Endokrynologia Polska
DOI: 10.5603/EP.2016.0068
Tom/Volume 67; Numer/Number 6/2016
ISSN 0423–104X

The influence of *INS* VNTR class III allele on auxological parameters, glucose, insulin, lipids, and adipocytokines secretion in prepubertal children born small for gestational age

Wpływ występowania allelu klasy III VNTR genu *INS* na parametry auksologiczne, stężenie glukozy, insuliny, lipidów i adipocytokin u przeddojrzewaniowych dzieci urodzonych z niską masą ciała

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Abstract

Introduction: The insulin gene variable number of tandem repeats (*INS* VNTR) class III allele has been implicated in lower birth weight, obesity, and insulin resistance. We assessed its influence on birth weight in the Polish population and on the current body mass and metabolic profile in prepubertal children born small for gestational age (SGA).

Material and methods: DNA for genotyping of *INS* VNTR was available for 123 subjects born SGA and 132 born appropriate for gestational age (AGA). We identified two alleles: class I and class III. Next, in 112 prepubertal (aged: 6.8 ± 1.38 years) SGA children, the auxological measurements, fasting serum C-peptide, triglycerides, cholesterol, ghrelin, leptin, adiponectin, resistin, cortisol, and insulin-like growth factor type I (IGF-I) concentrations, as well as glucose and insulin during oral glucose tolerance test (OGTT), were assessed and insulin resistance indices were calculated. The results were analysed depending on *INS* VNTR variants.

Results: The occurrence of individual *INS* VNTR variants were similar in the SGA and AGA groups. In prepubertal SGA children, we did not observe any statistical differences as regards birth weight, body mass, lipids, or adipocytokine concentrations among I/I, I/III, and III/III class groups. The concentration of insulin in 120′ of OGTT was significantly higher in class III homozygous than in class I homozygous individuals.

Conclusions: Variant *INS* VNTR class III was shown not to be associated in any essential way with birth weight in the Polish population. Among prepubertal SGA children, the presence of *INS* VNTR class III is related to higher insulin secretion during OGTT.

(Endokrynol Pol 2016; 67 (6): 585–591)

Key words: *INS VNTR; small for gestational age; children; obesity; insulin resistance; adipocytokines*

Streszczenie

Wstęp: Sugeruje się, że występowanie allelu klasy III fragmentu różnej liczby tandemowych powtórzeń (VNTR) genu insuliny (*INS*) przyczynia się do niższej masy urodzeniowej oraz rozwoju otyłości i insulinooporności. Autorzy ocenili wpływ tego wariantu na masę urodzeniową w populacji polskiej oraz na masę ciała i profil metaboliczny u przeddojrzewaniowych dzieci urodzonych ze zbyt niską masą ciała (SGA).

Materiały i metody: Oceniono polimorfizm *INS* VNTR u 123 osób z SGA i 132 urodzonych z prawidłową masą ciała (AGA). Identyfikowano dwa alelle VNTR: klasy I i klasy III. Następnie wyodrębiono grupę 112 przeddojrzewaniowych dzieci z SGA (w wieku 6,8 ± 1,38 lat), u których wykonano pomiary auksologiczne oraz oznaczono stężenie: C-peptydu, triglicerydów, cholesterolu, greliny, leptyny, adiponektyny, rezystyny, kortyzolu i insulinopodobnego czynnika wzrostu typu I (IGF-I), jak również oceniono stężenie glukozy i insuliny podczas doustnego testu tolernacji glukozy (OGTT). Obliczono wskaźniki insulinooporności. Wyniki przeanalizowano w zależności od występowania typu polimorfizmu *INS* VNTR.

Wyniki: Częstość występowania poszczególnych wariantów *INS* VNTR nie różniła się w grupie AGA i SGA. U przeddojrzewaniowych dzieci z SGA nie obserwowano istotnych różnic w odniesieniu do ich urodzeniowej masy ciała, aktualnej masy ciała, stężenia lipidów i adipocytokin w zależności od występowania klasy I/I, I/III czy III/III. Stężenie insuliny w 120. minucie OGTT było istotnie wyższe u homozygotycznych dzieci z klasą III niż u homozygotycznych dzieci z klasą I.

Wnioski: Wariant *INS* VNTR klasy III nie wydaje się być przyczyną niskiej urodzeniowej masy ciała (SGA) w populacji dzieci polskich. Wśród przeddojrzewaniowych dzieci z SGA, obecność klasy III *INS* VNTR wiąże się z wyższym wydzielaniem insuliny podczas OGTT.

