

Prevalence of autoantibodies against some selected growth and appetite-regulating neuropeptides in serum of short children exposed to *Candida albicans* colonization and/or *Helicobacter pylori* infection: the molecular mimicry phenomenon

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Abstract

OBJECTIVES: Many of peptides synthesized in gastrointestinal tract (GI) and adipose tissues, regulate growth and food intake. The GI microflora is an antigenic source. Based on the molecular mimicry hypothesis, intestinal microbe-derived antigens may trigger the production of autoantibodies cross-reacting with some neuropeptides.

DESIGN: The aim of the study was to assess whether in idiopathic short stature (ISS) children with *Candida albicans* (*C.albicans*) colonisation and/or *Helicobacter pylori* (*H.pylori*) infection the autoantibodies (in positive levels) against selected neuropeptides [anti-NP Abs(+)]: ghrelin, leptin, orexin A, αMSH are more prevalent than in Controls.

SETTING: The study group comprised 64 children with ISS and 36 children with normal height (Controls). In each child, IgG antibodies against *H.pylori*, ghrelin, leptin, orexin A and αMSH were assessed in serum, while presence of *C.albicans* – in stool samples.

RESULTS: The higher prevalence of anti-NP Abs(+) in ISS children with *C.albicans* and/or *H.pylori* than in normal height children with the colonization in question (34.4% vs 21.1%, $p<0.01$) was found. The prevalence of anti-NP Abs(+) in groups of children without *C.albicans* and *H.pylori* were low, anti-NP Abs(+) were detected in 9.4% of ISS children only, while in Controls they were not found.

CONCLUSIONS: In short children with *C. albicans* and/or *H. pylori* the incidence of autoantibodies against selected neuropeptides is high. It probably is connected with molecular mimicry between antigens of these microbiota and the mentioned peptides. It is tempting to speculate that presence of cross-reacting autoantibodies against regulatory neuropeptides may results in worse growth velocity. However, further studies are necessary to elucidate this issue.

INTRODUCTION

Idiopathic short stature (ISS) is defined as a condition in which the height of the individual is more than 2 standard deviations (SD) below the mean for age and sex, without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities (Wit *et al.* 2008; Pedicelli *et al.* 2009; Wit, 2011). However, it seems that many of ISS children do not represent familial short stature or constitutional delay of growth and puberty, and that the causes of short stature remain unknown. Although children with ISS show no evidence of malnutrition or gastrointestinal tract (GI) diseases, in many of them, a low body mass is observed (Thibault *et al.* 1993).

Recently, it has been proved that some peptide hormones synthesized in GI and adipose tissue regulate growth and body weight in children. One of them is ghrelin, a 28-amino-acid octanoylated peptide, predominantly produced by X/A cells in the gastric oxyntic mucosa, which – on the one hand – is a natural ligand of the type 1a growth hormone secretagogue receptor (Kojima *et al.* 1999; Sato *et al.* 2012), but – on the other hand – is an orexigenic peptide that regulates appetite and body weight (Nakazato *et al.* 2001). Also leptin, which is one of the adipokines (adipose tissue-derived peptide hormone) has an ability to transmit information on hunger or satiety state and energy storage to the brain. Next, these peptides interact with orexigenic neuropeptides such as neuropeptide Y (NPY) and orexin A or anorexigenic neuropeptides, e.g. alpha-melanocyte-stimulating hormone (α MSH) and regulate appetite and different homeostatic functions (Kalra *et al.* 1999).

In short children, a higher incidence of *Candida albicans* (*C. albicans*) colonisation and *Helicobacter pylori* (*H. pylori*) infection than in controls was described (Takahashi *et al.* 2002; Fialho *et al.* 2007; Stawerska *et al.* 2013). The GI microflora is an antigenic source. Based on the molecular mimicry hypothesis, intestinal microbe-derived antigens may trigger the production of autoantibodies cross-reacting with regulatory peptides and hormones and modify the functioning of the orexigenic/anorexigenic axis (Fetissov *et al.* 2008a).

Recently, the influence of these antibodies on the occurrence of autoimmune diseases and eating disor-

ders has been analyzed (Oldstone, 2005; Alcock *et al.* 2014). However, so far, there have been no studies on the incidence of autoantibodies against neuropeptides in children with short stature.

Thus, the aim of the study was the evaluation of the prevalence of autoantibodies (in positive levels) against some selected growth- and appetite-regulating peptides in short children, depending on *C. albicans* colonization and/or *H. pylori* infection.

