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Developmental programming of growth: Genetic variant in *GH2* gene encoding placental growth hormone contributes to adult height determination

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ABSTRACT

Introduction: Given the physiological role of placental growth hormone (PGH) during intrauterine development and growth, genetic variation in the coding *Growth hormone 2* (*GH2*) gene may modulate developmental programming of adult stature. Two major *GH2* variants were described worldwide, determined by single polymorphism (rs2006123; c.171 + 50C > A). We sought to study whether *GH2* variants may contribute to adult anthropometric measurements.
Methods: Genotyping of *GH2* SNP rs2006123 by RFLP, testing its genetic association with adult height and Body Mass Index (BMI) by linear regression analysis, and combining the results of three individual study samples in meta-analysis.
Study samples: HYPEST (Estonia), $n = 1464$ (506 men/958 women), CADCZ (Czech), $n = 871$ (518/353); UFA (Bashkortostan), $n = 954$ (655/299); meta-analysis, $n = 3289$ (1679/1610).
Results: Meta-analysis across HYPEST, CADCZ and UFA samples ($n = 3289$) resulted in significant association of *GH2* rs2006123 with height (recessive model: AA-homozygote effect: beta (SE) = 1.26 (0.46), $P = 5.90 \times 10^{-3}$; additive model: A-allele effect: beta (SE) = 0.45 (0.18), $P = 1.40 \times 10^{-2}$). Among men ($n = 1679$), the association of the A-allele with taller stature remained significant after multiple-testing correction (additive effect: beta = 0.86 (0.28), $P = 1.83 \times 10^{-3}$). No association was detected with BMI. Notably, rs2006123 was in strong LD ($r^2 \geq 0.87$) with SNPs significantly associated with height (rs2665838, rs7209435, rs11658329) and mapped near *GH2* in three independent meta-analyses of GWA studies.
Conclusions: This is the first study demonstrating a link between a placental gene variant and programming of growth potential in adulthood. The detected association between PGH encoding *GH2* and adult height promotes further research on the role of placental genes in prenatal programming of human metabolism.

1. Introduction

The human *Growth hormone/Chorionic somatomammotropin* (h*GH/CSH*) genes belong to a rapidly evolving primate-specific gene cluster spanning 48 kb at 17q22-24. In most mammals, a single *GH1* gene encodes the pituitary growth hormone (GH), whereas in primates, novel placenta-specific GH-related genes have arisen through gene duplications [1]. In humans, *GH/CSH* cluster consists of five highly homologous (91–97%) and structurally similar genes: *GH1*, *GH2*, *CSH1*, *CSH2* and *CSHL1* (*chorionic somatomammotropin-like 1*) [2]. It is well known that pituitary GH (also known as somatotropin) promotes postnatal growth of skeletal and soft tissue, and acts as an important regulator of immune function, bone turnover and muscle mass. GH deficiency (1:4000–1:10,000/births) is associated with short stature [3] and common *GH1* substitutions have been shown to contribute to height determination [4].

In the human placenta, the expression of four *GH/CSH* genes (*GH2*, *CSH1*, *CSH2*, *CSHL1*) is coordinately induced during fetal development

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Table 1
Anthropometric characteristics of the study samples.

Parameter[a]	HYPEST			CADCZ			UFA		
	All	Men	Women	All	Men	Women	All	Men	Women
No of subjects	1464	506	958	871	518	353	954	655	299
Age (y)	47.5 ± 13.4	45.3 ± 13.6	48.7 ± 13.2	50.0 ± 10.6	51.2 ± 9.8	48.1 ± 11.4	54.1 ± 17.1	50.3 ± 15.9	62.3 ± 17.0
Height (cm)	169.6 ± 9.3	178.9 ± 6.9	164.6 ± 6.1	172.4 ± 8.7	177.3 ± 6.9	165.3 ± 5.8	167.6 ± 8.2	169.1 ± 7.5	164.4 ± 8.6
BMI (kg/m^2)	26.3 ± 4.8	27.0 ± 3.9	26.0 ± 5.2	27.0 ± 4.4	27.7 ± 3.8	26.1 ± 5.0	27.9 ± 3.9	27.6 ± 3.7	28.7 ± 4.4

[a] For each parameter mean ± SD is shown; y, age in years; BMI, body mass index (kg/m^2).