(Endokrynol Pol 2016; 67 (6): 585–591)

Słowa kluczowe: *INS VNTR; niska masa urodzeniowa; dzieci; otyłość; insulinooporność; adipocytokiny*

The study was financially supported by funds from the Ministry of Scientific Research and Information Technology, the present name – the Ministry of Science and Higher Education of Poland, project No. 2751/P01/2007/32.

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Introduction

Approximately 3–10% of children are born small for gestational age (SGA). It means that their birth weight and/or birth length are below –2.0 standard deviation scores (SDS) for the mean value for gestational age (GA) [1, 2]. The causes of SGA include environmental factors and placental anomalies. However, in some cases they remain unexplained. Because familial recurrence and parental contribution to SGA have been observed, various genetic mutations are taken into consideration [3]. SGA individuals are characterised by a higher risk of obesity, insulin resistance (IR), and diabetes type 2 (T2DM) in adulthood [4]. However, we observed that certain symptoms can occur as early as in the first decade of life [5].

Insulin is crucial for both foetus growth and glucose metabolism. Variations in the insulin gene variable number of tandem repeats (*INS* VNTR) have been implicated in susceptibility to lower birth weight as well as to IR, T2DM, and obesity because it has been shown that they are influenced by pancreatic insulin gene transcription and altered insulin secretion, both in the foetus and the adult [6, 7]. According to the number of repeats, the alleles of *INS* VNTR are divided into: class I (with 26–63 repeats), class II (with 64–140 repeats), and class III (with 141–209 repeats); however, class II is very rare in the Caucasian population [8]. It was proven that both in foetal and in adult pancreas, lower insulin mRNA expression in *INS* VNTR class III homozygous than in class I homozygous individuals is observed [6, 7, 9]. However, the potential effect of class III allele on birth weight is still controversial [10–18]. On the other hand, class III is protective against diabetes mellitus type 1 (T1DM) and leads to an overexpression of insulin [19–21]. Thus, it may be responsible for obesity, IR, T2DM, and polycystic ovary syndrome (PCOS); however, the results regarding the influence of *INS* VNTR polymorphism on these disorders, both in the general population and in SGA individuals, are divergent [10, 13, 15, 17, 22–29]. The aim of the study was to verify whether *INS* VNTR class III allele is associated with low birth weight in the Polish population and to assess the influence of this variant on the current body mass index (BMI) as well as on the metabolic and hormonal profile in prepubertal SGA children.

Material and methods

In order to evaluate the incidence of *INS* VNTR gene class III, a genetic analysis was performed in 255 patients, including 123 (64 girls and 59 boys) subjects born SGA and 132 subjects (75 girls and 57 boys) born appropriate for gestational age (AGA). The children were qualified as SGA when their birth weight was below –2.0 SD for GA and sex, and as AGA when their birth weight was between –1.5 and +1.5 SD for GA and sex.

In order to enrol a group of children with SGA, written invitations were posted to the parents of all the children with birth weight below 2500 g which were born at the Polish Mother's Memorial Hospital-Research Institute (PMMH-RI) during the period 1999–2003. The exclusion criteria were applied to children from twin pregnancies, those with congenital abnormalities, and those with Silver-Russell syndrome, Turner syndrome, and other serious chronic diseases.

In order to have a group of children with AGA, blood samples were collected for genetic tests from children diagnosed at the Department of Endocrinology and Metabolic Diseases PMMH-RI for various reasons, as well as samples of umbilical blood from children born during the study at the PMMH-RI.

Genetic analysis

Genomic DNA was isolated from peripheral blood lymphocytes. The method of amplification was applied with the use of fluorescence-labelled probes, specific for allele variants. Fluorescence detection was performed by a 7900HT Fast Real-Time PCR System (Applied Biosystems, USA). We studied rs689 (also called 23HphI), a single nucleotide polymorphism in complete linkage disequilibrium with the *INS* VNTR locus, identifying two alleles: A and T. The rs689 A allele is representative of class I, whereas rs689 T allele is representative of class III of VNTR [30]. Then the correlation between birth weight (BW) and birth length (BL) was analysed with regards to the presence of I/I, I/III, and III/III variants. Birth weight and birth length were expressed by standard deviation score (SDS) for the gestational age and sex of the child (BW SDS and BL SDS), following the standards for the population of Polish children [31].

