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# Genetic analysis of the *PAPP-A2* gene and evaluation of free IGF-1, IGFBP-5, and ALS concentrations in a group of 22 patients with idiopathic short stature

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## Abstract

**Introduction:** Short stature is one of the main reasons for consultation in outpatient clinics and paediatric endocrinology departments and is defined as height below the 3<sup>rd</sup> centile or less than -2 standard deviations (SDs).

**Material and methods:** The study's overarching aim was to analyse the *PAPP-A2* gene at mutation sites described to date and at exons 3, 4, and 5, which encode the fragment of the catalytic domain with the active site of the pregnancy-associated plasma protein A2 (PAPP-A2) protein. The secondary aims of the study were clinical and auxological analysis of a group of patients with idiopathic short stature and biochemical analysis of growth hormone–insulin-like growth factor-1 (GH–IGF-1) axis parameters not assessed as part of the routine diagnosis of short stature, such as free IGF-1, insulin-like growth factor binding protein 5 (IGFBP-5), and acid-labile subunit (ALS) levels. Molecular analysis of the *PAPP-A2* gene was performed using polymerase chain reaction (PCR) and direct sequencing. Biochemical analysis of free IGF-1, IGFBP-5, and ALS was performed by enzyme-linked immunosorbent assay (ELISA).

**Results:** The mean height standard deviation score (HSDS) in the study group was -2.95. None of the patients exhibited previously described mutations in the *PAPP-A2* gene or mutations in exons 3, 4, and 5 encoding the fragment of catalytic domain with the active site of the PAPP-A2 protein. In 4 patients, the known, non-pathogenic, heterozygotic polymorphism c.2328C>T(rs10913241) in exon 5 was found.

**Conclusions:** Free IGF-1 levels correlate better with height and HSDS than total IGF-1 levels. The previously described mutations in the *PAPP-A2* gene and mutations in exons 3, 4, and 5 encoding the fragment of catalytic domain with the active site of the PAPP-A2 protein were not detected; only the known and non-pathogenic, heterozygotic polymorphism c.2328C>T(rs10913241) in exon 5 of the *PAPP-A2* gene was observed.

**Key words:** idiopathic short stature; *PAPP-A2*; IGF-1; free IGF-1

## Introduction

Idiopathic short stature (ISS) is currently defined as height < -2 standard deviations (SDS), normal birth size (birth weight and length > -2 SDS), absence of abnormal physical features, normal general screening investigations, normal body proportions, and absence of major dysmorphic features [1].

In 2016, Andrew Dauber et al. first described 2 unrelated families with a confirmed autosomal recessive mutation in the *PAPP-A2* protease gene [2]. In members of the described families, the progressive loss of growth velocity without a significant pubertal growth spurt, height below the target height, microcephaly, and long and thin bones were observed. In laboratory tests, a characteristically elevated concentration of growth hormone and growth hormone-dependent factors, such as total insulin-like growth factor (IGF-1),

insulin-like growth factor binding proteins (IGFBP): IGFBP-3, IGFBP-5, acid-labile subunit (ALS), and IGF-2, and decreased free IGF-1 were documented [2]. Evaluation of the parameters of the hypothalamic-pituitary-somatomedin axis, especially the observed disproportion between the concentrations of total and free IGF-1, indicates that pregnancy-associated plasma protein A2 (PAPP-A2) protease is the key regulator of the bio-availability of IGF-1 [3]. The bone age was consistent with the chronological age [2]. On the basis of changes in the proportions of total and free IGF-1, scientists assumed that treatment with recombinant human insulin-like growth factor 1 (rhIGF-1) should result in a change in the growth hormone (GH)–IGF-1 axis parameter balance, ultimately resulting in an increase in active IGF-1 and improving the growth rate in patients with a confirmed mutation in the *PAPP-A2* gene. Available data confirmed that rhIGF-1 therapy improved



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the patients' growth rate and height standard deviation score (HSDS) [4–6].

Short stature is a widely known topic of interest; however, subsequent reports have emphasised that further work is necessary for a broader understanding of this socially important problem. To date, 3 families with a confirmed mutation in the *PAPP-A2* gene have been described. No papers analysing the occurrence of mutations in the *PAPP-A2* gene in patients with ISS either in Poland or elsewhere have been published. The importance of this problem is additionally related to the availability of rhIGF-1, which could improve the final height of patients with a confirmed mutation in the *PAPP-A2* gene.

## Material and methods

### Aim of the study

The main aim of this study was to analyse the *PAPP-A2* gene in a group of 22 patients with ISS. The detailed aims were as follows:

- free IGF-1, IGFBP-5, and ALS analysis;
- analysis of the *PAPP-A2* gene (Gene ID: 60676, 1q25.2) at the sites of the mutations described to date, i.e. c.3098C> T, p.Ala1033Val in exon 8, and p.D643fs25\* in exon 3;
- analysis of exons 3, 4, and 5, which encode the fragment of catalytic domain with the active site of PAPP-A2 protein, in the absence of the known mutations.

### Study group

The study group consisted of 22 patients (16 girls, 6 boys) with ISS (HSDS < -2) diagnosed in the Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences. The study group was divided according to the stage of puberty into G1 (Th1) and G5 (Th5), with 12 and 10 patients, respectively. One girl was at Tanner stage 3 (during the analysis, she was included in the Tanner stage 5 group). Patients were enrolled according to the following recruitment criteria:

- height below 3<sup>rd</sup> centile or -1 SD below target height (TH);
- absence of chronic diseases that could be the cause of short stature;
- serum level of GH above 10 ng/mL in at least one of the 2 performed tests: after stimulation or after sleeping;
- Turner syndrome excluded.

The control group consisted of 9 patients: 2 girls in the Th1 stage, 2 girls in the Th5 stage, 3 boys in the G1 stage, and 2 boys in the G5 stage, who were recruited from patients at the Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences.

### Auxological analysis

The birth weight (BW) and the birth length (BL) were expressed as SDS using reference data by Fenton et al. [7]. Small for gestational age (SGA) was described as birth weight or birth length < -2 SD. The patient's postnatal height and weight were assessed by the attending physician. Height measurements were always taken in the same room using a Harpenden-type measuring device, with an accuracy of 1 mm; 3 measurements were taken, and the mean was calculated. The auxological analysis of height, weight, body mass index (BMI), and the ratio of weight to height referred to the Polish population growth chart developed by Palczewska and Niedzwiecka and were expressed as centile and SDS [8]. The target height was estimated according to the Tanner formula [9] and was expressed as height and target height SDS (TH SDS). The corrected height standard deviation score (corrHSDS) was

defined as the difference between HSDS and TH SDS. Bone age (BA) was assessed according to Greulich and Pyle [10], and predicted adult height (PAH) was estimated according to the Bayley and Pinneau formula [10]. IGF-1 and IGFBP-3 values were expressed as centile positions within the reference ranges reported by Blum [11]. Quantitative variables are presented as the mean and SDS, and qualitative variables are presented as proportions. Due to the size of the groups, quantitative variables were compared using the Mann-Whitney U test. The chi-square test and, where necessary, Fisher's exact test were used to compare qualitative variables. Correlations of 2 quantitative variables were performed using the Spearman method.  $P < 0.05$  was considered statistically significant. Statistical calculations were performed using the SPSS program (version 21, IBM, SPSS Statistics).