in the syncytiotrophoblasts from 5 to 8 weeks of pregnancy [5], and their expression profile exhibits pleiotropic effects at the maternal–fetal interface in regulating of fetal growth and modulating maternal metabolism [6–9]. Two of these, *CSH1* and *CSH2*, encode jointly an identical protein chorionic somatomammotropin (CSH, also known as placental lactogen, PL). *CSHL1* was originally considered as a pseudogene, although low levels of its expression in placenta have been reported [8]. *GH2* encodes placental GH (PGH), which progressively replaces maternal pituitary GH from mid-gestation onwards, peaking towards term [10,11]. Only 13 amino acid residues constitute the difference between PGH and GH. To execute its function, PGH binds to GH cell surface receptors (GHR) with similar affinity to pituitary GH [6]. Interestingly, secreted PGH is found predominantly, but not exclusively, in maternal circulation [12,13]. Maternal PGH serum levels have been positively correlated with infant birth weight [11,12,14]. Significantly lower placental expression of *GH2* and reduced levels of circulating PGH have been reported in women with fetal intrauterine growth retardation/small-for-gestational-age pregnancies [8,12,14].

We hypothesized that given the physiological role of PGH during intrauterine development, the genetic variation in the *GH2* gene may modulate growth *in utero* and in early infancy, therefore possibly affecting the developmental programming of human stature in adulthood. However, in contrast to the pituitary-expressed *GH1*, there are limited data on the impact of polymorphisms in the placental h*GH/CSH* genes on intrauterine growth and programming of the postnatal metabolism [15]. Detailed research on h*GH/CSH* cluster has been hindered by its complex genomic structure rich in repetitive genic and intergenic sequence fragments. Our pioneer study had revealed that the duplicated h*GH/CSH* genes exhibit substantial heterogeneity in diversity patterns and low linkage disequilibrium (LD) between allelic variants, driven by the interplay between active intergenic gene conversion and locus-specific selective pressures [16]. For the *GH2* gene, only two major gene variants were described, determined by the allelic status of one polymorphism (rs2006123; c.171 + 50C > A) located 50 bp from the donor splice site within intron 2 (original nomenclature [16], g.943C > A). *GH2* rs2006123 alleles were differentially distributed in studied populations: 92% of the Chinese Han individuals carried the ancestral C-allele, whereas the derived A-allele was enriched in African Mandenkalu (carrier frequency 95%). In European Estonians both alleles were commonly represented (C, 66%; A, 34%). As other h*GH/CSH* genes showed no or low intercontinental differentiation, it was suggested that the observed *GH2* variation pattern might reflect regional population-specific selection.

The present study aimed to test the association between human PGH coding *GH2* intron 2 polymorphism rs2006123 and anthropomorphic phenotypes (height, BMI) in three Eastern/Central European sample sets and in the subsequent meta-analysis (total sample size, $n = 3289$). We report significant association between *GH2* rs2006123 and adult height, and show that the studied *GH2* variant is in strong LD ($r^2 > 0.8$) with top hits from three independent meta-analysis of genome-wide association studies (GWAS) for height, mapped within the h*GH/CSH* gene cluster (rs2665838 [17]) or to its vicinity (<250 kb: rs7209435 [18]; rs11658329 [19]).

2. Materials and methods

2.1. Study groups

The analyzed sample collections HYPEST (Estonians), CADCZ (Czech) and UFA (Bashkirs and Tatars from Volga-Ural region, Russia) represent populations of Eastern/Central European origin and their basic characteristics are provided in Table 1. The recruitment of the three sample sets has been carried out in compliance with the Helsinki Declaration and participants have given the written informed consent. The HYPEST study has been approved by the Ethics Committee on Human Research of University of Tartu (permissions 122/13, 22.12.2003; 137/20, 25.04.2005). The CADCZ study has been approved by the Ethics Committee of Charles University—1st Faculty of Medicine (December 1996) and the UFA study by the Independent Ethics Committee of the Institute of Biochemistry and Genetics, Ufa Scientific Centre of Russian Academy of Sciences, Ufa, Russia (permission no. 5, 25.01.2005).