In turn, in the group of prepubertal children born SGA, we decided to establish the effect of the presence of allele class III variants on the occurrence of obesity and disturbances in glucose, insulin, C-peptide, lipids, and adipocytokines secretion rates. Thus, 112 prepubertal children (57 girls and 55 boys), aged 4.8 to 9.4 years (mean: 6.8 ± 1.38 years) were qualified into that part of the study. All of them were born small for gestational age (birth weight below –2.0 SD for sex and GA). In each of the children, anthropometric measurements were performed (body height, body mass, and waist circumference). Fasting triglycerides, total cholesterol and its fractions: HDL-cholesterol and LDL-cholesterol, C-peptide, ghrelin, leptin, adiponectin, resistin, cortisol, and IGF-I were assessed. Glucose and insulin concentrations were measured at fasting state and 60

and 120 minutes after the oral glucose tolerance test (OGTT), following glucose administration in the dose of 1.75 grams of glucose per kilogram of body mass, to a maximum dose of 75 g. On the basis of the obtained results, height SDS (HSDS), body mass index (BMI), and BMI SDS were calculated following the standards for the population of Polish children [32]. We also calculated the waist-to-height ratio (WHtR), which has recently been proposed for visceral obesity assessment in children [33] because it accounts for considerable height variations among children of the same age. Besides, the insulin resistance index (IRI) calculated using HOMA [34] and IRI calculated using Belfiore methods [35] were applied, according to the following formulas: $IRI_{HOMA} = [(GLU_{0'}/18.5) \times INS_{0'}]/22.5$, where $GLU_{0'}$ — fasting glucose concentration, expressed in mg/dL, $INS_{0'}$ — fasting insulin concentration, expressed in mIU/L; $IRI_{Belfiore} = 2/\{[1/(GLU_{AUC} \times INS_{AUC})] + 1\}$, where: $GLU_{AUC} = GLU_{AUCi}/GLU_{AUCmean,}$ while $INS_{AUC} = INS_{AUCi}/INS_{AUCmean}$, where GLU_{AUCi} and INS_{AUCi} — areas under respective glucose or insulin concentration curve during OGTT in a given patient, while $GLU_{AUCmean}$ and $INS_{AUCmean}$ — areas under respective glucose or insulin concentration curves during OGTT for an age group for our population (those values were calculated in our earlier work) [36].

The total ghrelin concentration was measured using the Millipore RIA kit (Linco Research) with sensitivity level: 100-10.000 pg/mL, intra-assay CV: 3.3–10.0%, and inter-assay CV: 14.7–17.8%.

The leptin, adiponectin, and resistin concentrations were measured using the Millipore ELISA kit (Linco Research). Sensitivity level, the intra-assay CV, and inter-assay CV were, respectively: 0.5–100 ng/mL, 1.4––4.9%, and 1.3–8.6% for leptin; from 0.78 ng/mL, 7.4%, and 2.4–8.4% for adiponectin and from 0.16 ng/mL, 3.2–7.0%, and 7.1–7.7% for resistin.

Plasma insulin concentration was measured using the DRG ELISA kit; sensitivity level 1.76–100 μIU/mL, the intra-assay CV: 1.8–2.6, and inter-assay CV: 2.9–6.0.

IGF-I was assessed by Immulite, DPC assays; WHO NIBSC 1st IRR 87/518 standard was applied, with an analytical sensitivity of 20 ng/mL, calibration range up to 1600 ng/mL, intra-assay CV — 3.1–4.3% and inter-assay CV — 5.8–8.4%. For comparison of children with different age and sex, IGF-I concentrations were expressed as IGF-I SDS, according to reference data.

In the evaluation of the obtained results, ANOVA statistical analysis was applied. For some statistical comparisons with variant I/I, subjects heterozygous I/III and homozygous III/III for the mutation were combined and referred to as I/III + III/III. Comparisons between genotypes were made on adjusted values using Mann-Whitney U test (I/I *vs.* I/III + III/III) or Kruskal-Wallis test for the three genotype groups. A p value < 0.05 was considered to be statistically significant.