MATERIAL AND METHODS

An approval for the study was obtained from the Bioethical Committee in the Polish Mother's Memorial Hospital – Research Institute (PMMH-RI) in Lodz.

We analyzed 100 children with short stature. In all patients, the body height was measured using a stadiometer and the height standard deviation score (HSDS) was calculated according to current population standards (Palczewska & Niedźwiecka 2001). Only children with HSDS below -2.0 were qualified into the study group. Next, based on the child's current percentile position, the height age (HA) was calculated (as the age ascribed to the 50th percentile for a given child's height). The body mass was assessed in all the qualified patients and that was followed by the calculation of the body mass index standard deviation score for chronological age (BMI SDS for CA) and for height age (BMI SDS for HA). BMI SDS for HA is better indicator for the assessment of nutrition status in short children than BMI SDS for CA and it is used for comparison of body mass in normal height and short stature children. Children with thyroid dysfunction, autoimmune diseases, eating disorders, suffering from chronic cardiovascular, respiratory or urinary system diseases, as well as girls with Turner's syndrome (diagnosed with the use of chromatin X and/or karyotype tests) were excluded from the study.

None of the children reported symptoms from the GI tract or previously had been diagnosed with or treated for GI diseases.

Growth hormone secretion assessment

In each individual, laboratory tests were performed as a part of the diagnostics of short stature conducted during hospitalisation at the Department of Endocrinology and Metabolic Diseases in PMMH-RI.

In order to assess the growth hormone (GH) secretion, in each child, a 3-hour nocturnal profile of GH secretion was recorded every half-hour, starting from the first hour after falling asleep. Next, two (2) stimulation tests were performed: one – after oral administration of clonidine (with the dose of 0.15 mg/m² of body surface and GH measurements at time 0 and at 30th, 60th, 90th and 120th minute of the test), and another – after intramuscular administration of glucagon (with the dose of 30 µg/kg body mass, with GH measurements at time 0 and at 90th, 120th, 150th and 180th minute of

the test). Peak GH concentration (GH_{\max}) was determined in both tests and after falling asleep. The children with GH_{\max} values $<10 \text{ ng/ml}$ were qualified as individuals with growth hormone deficiency (GHD), while in the patients with GH_{\max} value $\geq 10 \text{ ng/ml}$, ISS was diagnosed. For current analysis, only children with ISS were qualified.

The growth hormone levels were measured using the immunometric method. The measurements were performed with Immulite, DPC assay kits, calibrated to the WHO IRP 98/574 standard set, with the sensitivity level: 0.01 ng/ml , range: up to 40 ng/ml , the conversion index: $\text{ng/ml} \times 2.6 = \text{mIU/l}$, the intra-assay CV: 5.3–6.5% and inter-assay CV: 5.5–6.2%.

Study group

Finally, the study group consisted of 64 children with ISS (27 girls and 37 boys), aged from 4.1 to 17.8 years, mean \pm SD: 10.3 ± 3.44 years.

In each child, IgG antibodies against ghrelin (anti-ghrelin), leptin (anti-leptin), orexin A (anti-orexin A) and α MSH (anti- α MSH) were assessed, serologic tests for *H.pylori* were performed and stool samples for *Candida sp.* were taken.

Determination of IgG antibodies against ghrelin, leptin, orexin A and α MSH

Laboratory ELISA assays were used for the assessment of the IgG antibodies towards appetite regulating peptide hormones and neuropeptides. Concentrations of standard antigens and labeled antibodies were determined in preliminary studies. The standard peptide hormones and neuropeptides: ghrelin (Abbiotec, San Diego, USA), leptin (Bio Vendor, Brno, Czech Republic), orexin A and α MSH (Phoenix Pharmaceuticals, Inc., Burlingame, USA), were diluted in carbonate buffer pH 9.6 to the concentration of $2 \mu\text{g/ml}$ and were distributed into the wells of 96 well plate of MaxiSorp type (Nunc, Kastrup, Denmark), $100 \mu\text{l}/\text{well}$. The plates were incubated for 18 h, at 4°C , then washed three times with phosphate buffered saline (PBS) supplemented with 0.5% Tween 20 (PBS/Tween), $250 \mu\text{l}/\text{well}$. Excess binding sites were blocked for 2 h with 1% bovine serum albumin (BSA, fraction V, Sigma, St. Louis, Mo., U.S.A) in PBS/Tween ($300 \mu\text{l}/\text{well}$). After five (5) washings, the wells were supplemented with the serum samples diluted 1:100 in BSA/PBS/Tween ($100 \mu\text{l}/\text{well}$), and incubated for 1 h, in 37°C . Peroxidase conjugated rabbit antibodies against human IgG (Dako) were diluted 1:6000 in PBS/BSA/Tween and added into the wells for 1 h, 37°C . The colour reaction was developed as previously described. The optical density (OD) values were read at 450 nm wave length. In order to exclude the non-specific interactions, the control wells were included for each plate: the wells coated with the hormone and incubated with serum sample without secondary antibody, the wells coated with antigen and incubated with peroxidase conjugated