Originally, HYPEST subjects were recruited across Estonia, North-Eastern Europe during 2004–2007 (1966 individuals, age range 18–85 years) with the main aim to analyze genetic-epidemiological risk factors for cardiovascular disease in Estonian population [20]. In the current study, the total number of genotyped HYPEST subjects was $n = 1464$ (aged 47.5 ± 13.4 years) including 506 men (aged 45.3 ± 13.6 years) and 958 women (aged 48.7 ± 13.2 years). The CADCZ samples have been recruited across Czech Republic, Central Europe by the Cardiology Department of the 2nd Clinic of Internal Medicine, Faculty Hospital Královské Vinohrady in Prague with the main aim to study genetic factors relating to homocysteine metabolism in coronary artery disease and details are published elsewhere [21]. The number of CADCZ samples available for genotyping in the current study was 871 (aged 50.0 ± 10.6 years), including 518 men (aged 51.2 ± 9.8 years) and 353 women (aged 48.1 ± 11.4 years). UFA sample is comprised of subjects recruited in Bashkortostan, the Volga-Uralic region of Russia, located at the border of Eastern Europe and Asia (prof. E. Khusnutdinova and collaborators). In this study, 954 of Bashkortostan samples ($n = 464$ Tatars, $n = 490$ Bashkirs) were genotyped (aged 54.1 ± 17.1 years). Analyzed UFA samples included 655 men (aged 50.3 ± 15.9 years) and 299 women (aged 62.3 ± 17.0 years).

At the recruitment, anthropometric data (height in cm, weight in kg) of each participant of the three studies were documented. In order to consider in association testing both genders together, in addition to the direct height measurements, the transformed values were used as recommended [17]. Z-score (standard deviation score) for height was calculated as the subject's height minus the mean height in the sex appropriate subsample of each study divided by the standard deviation of that mean. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

2.2. Genotyping

In order to exclude amplification of other highly homologous h*GH/CSH* genes, the genotyping protocol of *GH2* rs2006123 SNP included long-range PCR to amplify specifically the entire *GH2* gene region (5634 bp product [16]; forward primer: 5′-AGGTCTGGAAAGGAGGAGACAAAAGAG-3′, reverse primer: 5′-TTGAATTA-GACTTGGGATTCTCCTGACA-3′), and allele-specific restriction fragment length polymorphism (RFLP) analysis (Supplemental Figs. S1–S2). In case the long-range PCR had resulted in insufficient product quantity for the RFLP detection, nested PCR (1855 bp product; forward primer: 5′-GCTTGTGGTCCGTGTCTGTTCC-3′, reverse primer: 5′-GGTGGCGATAGACGTTGCTGTCC-3′) was applied to further amplify the DNA fragment involving the targeted SNP. PCR conditions are described in Supplemental Text S1.

The amplified product was digested with FastDigest XapI restriction endonuclease according to manufacturer's recommendations (Thermo Fisher Scientific, Lithuania). Allele-specific RFLP products were separated by electrophoresis on a 1–2% agarose gel and 0.5xTris/Borate/EDTA buffer. The following fragments were detected in the RFLP analysis of (i) long-range PCR: 2658, 1626, 541, 484, 218, 107 bp (C-allele); 1626, 1614, 1044, 541, 484, 218, 107 bp (A-allele) (Supplemental Fig. S1A)

or (ii) nested PCR: 1546 and 309 bp (C-allele); 1045, 501, and 309 bp (A-allele) (Supplemental Fig. S1B). In developmental phase of the RFLP-based genotyping assay, a subset of samples were sequenced for the quality control and to verify few ambiguous genotypes using previously published sequencing primers and conditions [16].

2.3. Data analysis

Estimation of allele frequencies, conformance to Hardy–Weinberg Equilibrium (HWE; χ^2, $P > 0.05$) and association testing of the *GH2* rs2006123 with anthropometric characteristics using linear regression under additive and recessive genetic models was implemented in PLINK v1.07 software http://pngu.mgh.harvard.edu/purcell/plink/ [22]. Additive genetic model assumes that having two copies of the minor allele has twice the effect of having a single copy of a minor allele, while recessive model assumes that only having two copies of a minor allele has an effect on phenotype. In association testing in the full sample, adjustment for age and gender were applied; analyses among men or women, and in the full sample using transformed values (Z-score for height) were adjusted only for age. Bonferroni threshold for multiple testing was estimated for the discovery analysis $\alpha = 0.05/[2$ (models) $\times$ 2 (parameters: height, BMI) $\times$ 2 (gender)$] = 6.25 \times 10^{-3}$, and for the meta-analysis $\alpha = 0.05/[3$ (studies) $\times$ 2 (models) $\times$ 2 (parameters: height, BMI) $\times$ 2 (gender)$] = 2.08 \times 10^{-3}$. Results were combined in a meta-analysis using the inverse-variance method under fixed-effects model using R, ver. 2.13.1 (R Development Core Team 2011, http://www.r-project.org/). Presence of heterogeneity in the meta-analysis was assessed by Cochran's *Q* and I^2 statistics [23]. Power calculations for meta-analysis were performed in R (pwr package). Height distribution data was used to estimate f^2 values assuming additive genotype effects.