Table I. ***The incidence of individual variants (I/I, I/III, and III/III) of INS VNTR gene in the groups of children born small for gestational age or born appropriate for gestational age (SGA or AGA)***

Tabela I. ***Częstość występowania poszczególnych wariantów (I/I, I/III i III/III) VNTR genu INS w grupie dzieci urodzonych z niską i z prawidłową masą ciała (SGA i AGA)***

***INS* VNTR variant**	**I/I**	**I/III**	**III/III**
SGA = 123	68 (55.3%)	46 (37.4%)	9 (7.3%)
AGA = 132	71 (53.8%)	51 (38.6%)	10 (7.6%)
	p = 0.33	p = 0.39	p = 0.48

SGA — small for gestational age; AGA — appropriate for gestational age. The incidence distribution did not deviate from the Hardy-Weinberg equilibrium $\chi^2 = 1.16$; p = 0.71 for SGA and $\chi^2 = 0.59$; p = 0.55 for AGA. Odds ratio for III/III genotype is 0.88.

Results

We did not find any statistical differences in the occurrence of polymorphisms in the groups of children with SGA and AGA (Table I). The frequency distribution did not deviate from the Hardy-Weinberg equilibrium $\chi^2 = 1.16$; p = 0.71 for SGA and $\chi^2 = 0.59$; p = 0.55 for AGA.

In the analysed group of prepubertal SGA children we did not observe any statistical differences for birth weight and birth length among individual variants of *INS* VNTR gene groups (Table II).

In children with allele class III (I/III and III/III) group, the values of BMI and WHtR were higher than I/I but the differences were only on the border of statistical significance (Table III). In turn, we observed that triglyceride concentrations were significantly higher in the I/III+III/III group than in the I/I group, while we did not find any differences as regards cholesterol and its fractions between groups (Table IV). The correlation analysis showed that in the examined group of SGA children, triglycerides positively correlated with WHtR ($r = +0.31$, $p < 0.05$), BMI SDS ($r = +0.32$, $p < 0.05$), fasting insulin concentration ($r = +0.39$, $p < 0.05$), insulin at 60′ ($r = +0.22$, $p < 0.05$), insulin at 120′ ($r = +0.26$, $p < 0.05$), IRI_{HOMA} ($r = +0.37$, $p < 0.05$), $IRI_{Belfiore}$ ($r = +0.21$, $p < 0.05$), and leptin concentration ($r = +0.39$, $p < 0.05$).

We did not observe any statistical differences as regarded glucose concentrations among the groups. Nevertheless, we found that the fasting insulin concentration and IRI_{HOMA} was significantly lower in the III/III group than in the heterozygous I/III group, while insu-

Table II. *Perinatal data (mean ± SD) in individual variants of INS VNTR gene in the analysed group of prepubertal SGA children*

Tabela II. *Dane okołoporodowe (średnia ± SD) w zależności od rodzaju wariantu INS VNTR w analizowanej grupie przeddojrzewaniowych dzieci z SGA*

***INS* VNTR variant**	**I/I**	**I/III**	**III/III**	**I/I *vs.* I/III *vs.* III/III**	**(I/III + III/III)**	**I/I *vs.* I/III + III/III**
n =	61	42	9	*P value	51	**P value
Age (years)	6.9 ± 1.33	6.87 ± 1.43	6.8 ± 1.74	0.979535	6.85 ± 1.47	0.874067
GA (week)	38.34 ± 1.38	38.29 ± 1.31	37.89 ± 0.78	0.627259	38.22 ± 1.24	0.607538
BL [cm]	55 ± 17.11	53 ± 11.88	49.38 ± 2.62	0.54682	52.38 ± 10.94	0.370015
BL SDS	–0.15 ± 1.59	0.42 ± 1.49	–0.06 ± 1.36	0.231555	0.34 ± 1.46	0.129595
BW [g]	2318.2 ± 312.41	2314.5 ± 222.80	2383.3 ± 134.63	0.778081	2326.67 ± 210.49	0.869408
BW SDS	–2.02 ± 0.59	–2.11 ± 0.53	–2.04 ± 0.37	0.456618	–2.09 ± 0.52	0.717246

*p — according to Kruskal-Wallis test, **p — according to Mann-Whitney U test. GA — gestational age, BW — birth weight, BW SDS — birth weight standard deviation score, BL — birth length; BL SDS — birth length standard deviation score

Table III. *Auxological data (mean ± SD) in individual variants of INS VNTR gene in the analysed group of prepubertal SGA children*