antibody, the wells coated with antigen, followed by incubation with the substrate, and non-coated wells blocked with BSA/PBS/Tween, followed by the serum dilution and secondary antibody.

The ELISA cut off values were determined for wells coated with particular hormone and corresponding HRP-labeled antibodies + 2SD and were equal to 0.200. The absorbance readings exceeding the cut off values were considered positive.

H.pylori infection assessment

In each child, the serology test was performed to detect *H.pylori* infection. Specific antibodies to *H.pylori* antigens were detected with a laboratory enzyme-linked immunosorbent assay (ELISA), as previously described by Rechciński *et al.* (1997). The assay was conducted with a glycine acid extract (GE, 0.01 mg/ml) from the reference *H.pylori* strain CCUG 17874 (Culture Collection, University of Gothenborg, Sweden). The antigen was adjusted to the required concentration ($10 \mu\text{g/ml}$) in 0.05 M carbonate buffer (0.015 M Na_2CO_3 , 0.035 M NaHCO_3), pH 9.6. The serum samples were diluted from 1:500 to 1: 64 000 (to determine IgG), and from 1:100 to 1:6 400 (to determine IgA), peroxidase conjugated rabbit antibodies (Dako, Glostrup, Denmark) to human IgG were diluted 1:6 000 and to human IgA were diluted 1:1 000. For colour reaction the chromogen *o*-phenylenediamine dihydrochloride (Sigma, St. Louis, MO, USA) was used in the concentration of 1 mg/ml in 0.1 M citric phosphate buffer (0.1 M citric acid, H_2O , 0.067 M Na_2HPO_4 , 12 H_2O), pH 5.0, with 0.005 ml of 30% H_2O_2 per 1 ml of the buffer. The OD values were read at 450 nm wave length (1420 Victor 2, Oy, Turku, Finland). The panel of negative control sera was used to establish the cut off value between positive and negative ELISA results. The border value was defined as two standard deviations above the mean of control negative sera from subjects uninfected with *H.pylori*.

C.albicans colonization assessment

In order to diagnose *C.albicans* colonization, stool samples from patients were cultured for *Candida sp.* However, only significant levels of *C.albicans* were taken into consideration as *candidiasis mucosae* of GI.

Control group

The same tests (IgG antibodies anti-ghrelin, anti-leptin, anti-orexin A and anti- α MSH, serologic tests for *H.pylori* and analysis of stool samples for *Candida sp.*) were performed in 40 normal height children diagnosed in other departments of PMMH-RI for different reasons (however, not meeting the exclusion criteria). The control group was selected in such a manner that the prevalence of *H.pylori* and *C.albicans* was similar to the ISS group. Thus, the control group comprised 36 children with normal height (23 girls and 13 boys), aged from 4.5 to 15.8 years, mean \pm SD: 11.4 ± 2.7 years.

Statistics

Statistical analysis was performed with STATISTICA 5.0 PL programme. Mann-Whitney U test was used to verify the hypothesis that the two analyzed samples came from two statistically different populations. Chi-square (χ^2) test was used for the comparison of the prevalence of the analyzed parameters in the studied groups. In the case of a small number of data in the groups, the Yates' alteration was used.

RESULTS

In the ISS group, the *H.pylori* infection and/or *C.albicans* colonization were found in 32 out of 64 children (50%): *H.pylori* – in 13 out of 64 children (20.3%), *C.albicans* – in 29 out of 64 children (45.3%), while in 9 children (14.1%) both pathogens were observed.

Because we selected a control group in such a manner that the incidence of *H.pylori* and *C.albicans* should be similar to that in the ISS group, *H.pylori* infection and/or *C.albicans* colonization were found in 19 out of 36 children (52.8%): *H.pylori* – in 11 out of 36 children (30.5%), *C.albicans* – in 14 out of 36 children (38.9%), while in 6 children (16.7%) both pathogens were observed.