### Biochemical analysis

Growth hormone secretion was assessed after falling asleep and after stimuli. The DIASource HGH-IRMA, DIASource IGFBP-3-IRMA and DIASource IGF-1-IRMA DIASource Immuno Assays kits were used to assess the concentrations of growth hormones IGFBP-3 and IGF-1, respectively, using the immunoradiometric method according to the manufacturer's recommendations. The concentrations of free IGF-1, IGFBP-5, and ALS were evaluated with ELISA (free IGF-1, Ansh Labs; IGFBP-5, SunRed; and ALS, Mediagnost, Germany) according to the manufacturer's recommendations. Blood samples were collected from individuals after obtaining informed consent.

### Genetic analysis

Molecular tests were carried out in the Laboratory of Molecular Endocrinology, Department of Paediatric Endocrinology and Rheumatology of Poznan University of Medical Sciences and were approved by the Bioethics Committee (consent of the Bioethics Committee of the Poznan University of Medical Sciences, resolution no. 134/4 of 1 February 2018).

We performed genetic analysis with PCR and direct sequencing of the *PAPP-A2* gene (Gene ID: 60676, 1q25.2) at the sites of mutations described to date, i.e. c.3098C> T, p.Ala1033Val in exon 8, and p.D643fs25\* in exon 3 [2]. In the absence of these mutations, analysis of exons 3, 4, and 5, which encode fragment of catalytic domain with the active site of pappalysin A2, was performed. The oligonucleotides (Sigma Aldrich) used in PCR were designed using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>, access date: 10 April 2020), and their sequences and polymerase chain reaction (PCR) conditions are listed in Table 1. Received sequences (chromatograms) were analysed using Vector NIT 9.0 Software (Invitrogen) and compared to the reference wild-type sequence (NCBI Reference Sequence: NC\_000001.11). The identified DNA variants were analysed using Mutation Taster software (<http://www.mutationtaster.org/>, access date: 10/04/2020).

## Results

### Auxological data

The mean HSDS in the study group was -2.95, and most of the patients demonstrated severe short stature (Tab. 2). Detailed auxological data are presented in Table 2. Two patients were born prematurely. The birth weight of 2 other patients met the criteria for SGA. The birth length of all patients was within the normal range for gestational age. The mean SDS was -0.82 for birth weight and 0.9 for birth length. None of the patients was treated with growth hormone for short stature with SGA. The mean HSDS in the control group was -0.32.

**Table 1.** Oligonucleotide sequences and polymerase chain reaction (PCR) conditions used to amplify selected fragments of the PAPP-A2 gene

Oligonucleotide name	Sequence 5'→3'	Product size [bp]	Annealing temperature [°C]	Elongation [s]
PAPP-A2-E3F	CACCAGGTCCACAATTCCAC	391	60	30
PAPP-A2-E3R	TGTCTTATTCAGGGAGTGTGTA			
PAPP-A2-E8F	GCTGCTAAGGGCTACTCATTTT	698	60	60
PAPP-A2-E8R	TACGGTAAAATCCCCTCTGAAA			
PAPP-A2-E3D1F	AGCTCTGAGGATGGGCACTAT	588	60	40
PAPP-A2-E3D1R	ATAGCCTGTGAGTGGGTGCT			
PAPP-A2-E4D2F	CTGGGAGTGCCATTAGAATCTG	391	60	40
PAPP-A2-E4D2R	ACTAAGTGCCATTCTCAGCACA			
PAPP-A2-E5D3F	GGCAAGAAAGAAAAGCAATGAC	569	60	40
PAPP-A2-E5D3R	CTCCGACACAAATGACCACTTA			

**Table 2.** Detailed auxological data of the study group

Nr	Sex	CA [years]	BA [years]	Height [cm]	HSDS	TH [cm]	TH SDS	Corr HSDS	Weight [kg] [centile]	Weight-to-height ratio [centile]	BMI [centile]	Tanner stage
1	M	10 8/12	8	117.0	-4.23	160.0	-3.1	-0.58	25.0 [< 3]	75	18.3 (50-75)	G1
2	F	9 3/12	8	116.0	-3.5	147.0	-3.7	1.2	23.0 [3]	75	17.1 (50-75)	Th1
3	F	16 10/12	16	152.0	-2.24	172.0	1.6	-3.84	47.0 [10-25]	50-75	20.3 (25-50)	Th5
4	F	16 10/12	15	141.0	-4.17	152.0	-2.0	-2.12	32.0 [< 3]	25	16.1 (< 3)	Th5
5	F	11	11	130.9	-2.47	154.0	-1.8	-0.5	35.0 [50]	90-97	19.2 (75)	Th5
6	F	17	15	146.0	-3.3	157.0	-1.6	-1.47	36.0 [< 3]	25	16.9 (3)	Th5
7	M	16 9/12	17 1/12	159.0	-3.16	181.0	0.5	-3.66	51.5 [< 3]	50-75	20.2 (25)	G5
8	M	17 7/12	17	164.0	-2.40	184.0	0.7	-2.84	60.0 [10]	75	22.3 (50-75)	G5
9	F	17 8/12	17	146.0	-3.39	155.0	-1.8	-1.37	36.6 [< 3]	25-50	16.9 (< 3)	Th5
10	F	13 3/12	14	139.0	-3.31	156.5	-1.6	-1.74	57.0 [75-90]	> 97	29.5 (> 97)	Th4
11	K	12 6/12	11	139.4	-2.63	158.0	-1.2	-1.4	29.5 [< 3]	3-10	15.0 (3-10)	Th1
12	K	12 5/12	11	142.5	-2.00	162.0	-0.8	-2.14	29.0 [< 3]	10	14.4 (3)	Th2
13	M	6 11/12	7	107.2	-3.7	171.0	-1.0	-3.2	16.1 [< 3]	10	14.0 (10)	G1
14	M	9 11/12	7	121.1	-2.9	167.0	-1.8	-1.35	24.0 [3]	75	16.4 (25-50)	G1
15	K	11 6/12	11 6/12	136.8	-2.02	162.0	-0.9	-1.14	27.8 [< 3]	25	14.6 (3-10)	Th1
16	K	12 7/12	11 6/12	140.5	-2.48	160.0	-1.1	-1.06	40.5 [10-25]	75-90	20.4 (75)	Th3
17	K	12	12	136.1	-2.60	155.0	-1.7	-0.92	30.0 [< 3]	50	16.2 (25)	Th1
18	K	11 8/12	13	128.4	-3.47	155.0	-1.8	-1.7	24.0 [< 3]	50	14.6 (3-10)	Th2
19	K	15 9/12	16	151.9	-2.13	156.0	-1.45	-0.68	42.5 [3]	3-10	18.2 (10-25)	Th5
20	K	12 10/12	12 6/12	140.7	-2.74	151.0	-2.5	-0.24	42.0 [25]	> 90	21.5 (75-90)	Th5
21	M	6	3 3/12	102.5	-3.75	160.0	-3.1	-0.77	18.0 [< 3]	75-90	17.0 (75-90)	G1
22	K	9	7	114.5	-3.72	159.0	-1.2	-2.8	17.5 [< 3]	< 3	13.2 (3)	Th1