Tabela III. *Dane auksologiczne (średnia ± SD) w zależności od rodzaju wariantu INS VNTR w analizowanej grupie przeddojrzewaniowych dzieci z SGA*

***INS* VNTR variant**	**I/I**	**I/III**	**III/III**	**I/I *vs.* I/III *vs.* III/III**	**I/III + III/III**	**I/I *vs.* I/III + III/III**
n =	61	42	9	*P value	51	**P value
Height [cm]	122.58 ± 10.79	124.03 ± 12.06	122.03 ± 14.12	0.790229	123.68 ± 12.32	0.616653
H SDS	0.16 ± 1.06	0.41 ± 1.18	0.07 ± 1.09	0.4697	0.35 ± 1.16	0.364538
Body mass [kg]	23.86 ± 6.45	26.07 ± 8.81	25.91 ± 10.74	0.338907	26.04 ± 9.06	0.140630
BMI [kg/m^2]	15.61 ± 2.18	16.47 ± 2.61	16.65 ± 3.31	0.156875	16.50 ± 2.71	0.055113
BMI SDS	–0.1 ± 0.95	0.18 ± 1.01	0.07 ± 1.28	0.361165	0.16 ± 1.05	0.163606
Waist [cm]	56.45 ± 7.01	58.93 ± 9.47	59.39 ± 12.70	0.290574	59.01 ± 9.97	0.116657
WHtR	0.46 ± 0.04	0.47 ± 0.05	0.48 ± 0.06	0.18045	0.48 ± 0.05	0.075773

*p — according to Kruskal-Wallis test, **p — according to Mann-Whitney U test. H SDS — height standard deviation score, BMI — body mass index, BMI SDS — body mass index standard deviation score, WHtR — waist-to-height ratio

Table IV. *Triglycerides, total cholesterol, or HDL-cholesterol and LDL-cholesterol fractions concentrations (mean ± SD) in individual variants of INS VNTR gene in the analysed group of prepubertal SGA children*

Tabela IV. *Stężenie triglicerydów, cholestrolu całkowitego i jego frakcji HDL i LDL (średnia ± SD) w zależności od rodzaju wariantu INS VNTR w analizowanej grupie przeddojrzewaniowych dzieci z SGA*

***INS* VNTR variant**	**I/I**	**I/III**	**III/III**	**I/I *vs.* I/III *vs.* III/III**	**I/III + III/III**	**I/I *vs.* I/III + III/III**
n =	61	42	9	*P value	51	**P value
Total cholesterol [mg/dL]	173.72 ± 25.85	170.71 ± 27.81	167.44 ± 36.68	0.750451	170.14 ± 29.17	0.492183
Triglycerides [mg/dL]	66.25 ± 27.40[a]	79.76 ± 36.33	76.22 ± 42.06	0.108695	79.14 ± 36.98[a]	0.036594
HDL-cholesterol [mg/dL]	60.11 ± 14.26	57.95 ± 15.69	58.67 ± 11.87	0.758787	58.08 ± 14.99	0.463705
LDL-cholesterol [mg/dL]	99.66 ± 20.54	98.24 ± 26.69	94.67 ± 36.00	0.837841	97.61 ± 28.17	0.657896
HDL/total cholesterol ratio	0.35 ± 0.09	0.35 ± 0.10	0.36 ± 0.11	0.856165	0.35 ± 0.10	0.755334

*p — according to Kruskal-Wallis test, **p — according to Mann-Whitney U test, [a] — $p < 0.05$

lin concentrations at other time points were similar in individual analysed groups. However, the comparison of groups I/III and III/III together shows that in children with allele class III, the concentration of insulin in 120′ of OGTT is significantly higher than in children with both class I alleles (Table V).

Table V. *Glucose and insulin concentrations during OGTT and the calculated values of IRIs (mean ± SD) in individual variants of INS VNTR gene in the analysed group of prepubertal SGA children*

Tabela V. *Stężenie glukozy i insuliny podczas OGTT oraz wartości wskaźników insulinooporności (średnia ± SD) w zależności od rodzaju wariantu INS VNTR w analizowanej grupie przeddojrzewaniowych dzieci z SGA*

