



The polymorphism in insulin receptor substrate-1 gene and birth weight in neonates at term

Polimorfizm genu substratu 1 receptora insuliny a masa ciała u noworodków urodzonych o czasie

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Abstract

Background: The mutation of the *IRS-1* gene is one of the genetic risk factors which, it is speculated, is associated with insulin resistance or predisposition to type 2 diabetes. The aim of our study was to evaluate the association between the Gly972Arg polymorphism in the *IRS-1* gene and birth weight in newborn children with adequate gestational age.

Material and methods: 100 newborn children with adequate gestational age (38–42 weeks), whose mother had no disorders during pregnancy, were studied. Genomic DNA was extracted from umbilical cord blood leukocytes, and Gly972Arg polymorphism in the *IRS-1* gene was genotyped using the PCR-based method.

Results: Birth weight was significantly lower in the newborn with the *IRS-1* Gly972Arg polymorphism compared with a control group (3161.75 ± 380.86 g vs. 3427.92 ± 468.86 g). Body length and head circumference at birth were also lower in the neonates with that polymorphism (54.38 ± 3.13 cm vs. 52.69 ± 2.91 cm, and 34.08 ± 1.47 vs. 33.63 ± 0.81, respectively).

Conclusions: The results suggest that the Gly972Arg genotype is associated with lower birth weight, body length and head circumference in neonates with adequate gestational age. (*Pol J Endocrinol* 2008; 59 (3): 212–216)

Key words: *IRS-1*, Gly972Arg, birth weight, type 2 diabetes

Streszczenie

Wstęp: Mutacja genu substratu 1 receptora insuliny (*IRS-1*, *insulin receptor substrate-1*) jest jednym z genetycznych czynników ryzyka, przypuszczalnie związanych z występowaniem zjawiska oporności na insulinę lub predyspozycją do wystąpienia cukrzycy typu 2. Celem niniejszego badania była ocena potencjalnych zależności między polimorfizmem Gly972Arg w genie *IRS-1* a masą ciała u noworodków urodzonych o czasie.

Materiał i metody: W badaniu wzięło udział 100 noworodków urodzonych o czasie (38–42 tydzień ciąży), których matki nie chorowały podczas ciąży. Po wyekstrahowaniu genomowego DNA z leukocytów krwi pępowinowej przeprowadzono z użyciem metody PCR ocenę polimorfizmu Gly972Arg genu *IR-1*.

Wyniki: Urodzeniowa masa ciała była istotnie niższa u noworodków z polimorfizmem Gly972Arg genu *IRS-1* w porównaniu z grupą kontrolną (3161,75 ± 380,86 g vs. 3427,92 ± 468,86 g). U noworodków z tym polimorfizmem zaobserwowano również mniejszą długość ciała oraz mniejszy obwód głowy (odpowiednio: 54,38 ± 3,13 cm vs. 52,69 ± 2,91 cm oraz 34,08 ± 1,47 cm vs. 33,63 ± 0,81 cm).

Wnioski: Wyniki sugerują, że genotyp Gly972Arg u noworodków urodzonych o czasie wiąże się z niższą urodzeniową masą ciała, mniejszą długością ciała oraz mniejszym obwodem głowy. (*Endokrynol Pol* 2008; 59 (3): 212–216)

Słowa kluczowe: *IRS-1*, Gly972Arg, waga urodzeniowa, cukrzyca typu 2

Introduction

In recent years a number of studies have reported a genetic predisposition to the occurrence of type 2 diabetes. It is speculated that the mutation of the *IRS-1* gene is one of the genetic risk-factors associated with insulin

resistance or predisposition to type 2 diabetes [1]. There are around 11 known polymorphisms in the *IRS-1* gene, the most common variant being Gly→972→Arg replacement, which is a result of guanine by adenine substitution at the DNA chain [2, 3]. This polymorphism is more prevalent in patients with marked insulin resistan-



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ce, whether this is associated with type 2 diabetes or not [3, 4]. The Gly972Arg polymorphism in the *IRS-1* gene is associated with significant impairment of the insulin signalling pathway and may induce abnormalities in the functioning of the pancreatic beta-cells, such as a decrease in insulin secretion, a greater number of immature secretory granules and/or peripheral insulin resistance. This has been examined in type 2 diabetic subjects and controls from various populations, and in outcomes prevalence was higher in patients with type 2 diabetes than among healthy controls [2–6]. According to the meta-analysis conducted in 2003, carriers of the Gly972Arg variant of the *IRS-1* gene are at 25% increased risk of developing type 2 diabetes compared with non-carriers. These data suggest that this polymorphism represents a risk-factor for type 2 diabetes [7]. In contrast to this, Florez et al. [8] examined the genotype-phenotype correlation in three Caucasian samples of a total of 9000 individuals in order to evaluate the reproducibility of the model for the meta-analysis established by Jellema et al [7]. In their study Gly972Arg polymorphism was not associated with type 2 diabetes [8].