In the total group of children (ISS and Controls), the prevalence of the positive levels of analyzed autoantibodies was 18%. However, the prevalence of autoantibodies was significantly higher in ISS children than

in children from the control group (21.8% vs 11.1%, $p<0.05$), OR=1.96. Moreover, the prevalence of anti-NP Abs(+) was significantly higher in ISS children with *H.pylori* and/or *C.albicans* than in Controls with *H.pylori* and/or *C.albicans*. (34.4% vs 21.1%, $p<0.05$) (Table 1).

Prevalence of anti-NP Abs(+) in the groups of children without *C.albicans* and *H.pylori* were low, they were detected in 4 cases only (9.4%) of ISS children, while in the control group they were not found (Table 1).

Neither in ISS group nor in Controls, we found any statistical differences as regards growth deficiency (expressed by HSDS) and body mass (expressed by BMI SDS for HA) between children with *H.pylori* and/or *C.albicans* and children without *H.pylori* and/or *C.albicans* (Table 2). Next, an analysis of individual autoantibodies was conducted (Table 3).

Anti-ghrelin autoantibodies were observed in two children, both with ISS. In one of them, a significant colonization of *C.albicans* and *H.pylori* infection was observed at the same time, while in the other child none of the two analyzed pathogens was found.

Anti-leptin autoantibodies were observed both in ISS children (5 cases) and in Controls (2 cases). Thus, their prevalence was similar in ISS and in Controls (7.8% vs 5.5%; $p>0.05$). However, their presence was correlated with presence of microbiota, because they were confirmed in three (3) children with *C.albicans*, further

Tab. 1. Prevalence of autoantibodies against some selected growth and appetite-regulating hormones and neuropeptides in short children and children from control group, depending on *Candida albicans* colonization and/or *Helicobacter pylori* infection.

Groups	ISS, n=64		Controls, n=36		Total n=100
	with <i>H.pyl.</i> and/or <i>C.alb.</i> , n=32	without <i>H.pyl.</i> or <i>C.alb.</i> , n=32	with <i>H.pyl.</i> and/or <i>C.alb.</i> , n=19	without <i>H.pyl.</i> or <i>C.alb.</i> , n=17	
anti-ghrelin	1	1	0	0	2
anti-leptin	4*	1	2	0	7*
anti-orexin A	1	0	0	0	1
anti- α MSH	6*	1	2	0	9*
total	11 (34.4%)	3 (9.4%)	4 (21.1%)	0 (0%)	18 (18.0%)
Total in groups	14 (21.8%)		4 (11.1%)		

*One child was found to have 2 antibodies simultaneously

Tab. 2. Auxological parameters of children with short stature and Controls depending on *Candida albicans* colonization and/or *Helicobacter pylori* infection.

		age (years)	HSDS	BMI SDS for HA
ISS, n=64	with <i>H.pyl.</i> and/or <i>C.alb.</i> , n=32	10.09±3.39	-2.41±0.88	-0.28±1.56
	without <i>H.pyl.</i> and <i>C.alb.</i> , n=32	10.57±3.54	-2.47±0.56	-0.16±1.08
Controls, n=36	with <i>H.pyl.</i> and/or <i>C.alb.</i> , n=19	11.63±2.28	-0.23 ±0.90	0.07±1.25
	without <i>H.pyl.</i> and <i>C.alb.</i> , n=17	10.96±3.22	-0.25±0.99	0.67±1.54

No significant differences between the same parameters in ISS group and in control group. ISS – idiopathic short stature; HSDS – height standard deviation score; BMI SDS – body mass index standard deviation score; *C.alb.* – *Candida albicans*; *H.pyl.* – *Helicobacter pylori*

three (3) – in children with *H.pylori* and only further one (1) case – in child without *H.pylori* or *C.albicans*.

As regards anti-orexin A autoantibodies, they were found in one (1) child only, in whom ISS and *C.albicans* were simultaneously observed.

Anti- α MSH autoantibodies were most prevalent in the total examined group of children (9 cases). They were observed more frequently in the ISS group (7 cases, 10.9%) than in Controls (2 cases, 5.5%); $p<0.05$. Their occurrence coincided with the presence of *C.albicans* in all – beside one – reported cases. In two of those children (one from ISS group and one from the Control group), *H.pylori* infection additional was observed, besides *C.albicans*.