***INS* VNTR variant**	**I/I**	**I/III**	**III/III**	**I/I *vs.* I/III *vs.* III/III**	**I/III + III/III**	**I/I *vs.* I/III + III/III**
n =	61	42	9	*P value	51	**P value
Glucose 0'[mg/dL]	81.39 ± 8.23	82.81 ± 7.25	79.78 ± 6.04	0.474	82.27 ± 7.09	0.549
Glucose 60'[mg/dL]	113.77 ± 34.67	105.55 ± 25.21	122.67 ± 20.66	0.213	108.57 ± 25.16	0.375
Glucose 120'[mg/dL]	102.55 ± 20.88	107.07 ± 18.26	106.11 ± 13.14	0.499	106.90 ± 17.35	0.240
Insulin 0'[uIU/mL]	3.68 ± 2.34	4.56 ± 2.95[a]	2.58 ± 2.29[a]	0.026	4.21 ± 2.92	0.286
Insulin 60'[uIU/mL]	20.19 ± 17.05	21.97 ± 20.60	23.64 ± 22.92	0.823	22.27 ± 20.80	0.564
insulin 120'[uIU/mL]	17.23 ± 10.77[c]	23.37 ± 17.81	20.45 ± 17.03	0.107	22.85 ± 17.54[c]	0.041
IRI_{HOMA}	0.74 ± 0.51	0.95 ± 0.63[b]	0.52 ± 0.48[b]	0.0376	0.87 ± 0.62	0.212
$IRI_{BELFIORE}$	0.86 ± 0.30	0.89 ± 0.37	0.9 ± 0.38	0.580	0.89 ± 0.37	0.679
C-peptide [ng/mL]	2.37 ± 1.31	2.41 ± 1.26	2.12 ± 1.49	0.835	2.36 ± 1.29	0.964

*p – according to Kruskal-Wallis test, **p – according to Mann-Whitney U test. [a,b,c] — $p < 0.05$

Table VI. *Ghrelin, IGF-I, cortisol, leptin, resistin, and adiponectin concentrations (mean ± SD) in individual variants of INS VNTR gene in the analysed group of prepubertal SGA children*

Tabela VI. *Stężenie greliny, IGF-I, kortyzolu, leptyny, rezystyny i adiponektyny (średnia ± SD) w zależności od rodzaju wariantu INS VNTR w analizowanej grupie przeddojrzewaniowych dzieci z SGA*

***INS* VNTR variant**	**I/I**	**I/III**	**III/III**	**I/I *vs.* I/III *vs.* III/III**	**I/III + III/III**	**I/I *vs.* I/III + III/III**
n =	61	42	9	*P value	51	**P value
Ghrelin [pg/mL]	2819.1 ± 2316.8	2231.8 ± 1301.8	2575.5 ± 2526.7	0.398	2299.1 ± 1584.2	0.199
IGF-I [ng/mL]	199.01 ± 67.46	204.35 ± 93.02	175.6 ± 68.59	0.608	199.07 ± 89.11	0.997
IGF-I SDS	0.67 ± 0.72	0.7 ± 0.82	0.52 ± 0.67	0.811	0.67 ± 0.79	0.998
Cortisol [μg/dL]	11.88 ± 5.76	12.27 ± 5.36	14.06 ± 7.47	0.571	12.61 ± 5.76	0.517
Leptin [ng/mL]	6.39 ± 5.79	7.9 ± 9.37	6.84 ± 8.38	0.635	7.69 ± 9.10	0.381
Resistin [ng/mL]	9.92 ± 4.42	10.24 ± 3.52	11.21 ± 4.61	0.676	10.43 ± 3.72	0.534
Adinopectin [ng/mL]	25.41 ± 12.07	25.99 ± 12.21	23.29 ± 12.03	0.837092	25.45 ± 12.09	0.987

*p — according to Kruskal-Wallis test, **p — according to Mann-Whitney U test

Among the analysed group of SGA children, we did not find any differences as regards ghrelin, leptin, adiponectin, resistin, C-peptide, IGF-I, or cortisol concentration (Table VI).

Discussion

In our study, we found that the incidence of individual *INS* VNTR variants was similar in children born AGA and SGA, and the distribution of the alleles in both groups was analogous to the testing of large Caucasian populations [14, 17]. The frequency distribution in the AGA and in SGA groups did not deviate from the Hardy-Weinberg equilibrium; the odds ratio for genotypes III/III in SGA was 0.88.

Among SGA children, similarly to results provided by others [14–18], we did not find any relationship as regards the examined variants of *INS* VNTR and birth weight or birth length. Although Lindsay et al. [10] have reported that the class III allele is associated with a reduction of birth weight, we should be aware that the analysis was performed among Pima Indians, in whom high incidence of obesity, IR, and T2DM is observed, and the distribution of birth weight may be associated with these maternal factors.