Linkage disequilibrium (LD; r^2) in the h*GH/CSH* region was calculated using 1000GENOMES:pilot_1_CEU_low_coverage_panel dataset generated by sequencing the HapMap CEPH (Centre d'Etude du Polymorphisme Humain) samples representing Northern and Western European ancestry ($n = 60$; http://browser.1000genomes.org/index.html). The LD calculations using the above-mentioned data included also top SNPs associated with height within or in the vicinity of h*GH/CSH* region in the published meta-analysis of GWAS studies (rs2665838 [17]; rs7209435 [18]; rs11658329 [19]; rs2854160 [24]). Additionally, LD between *GH2* rs2006123 and SNPs within the *GH1* and *GH2* genes was estimated using published resequencing dataset from Ref. [16]. All LD plots were composed using the Haploview 4.2 program (www.broadinstitute/haploview/haploview) [25].

Sequence alignments were performed using the Web-based ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Statistical difference between men and women in the distribution of rs2006123 genotype frequencies was assessed using χ^2-test implicated in Genepop 4.2 software (http://kimura.univ-montp2.fr/~rousset/Genepop.htm; "population differentiation" option). The testing conditions: dememorization = 10,000, batches = 1000, iterations = 10,000.

3. Results

3.1. GH2 rs2006123 genotype and allele frequencies

The HYPEST ($n = 1464$), CADCZ ($n = 871$), and UFA ($n = 954$) samples were genotyped for the *GH2* rs2006123 polymorphism (intron 2; c.171 + 50C > A; original nomenclature g.943C > A [16]) using the combination of gene-specific long-range and nested PCR followed by allele-specific RFLP. In all sample sets, the distribution of rs2006123 genotypes was in accordance with HWE ($P > 0.05$; Table 2). Minor A-allele frequency of the *GH2* rs2006123 polymorphism exhibited a gradient from Volga-Ural to Central Europe (UFA 20.4%, HYPEST 25.4%, CADCZ 30.4%; Table 2). Genotype frequency distributions among male and female subjects in all three sample sets were statistically similar (χ^2-test, $P > 0.27$; Supplemental Table S1).

3.2. GH2 rs2006123 is associated with adult height

In the discovery analysis of HYPEST samples (Estonians), *GH2* rs2006123 SNP was significantly associated with male height under additive genetic model ($n = 506$; A-allele effect: beta (SE) = 1.52 (0.5); $P = 2.54 \times 10^{-3}$), whereas no association was detected in the female-only analysis ($n = 958$; Table 3). Although in the replication samples (UFA, Bashkortostan: $n = 655$ men, $n = 299$ women; CADCZ, Czech: $n = 518$ men, $n = 353$ women; Table 1) the association testing did not reach statistical significance, five of the six analyzed groups under the additive model and all groups under the recessive model showed consistent trend (positive beta) of association with height (Table 3).

Prior to meta-analysis, the consistency of effects across study groups was estimated. For all performed meta-analysis tests, the calculated heterogeneity statistic was indicative to the consistency of studies' results ($I^2 = 0\%$). In the meta-analysis across the HYPEST, CADCZ and UFA men ($n = 1679$), the association of the *GH2* rs2006123 A-allele with taller stature was enhanced (additive effect: beta = 0.86 (0.28), $P = 1.83 \times 10^{-3}$) and remained statistically significant after multiple testing correction (Table 3). In meta-analysis across women ($n = 1610$), the association was not statistically significant.

Based on direct height measurements, the HYPEST men carrying AA-genotype were in average 2.7 cm taller than the CC-homozygote men, and the mean height difference between AA- and CC-homozygote women was 1.6 cm. In the UFA sample, the AA- compared to CC-homozygotes were in average 2.4 cm and 1.5 cm taller among men and women, respectively; and in the CADCZ sample the estimated height difference was 1.8 cm (male) and 0.7 cm (female) (Table 4). Alternative testing of the full study sample based on the calculated Z-scores instead of raw height measurements (in cm) further supported the detected association between *GH2* rs2006123 and adult height (Table 5).