Nr — patient number; M — male; F — female; CA — calendar age; BA — bone age; HSDS — height standard deviation score; TH — target height; TH SDS — target height standard deviation score; corr HSDS — corrected height standard deviation score; BMI — body mass index; Th1 — Tanner stage 1 for girls; Th5 — Tanner stage 5 for girls; G1 — Tanner stage 1 for boys; G5 — Tanner stage 5 for boys

### Biochemical data

The mean concentration of growth hormone assessed after falling asleep in the study group was 15.4 (9.4) ng/mL, and in stimulation tests it was 13.0 (6.0)

ng/mL. Patient 21, despite having a concentration of growth hormone below 10 ng/mL in both tests, was qualified for the study group due to the qualification of 2 of his siblings and the short height of his parents

(HSDS  $-3.0$  in his mother and  $-3.36$  in his father). Patient 10, due to the progressive loss of growth rate despite rhGH treatment and the observed high concentrations of IGF-1, was also qualified for the study, although in the assessment of growth hormone secretion she had concentration values below  $10$  ng/mL. In Patient 3, the concentration of growth hormone was also below  $10$  ng/mL, but due to the patient's age and the completed process of sexual maturation during the qualification, the standards of growth hormone secretion of the European Society of Endocrinology for adult patients were referenced [12]. Detailed laboratory data are presented in Table 3.

The mean concentration of IGF-1 in the study group was  $330.5$  ( $178.0$ ) ng/mL and was insignificantly higher in girls than in boys ( $p = 0.09$ ). In the Tanner stage 1 group, a positive correlation was found between IGF-1 concentration and height ( $r = 0.682$ ,  $p = 0.021$ ). The mean concentration of IGFBP-3 in the study group was  $4849.6$  ( $1046.3$ ) ng/mL and was insignificantly higher in girls than in boys ( $p = 0.077$ ). In the Tanner stage

1 group, a positive correlation was found between IGFBP-3 concentration and height ( $r = 0.638$ ,  $p = 0.035$ ). In the Tanner 5 stage group, a negative correlation was found between IGFBP-3 concentration and height ( $r = -0.618$ ,  $p = 0.043$ ). The mean concentration of free IGF-1 was insignificantly higher in the study group than in the control group ( $p = 0.160$ ) and insignificantly higher in girls than in boys ( $p = 0.203$ ). Sex had a statistically significant impact on the concentration of free IGF-1 in the Tanner stage 1 group ( $p = 0.024$ ). In the Tanner 5 stage group, positive correlations were found between the concentration of free IGF-1 and BMI ( $p = 0.011$ ) and SDS of body weight ( $p = 0.036$ ). In addition, positive correlations were found between the concentration of free IGF-1 and height ( $r = 0.441$ ,  $p = 0.040$ ) and HSDS ( $r = 0.607$ ,  $p = 0.003$ ). The mean free IGF-1/IGF-1 ratio was insignificantly higher in the study group than in the control group ( $p = 0.667$ ). Similar average values were recorded in boys ( $0.071$ ) and girls ( $0.068$ ). The mean concentration of IGFBP-5 was significantly higher in the control group than in

Table 3. Biochemical parameters of the growth hormone–insulin-like growth factor 1 (GH–IGF-1) axis

Nr	Max GH after sleeping [ng/mL]	Max GH in stimulation test [ng/mL]	IGF-1 [ng/mL] [centile]	IGFBP-3 [ng/mL] [centile]	Free IGF-1 [ng/mL]*	Free IGF-1/IGF-1 ratio	IGFBP-5 [ng/ml]#	ALS [ng/mL]**
1	18.6	11.6	157.0 [35]	4487.0 [95]	11.1	0.07	73.6	6203.0
2	19.0	8.8	194.0 [50]	5379.0 [ $> 95$ ]	12.9	0.06	46.1	11781.0
3	1.9	8.6	363.0 [30]	4012.0 [80]	52.5	0.14	91.1	11023.0
4	29.0	7.0	295.0 [10]	4375.0 [90]	6.5	0.02	58.1	10421.0
5	26.8	-	941.0 [ $> 95$ ]	6036.0 [ $> 95$ ]	35.3	0.03	86.5	13944.0
6	-	13.6	196.0 [3]	3057.0 [50]	15.4	0.07	116.0	4941.0
7	11.5	10.5	231.0 [5]	3097.0 [70]	37.7	0.13	113.2	9101.0
8	30.7	16.3	456.0 [60]	4946.0 [95]	52.5	0.11	249.5	7940.0
9	28.3	-	341.0 [25]	3977.0 [75]	5.5	0.01	138.7	12107.0
10	2.3	3.4	398.0 [40]	5560.0 [70]	16.4	0.04	91.8	12849.0
11	5.1	12.6	397.0 [75]	6309.0 [95]	43.6	0.11	79.6	11412.0
12	4.7	28.3	270.0 [30]	5591.0 [90]	26.5	0.09	95.3	2653.0
13	7.6	19.8	162.0 [75]	5347.0 [ $> 95$ ]	10.5	0.06	206.4	9531.0
14	14.0	6.4	171.0 [40]	3311.0 [50]	4.3	0.02	201.3	8290.0
15	24.5	15.6	495.0 [90]	4620.0 [80]	16.6	0.03	103.9	4284.0
16	11.4	10.6	312.0 [ $> 50$ ]	4940.0 [ $> 95$ ]	38.1	0.12	209.9	14084.0
17	16.2	21.7	325.0 [60]	5073.0 [90]	52.5	0.16	157.8	14614.0
18	14.0	13.0	526.0 [90]	5843.0 [ $> 90$ ]	52.5	0.09	76.6	10029.0
19	-	15.3	364.0 [25]	6309.0 [ $> 95$ ]	14.2	0.03	122.7	9099.0
20	13.2	14.4	454.0 [85]	6439.0 [ $> 95$ ]	48.0	0.1	193.5	11897.0
21	4.6	5.0	126.0 [50]	3704.0 [ $> 90$ ]	3.1	0.02	146.5	6053.0
22	24.3	16.9	157.0 [30]	4279.0 [ $> 95$ ]	6.8	0.04	103.3	6285.0