One child (with ISS and *C.albicans*) was found to have 2 antibodies simultaneously (Table 3).

DISCUSSION

An optimization of the procedures used in the diagnosis of short stature in children is very important because there are still too many ISS cases where the cause of the condition remains unknown. Over the last few years, several new causes of short stature have been discovered, and genome-wide association studies (GWASs) have identified multiple genes involved in height variations in the normal population (Wit, 2011).

However, there are also other factors which affect the regulation of growth processes and food intake, such as peptides synthesized both centrally and peripherally in the GI tract or adipose tissue.

In our previous report (Stawerska *et al.* 2013), we proved that approximately 70% of children with ISS who had no symptoms or signs of GI diseases, were still diagnosed with a variety of GI disorders, particularly *H.pylori* infection and *C.albicans* colonization. So far, it has not been certain whether these microbiota may be responsible for growth disorders and low weight gain.

The microflora of the GI tract is a major source of antigens. Recently, Fetissov *et al.* (2008a, 2008b) published a series of reports concerning the molecular mimicry phenomenon between antigens of intestinal flora and some regulatory hormones and neuropeptides. In the serum of healthy people, the authors detected antibodies against 14 peptides involved in the regulation of food intake. Among others, the authors demonstrated the phenomenon of molecular mimicry between *H.pylori* and leptin and α MSH and between *C.albicans* and ghrelin, leptin, orexin, and α MSH. That is what sparked off our work.

To the best of our knowledge, so far there have been no studies on the presence of autoantibodies against neuropeptides or hormones derived from GI tract or

Tab. 3. Data of individual children in whom autoantibodies against selected peptides were found in both ISS and control group.

case	group	sex	CA (years)	HA (years)	HSDS	BMI SDS for HA	GHmax (clonidine)	GHmax (glucagon)	GHmax (nocturnal)	IGF-I SDS	C.alb.	H.pyl.	IgG anti-ghrelin	IgG anti-leptin	IgG anti-orexin A	IgG anti- α MSH
1	ISS	m	6.01	5.1	-2.10	-1.05	7.89	2.4	10.1	-1.70	+	+	+	-	-	-
2	ISS	m	14.33	10.9	-2.75	-1.01	7.1	10.3	1.7	0.44	-	-	+	-	-	-
3	ISS	f	5.87	4.0	-2.64	-2.01	16.8	4.4	12.0	-0.29	+	-	-	+	-	+
4	ISS	m	12.00	9.0	-3.00	1.99	5.58	10.94	5.26	-0.58	-	+	-	+	-	-
5	ISS	f	13.19	8.0	-4.30	0.00	16.4	13.3	16.4	-2.02	-	+	-	+	-	-
6	ISS	m	12.00	10.0	-2.2	-1.24	27.3	8.5	23.2	-1.51	+	-	-	+	-	-
7	ISS	m	8.85	7.1	-2.03	0.81	18	9.41	9.67	-1.54	-	-	-	+	-	-
8	ISS	m	10.57	10.0	-2.24	-2.01	5.9	10.6	12	-0.65	+	-	-	-	+	-
9	ISS	m	6.32	4.4	-3.07	-0.92	8.17	1.59	13.8	-	+	+	-	-	-	+
10	ISS	m	7.21	5.5	-2.43	-0.06	15.9	12.8	16.7	-1.29	+	-	-	-	-	+
11	ISS	m	9.56	7.1	-2.40	-0.92	19.3	6.51	10.1	-0.15	+	-	-	-	-	+
12	ISS	m	13.50	10.7	-2.26	-1.46	30.2	-	32.5	-	+	-	-	-	-	+
13	ISS	f	15.60	10.8	-2.95	0.74	4.25	18.7	18.3	-0.68	-	-	-	-	-	+
14	ISS	m	13.14	12.0	-2.10	-1.60	11.75	4.83	4.82	-1.48	+	-	-	-	-	+
15	Controls	f	11.90	12.5	0.57	-1.40					+	-	-	-	-	+
16	Controls	f	16.78	16.0	0.67	-0.71					+	+	-	-	-	+
17	Controls	f	13.90	12.3	-1.17	1.41					+	-	-	+	-	-
18	Controls	f	9.30	8.3	-1.00	0.19					-	+	-	+	-	-

ISS – idiopathic short stature; HSDS – height standard deviation score; BMI SDS – body mass index standard deviation score; C.alb. – *Candida albicans*; H.pyl. – *Helicobacter pylori*; GHmax – maximal growth hormone secretion; CA – chronological age; HA – height age

adipose tissue in patients with short stature and the GI infections mentioned above.