3.3. No association of GH2 rs2006123 with BMI

The *GH2* rs2006123 was not associated with BMI in any of the analyzed studies (HYPEST, UFA, CADCZ) either in the full sample or in sex-specific sub-samples (Supplemental Tables S2–S3).

3.4. GH2 rs2006123 is in strong LD with top hits from meta-analysis of GWA studies for height within or nearly the hGH/CSH region

Intergenic SNPs within the h*GH/CSH* cluster or in the vicinity have been associated with adult height in several independent meta-analysis of conducted genome-wide association studies

Table 2
Allele and genotype frequencies of the *GH2* rs2006123.

Population		*N*		Allele frequencies[a]		Genotype frequencies[a]			HWE[b]
				C	A	C/C	C/A	A/A	*P*-value
HYPEST	Estonia	1464	%	74.6	25.4	54.9	39.4	5.7	0.129
			(N)	(2185)	(743)	(804)	(577)	(83)	
CADCZ	Czech	871	%	69.6	30.4	47.9	43.5	8.6	0.471
			(N)	(1213)	(529)	(417)	(379)	(75)	
UFA	Bashkortostan	954	%	79.6	20.4	64.3	30.6	5.1	0.073
			(N)	(1518)	(390)	(613)	(292)	(49)	

N, number of genotyped subjects in each sample set.
[a] Data presented as percentage with number of allele/genotype carriers in brackets.
[b] HWE, *P*-value of the χ^2-test for Hardy-Weinberg-Equilibrium.

Table 3
Association testing of the *GH2* rs2006123 with measured adult height (in cm).

Study		*N*	Additive model			Recessive model		
			Beta (SE)	95% CI	*P*-value	Beta (SE)	95% CI	*P*-value
HYPEST	All	1464	0.43 (0.26)	[−0.07, 0.94]	9.09×10^{-2}	1.41 (0.67)	[0.10, 2.73]	$\mathbf{3.52 \times 10^{-2}}$
	Men	506	1.52 (0.50)	[0.54, 2.50]	$\mathbf{2.54 \times 10^{-3*}}$	2.07 (1.51)	[−0.89, 5.04]	1.72×10^{-1}
	Women	958	−0.05 (0.29)	[−0.63, 0.52]	8.53×10^{-1}	1.21 (0.73)	[−0.22, 2.63]	9.67×10^{-2}
CADCZ	All	871	0.27 (0.33)	[−0.37, 0.91]	4.06×10^{-1}	0.74 (0.74)	[−0.72, 2.20]	3.20×10^{-1}
	Men	518	0.37 (0.45)	[−0.51, 1.25]	4.16×10^{-1}	0.64 (1.03)	[−1.38, 2.66]	5.34×10^{-1}
	Women	353	0.08 (0.46)	[−0.82, 0.99]	8.58×10^{-1}	0.75 (1.05)	[−1.31, 2.80]	4.78×10^{-1}
UFA	All	954	0.81 (0.43)	[−0.04, 1.66]	6.25×10^{-2}	2.05 (1.15)	[−0.21, 4.31]	7.62×10^{-2}
	Men	655	0.83 (0.50)	[−0.14, 1.80]	9.47×10^{-2}	2.39 (1.32)	[−0.20, 4.97]	7.08×10^{-2}
	Women	299	0.66 (0.86)	[−1.03, 2.35]	4.46×10^{-1}	1.27 (2.29)	[−3.21, 5.75]	5.79×10^{-1}
Meta-analysis	All	3289	0.45 (0.18)	[0.09, 0.81]	$\mathbf{1.40 \times 10^{-2}}$	1.26 (0.46)	[0.36, 2.15]	$\mathbf{5.90 \times 10^{-3}}$
	Men	1679	0.86 (0.28)	[0.32, 1.41]	$\mathbf{1.83 \times 10^{-3**}}$	1.47 (0.71)	[0.07, 2.87]	$\mathbf{3.93 \times 10^{-2}}$
	Women	1610	0.04 (0.24)	[−0.43, 0.50]	8.79×10^{-1}	1.07 (0.58)	[−0.06, 2.20]	6.37×10^{-2}

Linear regression (age and gender as covariates) was used to test association with height under additive and recessive genetic models. Effect is given as beta, SE. $P < 0.05$ is highlighted in **bold** and *P*-values lower than Bonferroni threshold for multiple testing correction in the discovery analysis ($\alpha = 6.25 \times 10^{-3}$) and in the meta-analysis ($\alpha = 2.08 \times 10^{-3}$) are indicated with asterisks (*) or (**), respectively. Meta-analysis of across individual studies (HYPEST, CADCZ, UFA) was performed using an inverse-variance method under fixed-effect model. N, number of subjects; SE, standard error, CI, confidence interval.