Max — maximal; ALS — acid-labile subunit; \*normal range for free IGF-1; Tanner stage 1: 1.58–3.15, Tanner stage 5: 4.89–9.37; #normal range for IGFBP-5: Tanner stage 1: 211–700, Tanner stage 5: 293–1023; \*\*normal range for ALS: Tanner stage 1: 753–2634, Tanner stage 5: 1260–4030 [2]

the study group ( $p = 0.037$ ) and insignificantly higher in boys than in girls ( $p = 0.09$ ). In the Tanner 5 stage group, a positive correlation was found between IGFBP-5 concentration and height ( $r = 0.627$ ,  $p = 0.039$ ). In the Tanner stage 1 group, a negative correlation was found between the concentration of IGFBP-5 and IGF-1 ( $r = -0.645$ ,  $p = 0.032$ ). The mean concentration of ALS was insignificantly higher in the study group than in the control group ( $p = 0.151$ ) and insignificantly higher in girls than in boys ( $p = 0.083$ ). Comprehensive infor-

mation about the correlations within the study group and between the study group and the control group are presented in Tables 4 and 5.

### Genetic analysis

None of the patients in the study group carried the previously described mutations in the *PAPP-A2* gene or mutations in exons 3, 4, and 5 encoding the fragment of catalytic domain with the active site of the PAPP-A2 protein. In 4 patients (Patients 4, 8, 10,

**Table 4.** Correlations of selected biochemical and auxological parameters in the study group

	Th1 + G1 (n = 11)		Th5 + G5 (n = 11)		Study group (n = 22)	
	R	P	R	P	R	P
IGF-1 to height	0.682*	0.021*	-0.555	0.077	0.269	0.226
IGF-1 to HSDS	0.419	0.199	0.364	0.272	0.339	0.122
IGF-1 to GH	0.232	0.492	0.247	0.465	0.292	0.187
Free IGF-1 to height	0.736*	0.010*	0.160	0.639	0.441*	0.040*
Free IGF-1 to HSDS	0.627*	0.039*	0.615*	0.044*	0.607*	0.003*
Free IGF-1 to BMI	-0.132	0.699	0.731	0.011	0.334	0.129
Free IGF-1 to weight SDS	-0.119	0.728	0.635	0.036	0.418	0.053
Free IGF-1 to GH	0.073	0.831	-0.140	0.682	-0.105	0.642
Free IGF-1/IGF-1 to BMI	-0.099	0.773	0.566	0.069	0.247	0.267
Free IGF-1/IGF-1 to weight SDS	-0.221	0.514	0.354	0.286	0.262	0.238
Free IGF-1/IGF-1 to GH	-0.138	0.687	-0.318	0.340	-0.228	0.307
IGFBP-3 to height	0.638*	0.035*	-0.618*	0.043*	0.101	0.655
IGFBP-3 to HSDS	0.263	0.435	0.273	0.417	0.298	0.179
GH to height	0.073	0.831	-0.050	-0.898	0.002	0.995
GH to HSDS	0.000	1.000	-0.050	0.898	-0.044	0.855
IGFBP-5 to IGF-1	-0.645*	0.032*	0.118	0.729	-0.356	0.104
IGFBP-5 to free IGF-1	-0.327	0.326	0.282	0.400	0.047	0.836
IGFBP-5 to IGFBP-3	-0.464	0.151	0.155	0.650	-0.167	0.457
IGFBP-5 to GH	-0.232	0.492	0.142	0.678	-0.051	0.822
IGFBP-5 to weight SDS	-0.023	0.947	0.141	0.679	0.209	0.350
IGFBP-5 to BMI	-0.383	0.245	0.401	0.222	0.157	0.485
IGFBP-5 to height	-0.455	0.160	0.627	0.039	-0.143	0.0526
IGFBP-5 to HSDS	-0.128	0.709	0.409	0.212	0.103	0.647
ALS to IGF-1	0.318	0.340	0.091	0.790	0.302	0.172
ALS to free IGF-1	0.418	0.201	0.082	0.811	0.343	0.118
ALS to IGFBP-3	0.364	0.272	0.318	0.340	0.358	0.102
ALS to IGFBP-5	-0.082	0.811	-0.100	0.770	-0.003	0.990
ALS to GH	0.109	0.749	-0.333	0.316	-0.070	0.757
ALS to weight SDS	0.169	0.619	0.533	0.091	0.391	0.072
ALS to BMI	0.173	0.611	0.269	0.424	0.375	0.085
ALS to height	0.045	0.894	-0.518	0.102	-0.129	0.566
ALS to HSDS	-0.068	0.842	0.073	0.832	0.015	0.948

Th1 — Tanner stage 1 for girls; Th5 — Tanner stage 5 for girls; G1 — Tanner stage 1 for boys; G5 — Tanner stage 5 for boys; IGF-1 — insulin-like growth factor 1; HSDS — height standard deviation score; GH — growth hormone; BMI — body mass index; SDS — standard deviations; IGFBP — insulin-like growth factor binding protein; ALS — acid-labile subunit; \*statistically significant values

**Table 5.** Statistical differences in biochemical parameters within the study group according to the stage of puberty and sex (left part of the table) and statistical differences in the values of biochemical parameters between the study group and the control group according to the stage of puberty and sex (right part of the table)