Next, we analysed the influence of *INS* VNTR class IIII on auxological and hormonal parameters in a large group of prepubertal SGA children after the catch-up of growth phenomenon. There are few studies concerning the prevalence of obesity and IR depending on the

discussed variants in such a sublime population as children with SGA [15, 29]. Vu-Hong et al. [15] and Coletta et al. [29], similarly to us, did not find any significant differences between BMI and *INS* VNTR variants in SGA children.

In a general population, other authors also did not support associations between *INS* VNTR and body composition in childhood [25–28].

Generally, higher levels of insulin concentration and IRI_{HOMA} in SGA than AGA children are reported, especially in those of SGA in whom rapid weight gain in the first years of life was observed [37]. The authors suspected that it may be a risk factor for the development of IR and T2DM in later life. However, not all reports confirm this relationship [38]. In our study, we found that prepubertal SGA children with homozygous III/III had lower fasting insulin concentration and lower IRI_{HOMA} than children from other groups, whereas children with at least one allele T (class III), i.e. III/III or I/III, tended to have higher secretion of insulin at 120′ of OGTT than SGA children with homozygous I/I.

Vu-Hong et al. [15] reported that the INS VNTR class III allele was associated with higher fasting insulin concentration and a lower quantitative insulin sensitivity check index (QUICKY) in SGA subjects, but not in AGA once; however, the study was performed among young adults born SGA, with mean age 22 years. Other authors demonstrated that *INS* VNTR genotypes in the general population were not associated with fasting insulin secretion and also reported the lack of support for a role of *INS* VNTR in IR and T2DM [17, 23, 27]. In large-scale studies involving Danish Caucasians, young healthy subjects who were carriers of the *INS* VNTR class III/III had significantly decreased acute serum insulin and C-peptide responses during an IVGTT when compared with class I/I and heterozygous carriers. However, no impact of the class III allele on serum insulin and C-peptide responses to an oral glucose load could be demonstrated in middle-aged subjects [28].

Some researchers assessed insulin levels during OGTT in individual variants of *INS* VNTR in a group of obese children [39, 40]. They observed significantly higher insulin secretion in I/I obese children versus III carriers.

Thus, the relationship between the variants of *INS* VNTR and insulin concentration secretion and IR remains unclear.

We are aware of the limitations resulting from the cohort size, so further investigations are needed to elucidate the issue in question.

Low fasting insulin levels in homozygous III/III children is not surprising in the sense that class III alleles are associated with lower levels of insulin mRNA in vivo [7]. In turn, high levels of insulin at the 120th minute of OGTT in class III individuals indicates a greater functional reserve of beta cells in response to hyperglycaemic stimuli. Similar results obtained in a patient with familial background of T1DM were presented by Fendler et al. [21] Moreover, Fendler et al. [19] found that class I homozygosity is a significant risk factor of DM1 and acts independently from HLA haplotype in the actual risk of diabetes in children. Cejkova et al. [41] confirmed that the simultaneous effect of HLA and *INS* VNTR genotypes predispose to an increased risk of T1DM, LADA, or T2DM development. Similar conclusions regarding *INS* VNTR were presented by Zhang et al. [42]. In our study, apart from the insulin concentration, we also measured the concentration of C-peptide, which did not differ depending on the individual variant in the group of children analysed by us.

Also, we did not find any differences in $IRI_{BELFIORE}$ among individual variants of *INS* VNTR gene. Thus, even if the concentration of insulin is higher at the 120th minute of OGTT, it does not indicate the presence of IR. However, nutritional interventions may prevent IR in the future.

In our earlier work [43] we found that in prepubertal SGA children with catch-up, higher ghrelin and higher IGF-I concentration were observed than in SGA children without catch-up. Thus, we also analysed the differences in the concentrations of these hormones, depending on the genotypes. We should be aware that insulin concentration is negatively correlated with ghrelin concentration, and that insulin is permissive in nature for the production of IGF-I. However, in the group of children examined by us, we did not identify any significant differences in this respect.

In conclusion, variant *INS* VNTR class III was shown not to be associated in any essential way with birth weight in the Polish population. However, our data support the possible role of this gene in the development of insulin resistance in the later life of SGA individuals.

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