(GWAS) among Europeans and non-Europeans (rs2665838 [17]; rs7209435 [18]; rs11658329 [19]; rs2854160 [24]). We estimated the strength of LD between the *GH2* rs2006123 and the surrounding SNPs using the 1000Genomes dataset for the subjects of European origin (CEPH; $n = 60$). Consistent with the previous report in Estonians, Mandenkalu and Chinese Han [16], also in the 1000Genomes dataset the entire h*GH/CSH* gene region was characterized by weak LD (Supplemental Fig. S3). Notably, the *GH2* rs2006123 showed strong LD with three previously identified SNPs associated with adult height within or near the h*GH/CSH* cluster: rs2665838 ($r^2 = 0.87$, ~7.2 kb upstream from *GH2*), and rs7209435, rs11658329 ($r^2 = 0.92$, within the *MAP3K3* gene ~245 kb and ~195 kb upstream from rs2006123, respectively) (Fig. 1). The *GH2* rs2006123 was not in LD with the h*GH/CSH* cluster SNP rs2854160 detected in combined GWA results for height from 9 studies of individuals of African descent ($r^2 = 0.19$, *CSH1-CSHL1* intergenic region).

Importantly, in the 1000Genomes dataset the SNPs within or nearby the h*GH/CSH* region reported in four meta-analyses of GWAS data to be associated with height, completely lacked ($r^2 = 0$) or exhibited low LD ($r^2 \leq 0.3$) with SNPs in *GH1* encoding the pituitary growth hormone (Fig. 1, Supplemental Fig. S3). Also, in the published resequencing study of the h*GH/CSH* genes [16], almost no ($r^2 \leq 0.09$ for 14 of 18 SNPs) or low LD was estimated between the *GH2* rs2006123 and SNPs in the *GH1* gene (average $r^2 = 0.066$) or its upstream region ($r^2 = 0.19$), and other SNPs the *GH2* gene ($r^2 = 0.025$) (Supplemental Table S4).

4. Discussion

The current study reports the significant effect of the genetic variant (rs2006123; c.171 + 50C > A) in intron 2 of *GH2* gene encoding placental growth hormone (PGH) to modulate the adult height. To our knowledge, this is the first study to demonstrate a link between a polymorphism in a gene with known expression restricted to the placenta during prenatal period and the programming of the growth potential in adulthood. Humans with the derived *GH2* rs2006123 A-allele appeared to be taller and the effect was stronger among men. In meta-analysis across the three study samples of Eastern/Central European origin ($n = 3289$), the effect of the A-variant was detected 0.45 cm per allele ($P = 1.40 \times 10^{-2}$) and 1.26 cm for the AA-genotype ($P = 5.90 \times 10^{-3}$); whereas among men ($n = 1679$) the A-allele carrier effect was 0.86 cm per allele ($P = 1.83 \times 10^{-3}$) and 1.47 cm for AA-homozygotes ($P = 3.93 \times 10^{-2}$). The limitation of modest sample size possibly affected proper association testing with female height (Supplemental Fig. S4). Still, the observed effect sizes in women showed consistent non-significant trend (positive beta) of association with height under the recessive model (meta-analysis: beta (SE) = 1.07 (0.58); $P = 6.37 \times 10^{-2}$; Table 3). Unfortunately, further postnatal influences were not known for the study groups and thus, could not be ruled out as additional modifiers of the study outcome. Overall, the present results contribute to a larger array of genetic effects reported to be involved in human adult height determination [17–19,24].

The studied *GH2* polymorphism rs2006123 (C/A; located in intron 2, 50 bp from donor splice site) is determining the two main *GH2* variants in humans [16]. It shows high allelic differentiation among world populations and possible balancing selection acting on its genetic variation. Whereas in Asia the prevalence of the derived A-allele is low (≤8% in Han Chinese and Japanese; dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2006123), it represents the major *GH2* variant in Africa (Senegalese Mandenkalu 85%, Nigerian Yorubans 79%). In Europe, there appears to be a West-East gradient of the A-allele frequency, from 30% in Central-Europe to 20% at the Volga-Ural region bordering Europe and Asia. In human-chimpanzee comparison, *GH2* is the most conserved gene in the *GH/CSH* cluster and so far only chromosomes with C-allele at the position c.171 + 50C > A have been described [26]. Also, all the rest of the highly homologous genes in

Table 4
Measured height in subgroups carrying the alternative *GH2* rs2006123 genotypes.