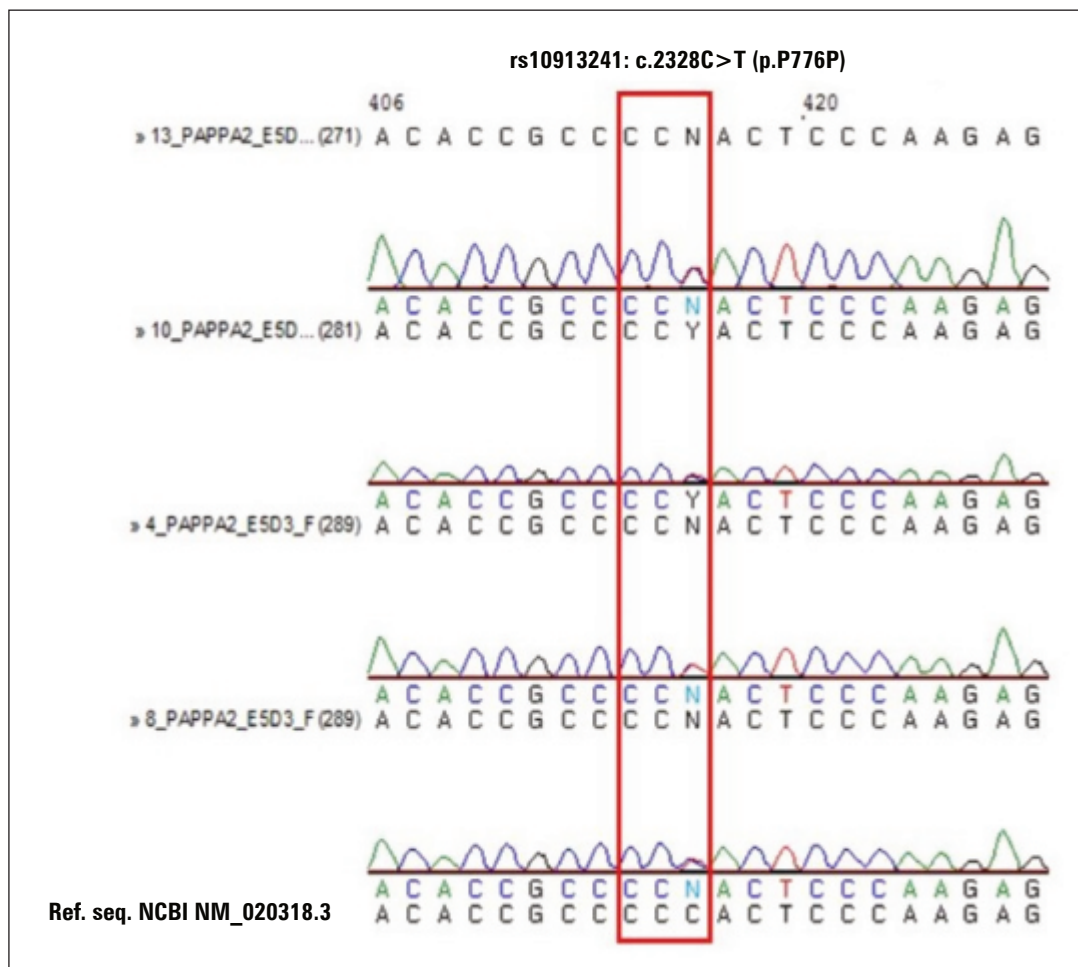
	Girls (n = 16)	Boys (n = 6)	p	Study group (n = 22)	Control group (n = 9)	p
ALS [ng/mL]	10,088.9 ± 3676.9	7853.0 ± 1451.5	0.083	94,791.0 ± 3346.2	7268.3 ± 3867.6	0.151
IGFBP-5 [ng/mL]	110.6 ± 45.0	165.0 ± 65.5	0.098	125.5 ± 55.7	209.7 ± 120.6	0.037
Free IGF-1 [ng/mL]	27.4 ± 17.6	18.9 ± 19.4	0.203	25.1 ± 18.1	14.5 ± 8.9	0.160
Free IGF-1/IGF-1	0.069 ± 0.05	0.071 ± 0.05	0.858	0.07 ± 0.04	0.05 ± 0.02	0.203
	Th1 (n = 7)	G1 (n = 4)	p	Study group Th1 (n = 7)	Control group Th1 (n = 2)	p
ALS [ng/mL]	8722.5 ± 4387.1	7519.2 ± 1685.5	0.527	8722.6 ± 4387.2	12,488.0 ± 2439.5	0.500
IGFBP-5 [ng/mL]	94.5 ± 33.9	156.9 ± 61.8	0.230	94.5 ± 34.0	108.1 ± 2.2	0.222
Free IGF-1 [ng/mL]	29.6 ± 18.4	7.3 ± 4.1	0.024	29.6 ± 18.4	16.4 ± 8.2	0.500
Free IGF-1/IGF-1	0.08 ± 0.04	0.04 ± 0.03	0.109	0.08 ± 0.04	0.07 ± 0.01	0.667
	Th5 (n = 9)	G5 (n = 2)	p	Study group Th5 (n = 9)	Control group Th5 (n = 2)	p
ALS [ng/mL]	11,151.0 ± 2830.2	8520.5 ± 820.9	0.218	11,151.7 ± 2830.2	4280.5 ± 4636.5	0.073
IGFBP-5 [ng/mL]	123.1 ± 50.4	181.3 ± 96.3	0.436	123.1 ± 50.4	142.2 ± 59.3	0.727
Free IGF-1 [ng/mL]	25.8 ± 17.9	42.1 ± 14.7	0.327	25.8 ± 17.9	19.0 ± 14.6	0.727
Free IGF-1/IGF-1	0.06 ± 0.05	0.12 ± 0.01	0.218	0.06 ± 0.05	0.05 ± 0.03	0.909
	Th1 + G1 (n = 11)	Th5 + G5 (n = 11)	p	Study group G1 (n = 4)	Control group G1 (n = 3)	p
ALS [ng/mL]	8285.0 ± 3573.4	10673.3 ± 2758.3	0.116	7519.3 ± 1685.6	7583.7 ± 1445.2	0.857
IGFBP-5 [ng/mL]	117.2 ± 53.2	133.7 ± 59.3	0.847	157.0 ± 61.8	279.1 ± 159.1	0.400
Free IGF-1 [ng/mL]	21.4 ± 18.3	28.7 ± 17.9	0.300	7.3 ± 4.1	9.8 ± 4.7	0.629
Free IGF-1/IGF-1	0.07 ± 0.04	0.07 ± 0.05	0.797	0.04 ± 0.03	0.04 ± 0.03	0.857
	Study group Th1 (n = 7)	Study group Th5 (n = 9)	p	Study group G5 (n = 2)	Control group G5 (n = 2)	p
ALS [ng/mL]	8722.6 ± 4387.2	11151.7 ± 2830.2	0.252	8520.5 ± 821.0	4563.5 ± 874.7	0.333
IGFBP-5 [ng/mL]	94.5 ± 34.0	123.1 ± 50.4	0.299	181.4 ± 96.4	274.7 ± 97.3	0.667
Free IGF-1	29.6 ± 18.4	25.8 ± 17.9	0.606	42.1 ± 14.7	15.1 ± 14.1	0.333
Free IGF-1/IGF-1	0.08 ± 0.04	0.06 ± 0.05	0.351	0.12 ± 0.01	0.03 ± 0.03	0.333
	Study group G1 (n = 4)	Study group G5 (n = 2)	p			
ALS [ng/mL]	7519.3 ± 1685.6	8520.5 ± 821.0	0.800			
IGFBP-5 [ng/mL]	157.0 ± 61.8	181.4 ± 96.4	0.800			
Free IGF-1	7.3 ± 4.1	42.1 ± 14.7	0.133			
Free IGF-1/IGF-1	0.04 ± 0.03	0.12 ± 0.01	0.133			

ALS — acid-labile subunit; IGFBP — insulin-like growth factor binding protein; IGF-1 — insulin-like growth factor 1; Th1 — Tanner stage 1 for girls; Th5 — Tanner stage 5 for girls; G1 — Tanner stage 1 for boys; G5 — Tanner stage 5 for boys;

and 13), the known, non-pathogenic, heterozygotic polymorphism c.2328C>T(rs10913241) in exon 5 outside the coding site of the catalytic domain (Fig. 1) was detected. This polymorphism has been deposited in the ExAC database (<https://gnomad.broadinstitute.org/>, access date: 10 April 2020) and 1000G (<http://www.internationalgenome.org/1000-genomes-browsers/>, access date: 10 April 2020) and has no clinical significance.

