin resistance type A features, dental dysplasia, pituitary hyperplasia, physical growth disorders, diabetes mellitus, precocious puberty, growth retardation, renal disorders and malfunction, death in the first or second decade of life due to the developing diabetic ketoacidosis	Insulin receptor gene mutations (autosomal recessive inheritance) or other target-cell defects in insulin action; other defects also possible
Donohue Syndrome (leprechaunism)	The most severe form of insulin resistance, intrauterine growth retardation, elf-like face features, hypertrichosis, depletion of adipose tissue, sexual organs enlargement, diabetes mellitus due to hyperinsulinism, cardiomyopathy, liver, spleen and ovaries hypertrophy, hyperglycemia, mental retardation, lack of diabetic ketoacidosis, death in the first several years of life during infancy and in the early childhood	Insulin receptor gene mutations (autosomal recessive inheritance), mutations in other genes encoding for growth factors receptors or signal transduction proteins of common pathways in which growth factors also contributes are also possible
Congenital generalized lipodystrophy (lipoatrophy)	Total lipoatrophy, insulin resistance type A features possible, liver and spleen hypertrophy, cardiomyopathy, features of acromegaly, mental retardation, hypertriglyceridaemia, hyperandrogenism, bone cysts	Mutations in <i>AGPAT2</i> and <i>seipin</i> genes, due to lack of these mutations in some patients, other mutations in other genes are also possible

SYNDROME	CLINICAL FEATURES	MOLECULAR MECHANISM
Congenital partial lipodystrophy	Changes in adipose tissue distribution associated with lipoatrophy or lipohypertrophy, insulin resistance type A features also possible, liver and spleen hypertrophy, cardiomyopathy, muscle dystrophy, disorders with nervous signalling, features of acromegaly or hypertriglyceridaemia	Mutations in <i>LMNA</i> gene encoding for nuclear lamina proteins – A and C lamin (autosomal dominant inheritance), in <i>ZMPSTE24</i> gene encoding for zinc metalloproteinase or in <i>PPARγ</i> gene encoding for nuclear receptor activated by peroxisome proliferators

One of such syndromes is **Rabson-Mendenhall syndrome (RMS)**, which was first described in 1956 [113, 114] and since then, only several cases have subsequently been described. While the genetic background of this extremely rare, autosomal recessive disorder is not fully understood, several cases are linked to mutations in insulin receptor gene. The characteristic features of Rabson-Mendenhall syndrome include severe insulin resistance developing early in childhood, diabetes mellitus, acanthosis nigricans, polycystic ovaries and virilisation in female patients. Other manifestations include leprechaunism [78], dental dysplasia, abnormal physical development, precocious puberty, pineal hyperplasia with melatonin secretion changes [22, 49, 68]. Moreover, these symptoms are associated with postnatal growth retardation, motor skills difficulties, speech and hearing problems, fast growing nails, hypertrichosis and other defects such as renal abnormalities [50]. Due to this extremely severe form of insulin resistance and, in turn, diabetic ketoacidosis, such patients die in the first or second decade of their life, before age of 16 [152].

Numerous homozygous and heterozygous mutations in the *INSR* gene have been described for patients diagnosed for Rabson-Mendenhall syndrome which result in receptor dysfunction in extracellular ligand binding domain and in intracellular domain possessing tyrosine kinase activity. In one case, the affinity of insulin binding is lowered or even completely inhibited, whereas in another case, the mutation lead to the insulin transduction defects inside the cell.

Apart from Rabson-Mendenhall syndrome, other forms of insulin resistance which differ in their severity are also connected with *INSR* gene mutations (Table 1).

In the case of **insulin resistance type A**, observed in women, diabetes mellitus may develop as a result of insulin receptor down-regulation or defects in its function [150]. Characteristic features, apart from diabetes mellitus, additional disease symptoms include: hyperandrogenism, virilisation, hirsutism, menstrual cycle and fertility disorders [62, 97].

In turn, antibodies raised against insulin receptor are responsible for **insulin resistance type B**. This form of insulin resistance may coincide with other disorders such as systemic lupus erythematosus [6, 117].

Another example of the most severe form of insulin resistance associated with a lack of insulin binding by its receptor is **leprechaunism** (Donohue syndrome), which is an autosomal recessive disease, similar to RMS [2, 7, 26, 59, 78]. Features characteristic for this syndrome include: disorders with glucose metabolism, diabetes mellitus, growth and mental retardation, polycystic ovaries in females, hypertrichosis, adipose tissue depletion and acanthosis nigricans. Children suffering from leprechaunism usually die within the first few several years after birth.

Pathogenetic relationships of lipodystrophies syndromes with insulin resistance

Congenital lipodystrophies are rare autosomal recessive or dominant genetic disorders which are characterized by the selective differing in its severity of adipose tissue depletion. A common feature of such disorders - extreme hypertriglyceridaemia may indicate a role of adipose tissue in lipid homeostasis. As hypertriglyceridaemia results from an increase in very-low-density-lipoproteins synthesis in liver, low-fatty diets are beneficial in patients with congenital lipodystrophy [138].

Lipodystrophy may also be frequently associated with severe insulin resistance, diabetes mellitus and liver steatosis. Lipodystrophy may be congenital which includes congenital generalized lipodystrophy (CGL) (lipoatrophy) and familial partial lipodystrophy (FPL), or acquired. In recent years, three *loci* responsible for congenital generalized lipodystrophies have been discovered which include *AGPAT2*, a gene encoding 1-acylglycerol-3-phosphate-O-acyltransferase 2 and *BSCL2* [40].

Berardinelli-Seip Congenital Lipodystrophy type 2 (BSCL2) is the most severe form of lipodystrophy. This recessive disorder with almost total lipoatrophy is due to a mutation in the cell-autonomous regulator of adipogenesis, *BSCL2* [147, 111]. Interestingly, four other *loci* were connected with partial congenital lipodystrophies including *LMNA* a nuclear lamin protein – A and C lamin), *PPARG* (*PPARγ*) a peroxisome proliferator-activated receptor gamma, and *ZMPSTE24* a zinc metalloproteinase [40].

In patients with Dunnigan-type Familial Partial Lipodystrophy (FPLD; OMIM ID: 151660) a missense mutation (R482Q) in *LMNA* was shown to be correlated with hyperinsulinism and disorders in blood plasma lipids (increase in blood plasma triglycerids, decreased HDL cholesterol and no changes in general and LDL cholesterol concentration) preceding blood plasma glucose disorders [51].

SUMMARY

In summary, various disorders including severe and extremely rare forms of insulin resistance such as Rabson-Mendenhall syndrome or Donohue syndrome may be a result from mutations in insulin receptor gene. The effects of disruptions to this important protein required to maintain homeostasis may include a decrease in insulin binding, changes in tyrosine kinase activity of the β subunit, accompanied by signal transduction defects, defects in receptor maturation and folding, improper distribution of insulin receptor in plasma membrane [151], or disorders with receptor internalisation and/or its accelerated degradation.

Molecular studies including genetic analysis of insulin receptor gene and its transcript are a powerful tool in prenatal diagnostics and in pregnancy planning (testing for a mutation carrier). It is possible that additional analysis of proteins involved in insulin response and factors regulating insulin receptor gene expression as well as those involved in transcript maturation (including yet unidentified microRNAs) will aid in the understanding of the molecular mechanisms underlying various forms of insulin resistance as well as to better treatment.

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