Genotype	HYPEST		CADCZ		UFA	
	Men	Women	Men	Women	Men	Women
N: CC, CA, AA	281, 206, 19	523, 371, 64	255, 218, 45	162, 161, 30	413, 208, 34	200, 84, 15
CC	178.1 ± 6.8[a]	164.7 ± 5.8	176.9 ± 7.4	165.3 ± 5.7	168.8 ± 7.7	164.2 ± 8.8
CA	179.8 ± 7.0	164.2 ± 6.3	177.4 ± 6 .3	165.1 ± 5.7	169.3 ± 7.1	164.8 ± 8.5
AA	180.8 ± 5.0	166.3 ± 6.3	178.7 ± 6.5	166.0 ± 7.1	171.2 ± 7.8	165.7 ± 7.4

[a] Data is provided as mean ± SD of height in centimeters (cm); *N*, number of subjects in each genotype group (CC, CA, AA).

Table 5
Association testing of the *GH2* rs2006123 with adult height based on calculated Z-scores (Standard deviation scores).

Study	*N*	Additive model			Recessive model		
		Beta (SE)	95% CI	*P*-value	Beta (SE)	95% CI	*P*-value
HYPEST	1464	0.066 (0.040)	[−0.012, 0.145]	1.01×10^{-1}	0.238 (0.105)	[0.032, 0.444]	$\mathbf{2.36 \times 10^{-2}}$
CADCZ	871	0.038 (0.050)	[−0.061, 0.136]	4.56×10^{-1}	0.113 (0.115)	[−0.113, 0.338]	3.27×10^{-1}
UFA	954	0.103 (0.055)	[−0.005, 0.210]	6.20×10^{-2}	0.264 (0.146)	[−0.023, 0.550]	7.13×10^{-2}
Meta-analysis	3289	0.067 (0.027)	[0.013, 0.120]	$\mathbf{1.44 \times 10^{-2}}$	0.199 (0.069)	[0.065, 0.333]	$\mathbf{3.64 \times 10^{-3}}$

Linear regression (age as covariate) was used to test association with height under additive and recessive genetic models. Effect is given as beta, SE. $P < 0.05$ is highlighted in **bold**. Meta-analysis of across individual studies (HYPEST, CADCZ, UFA) was performed using an inverse-variance method under fixed-effect model.
N, number of subjects; SE, standard error, CI, confidence interval.

the h*GH/CSH* cluster (*GH1*, *CSH1*, *CSH2*, *CSHL1*) are monomorphic for the C-nucleotide at this position (Supplemental Fig. S5). In case the C-allele is considered as ancestral, the derived A-allele associated with a taller statue, arose as a novel mutation among humans.

PGH secreted by syncytiotrophoblast functions as an insulin antagonist, controls maternal Insulin Growth Factor 1 (IGF-1) production and glucose utilization in pregnancy [6,7]. The induction of PGH by glucose deprivation provides a feedback loop to ensure a delivery of nutrients to developing fetus. Multiple studies have provided convincing evidence for a positive correlation between maternal serum PGH concentration and birth-weight [11,12,14]. In addition, it has been shown that transgenic mice over-expressing the gene encoding for PGH became larger than their normal littermates and they are at risk to develop insulin resistance in later life [27]. The observed effects of PGH speak for its influence on growth process, thus explaining the association found with the adult height, but the exact molecular mechanisms of the effect are yet to be discovered. However, based on the accumulated evidence, it has been concluded that altered metabolism of PGH in humans may lead to consequences not only in fetal growth and gestational metabolism, but also in programming the birth-weight of a newborn and long-term metabolic function [7]. Unfortunately, the