## Discussion

The diagnosis of ISS is based on the exclusion of known causes. Endocrinologists agree that Turner syndrome should be excluded in all girls with ISS, even those without characteristic phenotypic features. Additionally, it is recommended to rule out chromosomal aberrations in boys with atypically developed external genitalia [13].



**Figure 1.** Chromatogram showing the polymorphism of the PAPP-A2 gene (rs10913241: c.2328C>T) detected in Patients 4, 8, 10, and 13

In the case of familial short stature, the probability of finding a genetic cause is low [14]. When the height of one of the parents is more than  $-2$  SD, the possibility of autosomal dominant mutations in the family should be considered. A recent study conducted on a group of patients meeting the criteria for SGA or growth hormone deficiency with concomitant severe familial short stature revealed a monogenic disorder in 52% (17/33 patients) [15]. In our study, patients whose height was  $-2.0$  SD or less were included; in 13 patients, the height of one parent was  $-2.0$  SD or less.

Evaluation of IGF-1 in the initial assessment of a patient with short stature and during rhGH therapy remains a crucial indicator of treatment effectiveness and safety. When we estimate the concentration of IGF-1, we evaluate its total concentration, which includes inactive IGF-1. The advantage of the evaluation of free IGF-1 over total IGF-1 has not been demonstrated; therefore, there are no reasons to include the evaluation of free IGF-1 in the routine diagnosis of short stature [16]. In patients with obesity, the concentration of

free IGF-1 may be higher due to the suppressive effect of insulin on the concentration of IGFBP-1, and according to reports, the concentration of IGF-1 may be reduced, elevated, or within normal limits [17, 18]. In our study group, one patient was diagnosed as obese; her free IGF-1 concentration was elevated (Tab. 3), and the ratio of free IGF-1 to total IGF-1 was only slightly elevated (0.04) compared to the reference value (0.02). Kamoda et al., in their study assessing the concentrations of free IGF-1, total IGF-1, and IGFBP-1 in a group of 19 patients with ISS, obtained significantly lower concentrations of free IGF-1 ( $p < 0.05$ ), and the concentration of total IGF-1 was insignificantly lower compared to the control group [19]. Jull et al. analysed the concentration of free IGF-1 in a group of 1430 healthy children and adults and showed that the concentration of free IGF-1 remains low during infancy and early childhood, increases to the highest average concentration during puberty, and then returns to prepubertal values without further changing with age [20]. In the prepubertal group, 3.3% of patients had a concentration of free

IGF-1 below the detection threshold, although they were not diagnosed with growth hormone deficiency [20]. In the paediatric group, there were differences according to sex. In girls, an increase in the concentration of free IGF-1 was observed 1–2 years earlier than in boys, which coincides with the physiological course of sexual maturation. In addition, girls had significantly higher concentrations of free IGF-1 than boys. These sex differences were not observed in the adult population [20]. Kawai et al. observed that in infancy, the ratio of free IGF-1/IGF-1 was significantly higher [21]. In our study group, as in the available reports, higher values of free IGF-1 were measured in girls in both the study group and in the control group, except for the Tanner stage 5 subgroup, where higher values were noted in boys. However, the group of boys at this stage of puberty consisted of only 2 patients, so further research on a larger group is needed to draw proper conclusions. Sex showed a statistically significant effect on the concentration of free IGF-1 in Tanner stage 1 patients. A positive correlation was found between the concentration of free IGF-1 and height and HSDS. In the study group, the concentration of free IGF-1 correlated better with height and HSDS than the concentration of IGF-1, and this correlation can be used in the analysis of the growth curve and to estimate the final height of patients with ISS. Jull et al. assessed the concentration of free IGF-1 using the immunoradiometric method (Diagnostic System Laboratories, Webster TX). However, this cannot be the reason for the differences in the obtained results, especially the differences between sexes. Jull et al. evaluated the concentration of free IGF-1 in a group of approximately 800 healthy children, and our control and study groups were small. Research on larger, more representative groups is necessary. Two patients (Patients 4 and 9) from the Tanner stage 5 group and one (Patient 21) from the Tanner stage 1 group exhibited values of free IGF-1 and the free IGF-1/IGF-1 ratio in the normal range. The remaining patients from the study group had values above the reference range for the appropriate stage of puberty. Despite free IGF-1 values greater than the reference range for a given stage of puberty, Patient 14 had a free IGF-1/IGF-1 ratio within the described reference range, which suggests a very high concentration of IGF-1. Three patients (Patient 15 from the Tanner stage 1 group and Patients 5 and 19 from the Tanner stage 5 group) had values of free IGF-1 above the reference range for a given stage of puberty and free IGF-1/IGF-1 ratios slightly above the reference range (0.03). Nineteen (19/22) patients had free IGF-1 levels above the reference values for puberty. In 4 (4/22) patients, these values exceeded the reference value by less than one-fold. Eight patients (8/22) exceeded it

by less than 5-fold. In 4 patients (4/22), the measured values exceeded the reference value by 5- to 10-fold. Three patients (3/22) had values exceeding the reference range by 10-fold, and all 3 patients were female patients in the Tanner stage 1 group. In this group, the IGF-1 concentration was above the 50<sup>th</sup> centile, and the maximum values of the growth hormone concentration were 12.6 ng/mL, 21.7 ng/mL, and 14.0 ng/mL (Tab. 3). Due to the nature of the study, the progressive loss of growth rate, and severe ISS, the measured values of free IGF-1 concentration may indicate the resistance of peripheral tissues to IGF-1, which can be confirmed by high concentrations of IGF-1 and free IGF-1 and inadequate growth rate. Mutations in the IGF-1 receptor (*IGF-1R*) gene are very rare and are characterized by severely decreased birth weight and length, microcephaly and severe short stature with an HSDS of –7 [22]. None of our patients presented with an extremely short stature. However, this does not rule out the possibility of a mutation in the *IGF-1R* gene or the occurrence of a milder variant. It is also possible that the process of postreceptor signal transduction was disturbed in patients in the study group.