Since *IRS-1* function is also related to stimulation of the IGF-1 receptor, the *IRS-1* gene polymorphism might also influence IGF-1 receptor function. A reduction in IGF-1 insulin signalling pathway and also in insulin secretion may contribute to lower birth weight [9, 10].

Material and methods

The purpose of this study was to determine whether the Gly972Arg variant of the *IRS-1* gene is associated with decreased birth weight. We studied 100 newborn children with adequate gestational age (38–42 weeks), delivered after uncomplicated pregnancies (mothers had no documented gestational diabetes, type 2 diabetes, impaired glucose tolerance, hypertension, were not cigarette smokers and were not obese before pregnancy (BMI < 30 kg/m²). The gender of the neonates, birth weight, body length and head circumference were taken from standard hospital records after delivery. Genomic DNA was extracted from umbilical cord blood leukocytes by standard techniques. The Gly972Arg genotype was obtained by the PCR-Restriction Fragment Length Polymorphism (RFLP). The product of amplification was digested with restriction enzyme *Eco881*, recognising the 5' CZ(C/T)CG(A/G)G 3' sequence. Amplified fragments of the *IRS-1* gene (homozygote GG genotype: 169 bp and 29 bp fragments, homozygote AA: 198 bp, and heterozygote GA: 198 bp, 169 bp and 29 bp) were run on 4% agarose gel and visualised under ultraviolet illumination. DNA molecular weight marker XIII (Roche) was used as a DNA reference standard. Written informed consent was obtained from the pregnant

mothers, and the study was approved by the Ethics Committee of the Silesian School of Medicine in Katowice, Poland.

In statistical analysis the multivariate analysis, which included Gly972Arg polymorphism and demographic parameters of the mothers (age, gestational age, height and weight before pregnancy), was performed in order to determine the independent impact of the polymorphism examined. All data are presented as means \pm standard deviation (SD). P values less than 0.05 were considered significant. The neonates were divided into three groups on the basis of *IRS-1* genotype: 1 — homozygote GG, 2 — homozygote AA, 3 — heterozygote GA. The U test and χ^2 test were used for assessing differences between groups.

Results

Table 1 presents the main characteristics of the study population. Genotype frequencies were: GG — 84%, GA — 15%, AA — 1%, and distribution was within the Hardy-Weinberg equilibrium. Birth weight was significantly lower in the newborn carrying the *IRS-1* Gly972Arg variant than in non-carriers. Body length and head circumference at birth were also lower in the neonates carrying the Gly972Arg variant (Table 1). Mothers whose children were carriers of the Gly972Arg variant were found to have had lower body weight (57.69 ± 7.43 kg) than mothers in the control group (60.19 ± 10.24 kg), whose children were not carriers of the polymorphism. The difference was borderline significant ($p = 0.06$) (Table I).

There were no significant differences between the groups with regard to the gestational age of the neonates, the age of the mothers or their height and BMI. The multifactorial analysis showed that there was a significant independent influence of the examined polymorphism on birth weight ($p < 0.05$).

Discussion

The results of the present study suggest that the Gly972Arg polymorphism in the *IRS-1* gene leads to decreased birth weight, body length and head circumference in children born at adequate gestational age. The occurrence of this polymorphism is a significant independent risk factor for lower birth body weight. The *IRS-1* represents the main candidate potentially impairing insulin signalling [11, 12]. *In vitro* studies have shown that this variant has been associated with decreased insulin secretion, reduced insulin sensitivity of the peripheral tissues and increased beta-cells apoptosis [11–14]. Moreover, similar abnormalities have been observed in *in vivo* settings [6] but were not entirely reproducible [2, 5,

Table I. *IRS-1* gene polymorphism and demographic characteristics of the study groupsTabela I. Polimorfizm genu *IRS-1* a demograficzna charakterystyka badanych grup

	Homozygote GG	Homozygote AA and heterozygote GA	p
n	84	16	
Mother's age at delivery	27.06 ± 4.81*	26.63 ± 4.11	p = 0.18
Mother's weight before pregnancy [kg]	60.19 ± 10.24	57.69 ± 7.43	p = 0.06
Mother's body length [m]	1.66 ± 0.06	1.65 ± 0.07	p = 0.15
Mother's BMI	21.88 ± 3.51	21.27 ± 2.89	p = 0.11
Gestation weeks	39.15 ± 0.88	38.94 ± 0.85	p = 0.09
Number of gestation	1.85 ± 0.99	2.44 ± 2.73	P = 0.90
Sex of neonate (%)	54/46	69/31	p = 0.94
Birth weight [g]	3427.92 ± 468.86	3161.75 ± 380.86	p = 0.0034
Birth height [cm]	54.38 ± 3.13	52.69 ± 2.91	p = 0.01
Head circumference [cm]	34.08 ± 1.47	33.63 ± 0.81	p = 0.02

15–22]. Stumvoll et al. have investigated the association between insulin secretion in well-matched normal glucose-tolerant patients and the *IRS-1* gene marker and found decreased glucose-stimulated insulin secretion during the Oral Glucose Tolerance Test (OGTT) during hyperglycaemic clamp and after maximal stimulation with arginine. These data suggest that the Gly972Arg polymorphism in the *IRS-1* gene might be associated with decreased insulin secretion in response to glucose but not directly with insulin sensitivity [21].