In the present study, the significantly higher prevalence of cases with the positive levels of autoantibodies against selected neuropeptides among ISS children with *C. albicans* and/or *H. pylori* than in normal height children and in children without *C. albicans* and *H. pylori* was found. However, we still have no evidence that their presence may have an impact on the growth process and food intake disorders in children.

It is well known that in patients with *H. pylori* infection serum ghrelin concentration is reduced (Ozen *et al.* 2011). What is interesting, in some children with short stature, ghrelin concentration is elevated (Stawarska *et al.* 2012). Takagi *et al.* (2013) proved that ghrelin-reactive immunoglobulins protect ghrelin from degradation and may enhance its orexigenic effect, which was increased in obese humans. However, the potential influence of these autoantibodies on ghrelin mediated stimulation of GH secretion remains unknown. In the group of children examined by us, we found anti-ghrelin autoantibodies in two children only – in one of them both *C. albicans* colonization and *H. pylori* infection was observed, while in the other one neither the *C. albicans* nor *H. pylori* was found. Thus, it is difficult to confirm any relationship between short stature, presence of mentioned microbiota and anti-ghrelin autoantibodies.

Leptin is well known protein which regulates food intake and strongly positively correlates with body mass. In children with cerebral palsy and decreased subcutaneous fat, the low leptin concentrations were observed (Yakut *et al.* 2006). Hypoleptinaemia is also connected with disturbed control of appetite and physical hyperactivity in girls with anorexia nervosa (Baranowska *et al.*, 2008). It has previously been reported that lower leptin levels in patients with *H. pylori* infection are observed (Pacifico *et al.*, 2008). Moreover, Carro *et al.* (1997) have suggested that leptin is also metabolic signal that regulated GH secretion. Since Fettisov *et al.* (2008a) found the molecular mimicry between *H. pylori* and leptin and between *C. albicans* and leptin, we tested the hypothesis that anti-leptin autoantibodies might modify the functioning of the anorexigenic and orexigenic axis. We found anti-leptin autoantibodies in 3 children with *H. pylori*, in 3 other children with *C. albicans* and in only 1 child without any of the examined pathogens. Thus, it is possible that there is a relationship between *H. pylori* and *C. albicans* and the abovementioned autoantibodies. However, because they are observed both in short and in normal height children, it is difficult to make any conclusions.

It was proved that intestinal inflammation might influence food intake and nutritional status by modulation of αMSH by their autoantibodies (Coquerel *et al.* 2012). In current study, anti-αMSH autoantibodies were found in six (6) children with *C. albicans* and in two (2) with *C. albicans* and *H. pylori* at the same time, mainly from the ISS groups. Thus, we confirmed the

occurrence of anti-αMSH autoantibodies in 8 out of 43 children with *C. albicans* (18.6%). Even if there is a causal relationship between the presence of *C. albicans* and the occurrence of the anti-αMSH autoantibodies, it is not certain that they affect growth velocity in children.

Certainly, we are aware of the limitations of the results we obtained. First, the number of children from the control group is not large. Secondly, there is no strong evidence to suggest that the observed autoantibodies are closely associated with the presence of *H. pylori* and *C. albicans* because – as proven by Fettisov *et al.* (2008a) – many other pathogens also cross-react with these hormones. Therefore, further research is planned. We intend to draw attention to the specific antigens of *H. pylori* and *C. albicans* that induce the potentially autoreactive response.

Proving the importance of the molecular mimicry phenomenon for the regulatory functions of neuropeptides and hormones in short children indicates the need for *H. pylori* and *C. albicans* diagnosis, followed by eradication in any child with growth and weight disorders. It should be emphasized that both screening for *H. pylori* infection and testing for *C. albicans* colonization do not require specialized diagnostic tools. Moreover, the treatment of both infections is commonly known and generally successful.

Summing up, a significantly higher incidence of autoantibodies against selected neuropeptides observed in children with idiopathic short stature and the presence of *C. albicans* colonization and/or *H. pylori* infection, suggest that autoimmunization, which is a result of the molecular mimicry phenomenon and cross-reaction with peptide hormones, may have an impact on the growth process and food intake disorders in children. However, further studies are necessary to elucidate this issue.

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Conflict of interest statement. The authors declare no conflict of interest.

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