According to previous studies, a decrease in IGFBP-5 concentration is observed with age. Positive correlations between the concentration of IGFBP-5 and IGF-1, IGF-2, and ALS were previously described [23–25]. In the study group, a negative correlation between the concentration of IGFBP-5 and IGF-1 was found, and no correlations were found between the concentration of IGFBP-5 and free IGF-1, IGFBP-3, and ALS. In all patients from the study group, the concentration of IGFBP-5 was below the normal range.

The ALS concentration gradually increases during childhood, peaking during puberty. A statistically significant difference in ALS concentration by sex has been described, with significantly higher ALS concentrations in girls [26]. In the study group, higher values were observed among girls, similarly to previous reports, but no statistical significance was noted.

The genetic analysis of previously described mutations in the *PAPP-A2* gene and the fragment of the *PAPP-A2* gene encoding the fragment of catalytic domain with the active site of PAPP-A2 detected the known heterozygous polymorphism c.2328C>T (rs10913241) in exon 5, outside the coding site of the catalytic domain, in patients 4, 8, 10, and 13. According to data from the ExAC database (<https://gnomad.broadinstitute.org/>, access date: 20 February 2021), in patients under 30 years of age, 701 out of 2547 subjects were heterozygous carriers of the variant, which is a frequency of 0.27. In the European population, excluding the Finnish population, the rs10913241 polymorphism in the *PAPP-A2* gene was detected in 20,498 cases out of 112,964 analysed, of which 1891 were homo-



zygous, which is an incidence of 0.18. The frequency of this polymorphism varies depending on the population studied. In the study group, 4 out of 22 patients exhibited this polymorphism, which had a frequency of 0.18, consistent with that reported for the European population excluding the Finnish population.

The main limitation of this study is undoubtedly the size of the study and control groups. Previous studies suggest that a large study population is needed to identify new, rare causes of ISS using next-generation sequencing. Our sample size was small, and genetic analysis of only one gene (*PAPP-A2*) was performed, which focused only on mutations previously described in the literature and in the gene region encoding the fragment of catalytic domain with the active site of the PAPP-A2 protein. Further research should be carried out in a larger study group with the use of whole-exome sequencing. Adding a reduced concentration of free IGF-1 to the inclusion criteria allows researchers to focus only on the analysis of the *PAPP-A2* gene.

## Conclusions

Free IGF-1 correlates better with height and HSDS than total IGF-1. The free IGF-1/IGF-1 ratio and IGFBP-5 concentration may be helpful parameters for the prediction of final height in patients with ISS. The previously described mutations in the *PAPP-A2* gene and mutations in exons 3, 4, and 5 encoding the fragment of catalytic domain with the active site of the PAPP-A2 protein were not detected; only the known, non-pathogenic, heterozygotic polymorphism c.2328C>T(rs10913241) in exon 5 of the *PAPP-A2* gene was detected. Preliminary determination of free IGF-1 levels in a larger study group is necessary to estimate the potential risk of *PAPP-A2* gene mutation occurrence in patients with ISS.

## Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Ethics statement

Research was approved by the Bioethics Committee (consent of the Bioethics Committee of the Poznan University of Medical Sciences, resolution no 134/4 of 1 February 2018).

## Author contributions

Conceptualisation: M.N., M.B.Z., A.R. Patient care and data collection: M.B.-Z., M.N. Methodology and data analysis: M.B.Z., M.N., A.R. Analysis and Interpretation: M.N., M.B.Z., A.R. Literature Search: M.B.Z., A.R. Writing — original draft: M.B.Z. Writing — review and editing: M.N., A.R. All authors have read and agreed to the published version of the manuscript.

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## Conflict of interest

The authors declare no conflict of interests.