The impact of the Gly972Arg polymorphism in the *IRS-1* gene on insulin action in diabetic patients was discovered in 1994 [20]. Laakso et al. reported that this polymorphism could cause impairment of insulin signalling, insulin resistance and reduction in insulin secretion in subjects with and without diabetes; such a relationship was confirmed in subsequent studies [11, 23]. Subjects carrying this *IRS-1* gene variant had lower fasting C-peptide concentrations compared to control groups [16, 24]. They also expressed decreased basic and insulin-stimulated glucose transport due to a defect in the translocation of the glucose transporters GLUT-1 and GLUT-4 to the plasma membrane [13].

The carriers of the Gly972Arg *IRS-1* polymorphism presented features of metabolic syndrome: hyperglycaemia, insulin resistance, increased triglyceride and free fatty acid levels, microalbuminuria, hypertension and an increase in intima-media thickness. It can be suspected, that the *IRS-1* gene marker represents a risk factor of atherosclerosis and cardiovascular disease [25].

The presence of the Gly972Arg polymorphism in patients with type 2 diabetes was also evaluated in the United Kingdom Prospective Diabetes Study (UKPDS) study, but there was no observed increase in the preva-

lence of type 2 diabetes among carriers. It was noted, however, that the Gly972Arg variant was more common among subjects with diabetes and profound insulin resistance [6]. On the other hand, according to Florez et al. there was no association between the Gly972Arg variant and type 2 diabetes in populations of European descent [26].

The influence of the polymorphism described on the development of diabetes depends on numerous factors such as body mass [15, 16], phenotype of disease [7] and ethnicity, type 2 diabetes occurring more frequently in the Caucasian population than others [14].

Recent studies present the relationship between lower birth weight and increased risk of impaired glucose tolerance and diabetes in adults [27–30]. One study, that by Hales et al., suggests that this is a result of “metabolic programming” during intrauterine growth, or changes in the gene expression associated with an unfavourable intrauterine environment [31]. Such early adaptations to a nutritional environment could cause metabolism disturbances in later life.

The Gly972Arg polymorphism may thus have led to a change in the physiology of fuel metabolism and a decrease in insulin secretion and peripheral insulin resistance. In the present study we analysed the relationship between the Gly972Arg genotype, body length at birth and head circumference. A reduction in birth weight may be associated with insulin resistance resulting from genetic defects that influence insulin action during embryogenesis.

The importance of *IRS-1* has been demonstrated in the *IRS-1* knockout mice. *IRS-1* deficiency mice showed impaired glucose tolerance, a decrease in insulin-stimulated glucose uptake and a 50% reduction in intrauterine

growth [32]. A lack of the *IRS-1* gene and glucokinase gene both lead to reduced birth weight and body size. Transgenic mice also exhibited resistance to the metabolic effects of insulin in skeletal muscle and adipose tissue [33]. A mutation in the glucokinase gene does seem to reduce birth weight and to cause hyperglycemia after birth, perhaps because of decreased secretion in response to maternal glucose levels.

In 1992, Hales et al. hypothesised that lower birth weight predisposes to the development of diabetes in later life. The diabetes could result from the incorrect development of organs during intrauterine life and their subsequent dysfunction in the adult. It was suggested in this study that type 2 diabetes is mainly the result of intrauterine environmental factors, where only a minor role should be attributed to genetic factors [31]. However, it is now widely accepted that both genetic and environmental factors are engaged in the pathogenesis of type 2 diabetes. It is also postulated that the same genetic factors which cause impairment in insulin secretion or lead to insulin resistance might change intrauterine growth and glucose tolerance in later life [34].

Recently studies by Mason in the United Kingdom [35] and by Rasmussen in Denmark [36] found no differences in body weight at birth between carriers of the Gly972Arg variant and a control group, whereas a study in Brazil of neonates showed that body size at birth was lower in a group of Gly972Arg carriers than in a control group [37].

Conclusion

Our findings seem to suggest an independent association of the Gly972Arg genotype in the *IRS-1* gene with decreased intrauterine growth. The results were arrived at through the applications of statistical analysis alone. They should be treated as preliminary research and need to be corroborated by clinical findings in the future.

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