## References

1. Ranke MB. Towards a consensus on the definition of idiopathic short stature. *Horm Res.* 1996; 45 Suppl 2: 64–66, doi: [10.1159/000184851](https://doi.org/10.1159/000184851), indexed in Pubmed: [8805048](https://pubmed.ncbi.nlm.nih.gov/8805048/).
2. Dauber A, Muñoz-Calvo MT, Barrios V, et al. Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability. *EMBO Mol Med.* 2016; 8(4): 363–374, doi: [10.15252/emmm.201506106](https://doi.org/10.15252/emmm.201506106), indexed in Pubmed: [26902202](https://pubmed.ncbi.nlm.nih.gov/26902202/).
3. Banaszak-Ziemska M, Niedziela M. PAPP-A2 a new key regulator of growth. *Endokrynol Pol.* 2017; 68(6): 682–691, doi: [10.5603/EPa.2017.0060](https://doi.org/10.5603/EPa.2017.0060), indexed in Pubmed: [29238946](https://pubmed.ncbi.nlm.nih.gov/29238946/).
4. Muñoz-Calvo MT, Barrios V, Pozo J, et al. Treatment With Recombinant Human Insulin-Like Growth Factor-1 Improves Growth in Patients With PAPP-A2 Deficiency. *J Clin Endocrinol Metab.* 2016; 101(11): 3879–3883, doi: [10.1210/jc.2016-2751](https://doi.org/10.1210/jc.2016-2751), indexed in Pubmed: [27648969](https://pubmed.ncbi.nlm.nih.gov/27648969/).
5. Cabrera-Salcedo C, Mizuno T, Tyzinski L, et al. Pharmacokinetics of IGF-1 in PAPP-A2-Deficient Patients, Growth Response, and Effects on Glucose and Bone Density. *J Clin Endocrinol Metab.* 2017; 102(12): 4568–4577, doi: [10.1210/jc.2017-01411](https://doi.org/10.1210/jc.2017-01411), indexed in Pubmed: [29029190](https://pubmed.ncbi.nlm.nih.gov/29029190/).
6. Babiker A, Al No, Al Sw, et al. Short stature with low insulin-like growth factor 1 availability due to pregnancy-associated plasma protein A2 deficiency in a Saudi family. *Clin Genet.* 2021; 100(5): 601–6.
7. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr.* 2013; 13: 59, doi: [10.1186/1471-2431-13-59](https://doi.org/10.1186/1471-2431-13-59), indexed in Pubmed: [23601190](https://pubmed.ncbi.nlm.nih.gov/23601190/).
8. Palczewska I, Niedźwiecka Z. Wskaźniki rozwoju somatycznego dzieci i młodzieży warszawskiej. *Med Wieku Rozwoj.* 2001; 5: 17–118.
9. Tanner JM, Goldstein H, Whitehouse RH. Standards for children's height at ages 2-9 years allowing for heights of parents. *Arch Dis Child.* 1970; 45(244): 755–762, doi: [10.1136/adc.45.244.755](https://doi.org/10.1136/adc.45.244.755), indexed in Pubmed: [5491878](https://pubmed.ncbi.nlm.nih.gov/5491878/).
10. BAYLEY N, PINNEAU SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. *J Pediatr.* 1952; 40(4): 423–441, doi: [10.1016/s0022-3476\(52\)80205-7](https://doi.org/10.1016/s0022-3476(52)80205-7), indexed in Pubmed: [14918032](https://pubmed.ncbi.nlm.nih.gov/14918032/).
11. Blum W, Breier B. Radioimmunoassays for IGFs and IGFBPs. *Growth Regul.* 1994; 4(Suppl 1): 11–9.
12. Fleseriu M, Hashim IA, Karavitaki N, et al. Hormonal Replacement in Hypopituitarism in Adults: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2016; 101(11): 3888–3921, doi: [10.1210/jc.2016-2118](https://doi.org/10.1210/jc.2016-2118), indexed in Pubmed: [27736313](https://pubmed.ncbi.nlm.nih.gov/27736313/).
13. Cohen P, Rogol AD, Deal CL, et al. Consensus Statement on the Diagnosis and Treatment of Children with Idiopathic Short Stature: A Summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab.* 2008; 93(11): 4210–7, doi: [10.1210/jc.2008-0509](https://doi.org/10.1210/jc.2008-0509), indexed in Pubmed: [18782877](https://pubmed.ncbi.nlm.nih.gov/18782877/).
14. Wit JM, Clayton PE, Rogol AD, et al. Idiopathic short stature: definition, epidemiology, and diagnostic evaluation. *Growth Horm IGF Res.* 2008; 18(2): 89–110, doi: [10.1016/j.ghir.2007.11.004](https://doi.org/10.1016/j.ghir.2007.11.004), indexed in Pubmed: [18182313](https://pubmed.ncbi.nlm.nih.gov/18182313/).
15. Plachy L, Strakova V, Elblova L, et al. High Prevalence of Growth Plate Gene Variants in Children With Familial Short Stature Treated With GH. *J Clin Endocrinol Metab.* 2019; 104(10): 4273–4281, doi: [10.1210/jc.2018-02288](https://doi.org/10.1210/jc.2018-02288), indexed in Pubmed: [30753492](https://pubmed.ncbi.nlm.nih.gov/30753492/).
16. Frystyk J. Utility of free IGF-I measurements. *Pituitary.* 2007; 10(2): 181–187, doi: [10.1007/s11102-007-0025-y](https://doi.org/10.1007/s11102-007-0025-y), indexed in Pubmed: [17429595](https://pubmed.ncbi.nlm.nih.gov/17429595/).
17. Scacchi M, Pincelli AI, Cavagnini F. Growth hormone in obesity. *Int J Obes Relat Metab Disord.* 1999; 23(3): 260–271, doi: [10.1038/sj.sjo.0800807](https://doi.org/10.1038/sj.sjo.0800807), indexed in Pubmed: [10193871](https://pubmed.ncbi.nlm.nih.gov/10193871/).
18. Frystyk J, Vestbo E, Skjaerbaek C, et al. Free insulin-like growth factors in human obesity. *Metabolism.* 1995; 44(10 Suppl 4): 37–44, doi: [10.1016/0026-0495\(95\)90219-8](https://doi.org/10.1016/0026-0495(95)90219-8), indexed in Pubmed: [7476310](https://pubmed.ncbi.nlm.nih.gov/7476310/).
19. Kamoda T, Saitoh H, Hirano T, et al. Serum levels of free insulin-like growth factor (IGF)-I and IGF-binding protein-1 in prepubertal children with idiopathic short stature. *Clin Endocrinol (Oxf).* 2000; 53(6): 683–688, doi: [10.1046/j.1365-2265.2000.01145.x](https://doi.org/10.1046/j.1365-2265.2000.01145.x), indexed in Pubmed: [11155089](https://pubmed.ncbi.nlm.nih.gov/11155089/).
20. Juul A, Holm K, Kastrup KW, et al. Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in

- patients suspected of growth hormone deficiency. *J Clin Endocrinol Metab.* 1997; 82(8): 2497–2502, doi: [10.1210/jcem.82.8.4137](https://doi.org/10.1210/jcem.82.8.4137), indexed in Pubmed: [9253324](https://pubmed.ncbi.nlm.nih.gov/9253324/).
21. Kawai N, Kanzaki S, Takano-Watou S, et al. Serum free insulin-like growth factor I (IGF-I), total IGF-I, and IGF-binding protein-3 concentrations in normal children and children with growth hormone deficiency. *J Clin Endocrinol Metab.* 1999; 84(1): 82–89, doi: [10.1210/jcem.84.1.5407](https://doi.org/10.1210/jcem.84.1.5407), indexed in Pubmed: [9920066](https://pubmed.ncbi.nlm.nih.gov/9920066/).
  22. Walenkamp MJE, Losekoot M, Wit JM. Molecular IGF-1 and IGF-1 receptor defects: from genetics to clinical management. *Endocr Dev.* 2013; 24: 128–137, doi: [10.1159/000342841](https://doi.org/10.1159/000342841), indexed in Pubmed: [23392101](https://pubmed.ncbi.nlm.nih.gov/23392101/).
  23. Baxter RC, Meka S, Firth SM. Molecular distribution of IGF binding protein-5 in human serum. *J Clin Endocrinol Metab.* 2002; 87(1): 271–276, doi: [10.1210/jcem.87.1.8151](https://doi.org/10.1210/jcem.87.1.8151), indexed in Pubmed: [11788658](https://pubmed.ncbi.nlm.nih.gov/11788658/).
  24. Mohan S, Libanati C, Dony C, et al. Development, validation, and application of a radioimmunoassay for insulin-like growth factor binding protein-5 in human serum and other biological fluids. *J Clin Endocrinol Metab.* 1995; 80(9): 2638–2645, doi: [10.1210/jcem.80.9.7545694](https://doi.org/10.1210/jcem.80.9.7545694), indexed in Pubmed: [7545694](https://pubmed.ncbi.nlm.nih.gov/7545694/).
  25. Bach LA. Insulin-like growth factor binding proteins 4-6. *Best Pract Res Clin Endocrinol Metab.* 2015; 29(5): 713–722, doi: [10.1016/j.beem.2015.06.002](https://doi.org/10.1016/j.beem.2015.06.002), indexed in Pubmed: [26522456](https://pubmed.ncbi.nlm.nih.gov/26522456/).
  26. Juul A, Møller S, Mosfeldt-Laursen E, et al. The acid-labile subunit of human ternary insulin-like growth factor binding protein complex in serum: hepatosplanchnic release, diurnal variation, circulating concentrations in healthy subjects, and diagnostic use in patients with growth hormone deficiency. *J Clin Endocrinol Metab.* 1998; 83(12): 4408–4415, doi: [10.1210/jcem.83.12.5311](https://doi.org/10.1210/jcem.83.12.5311), indexed in Pubmed: [9851786](https://pubmed.ncbi.nlm.nih.gov/9851786/).