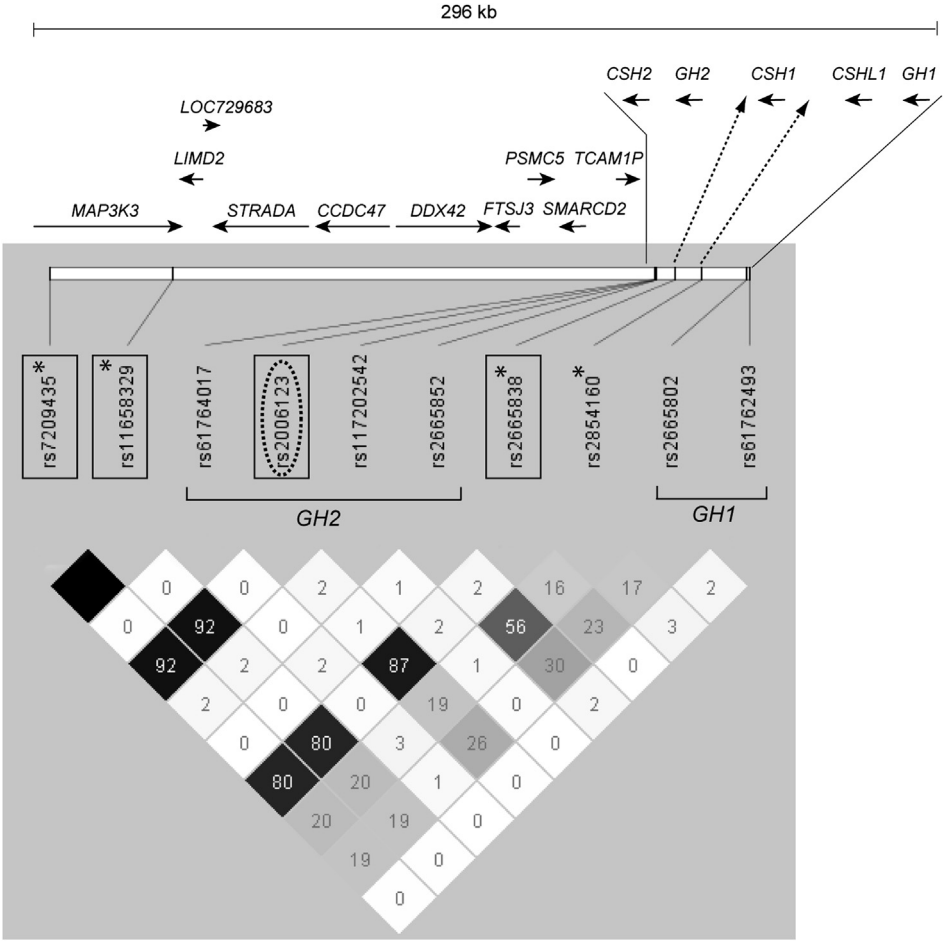

Fig. 1. Pairwise LD plot (296 kb region; 17q23.3) including SNPs in the *GH1* and *GH2* genes and top-hits from four independent meta-analysis of GWA studies of adult height mapped within or nearby the h*GH/CSH* genome cluster (rs2665838 [17], rs7209435 [18], rs11658329 [19], rs2854160 [24]). The *GH2* rs2006123 is indicated with an oval dashed line and the GWAS top-SNPs are marked with an asterisk. SNPs in strong LD ($r^2 > 0.8$) are boxed. The genomic context of the region is shown above the LD plot. Solid arrows indicate to the gene location and transcriptional orientation. The location of SNPs rs2665838 and rs2854160 within the zoomed-in h*GH/CSH* gene cluster is shown with dashed arrows. LD (r^2) was calculated based on 1000GENOMES:pilot_1_CEU_low_coverage_panel dataset containing HapMap individuals with European ancestry (CEPH; $n = 60$). Numbers shown on the individual squares of the LD triangle represent the estimated r^2 (%) values between SNP pairs, scaling from no LD (white squares, $r^2 = 0$) to complete allelic association (black squares, $r^2 = 100\%$).

datasets of the current study did not allow addressing the association of *GH2* polymorphism rs2006123 with birth weight and height.

The secretion of PGH is inhibited by glucose *in vitro* and *in vivo* [28], indicating that maternal nutrition, food availability and diet may affect PGH synthesis. Different *GH2* expressional variants could have had a selective advantage to guarantee the optimal birth weight for a given population environment and life-style. However, as human dietary habits and lifestyle have changed tremendously in past centuries, the genetic composition evolved to support normal growth axis even in nutrient-limited situations may contribute to the programming of accelerated growth in a nutrient-abundant environment. Notably, a recent study showed that frequencies of SNP alleles associated with increased height were systematically elevated in Northern compared with Southern Europeans mirroring the intra-European height differences and being consistent with weak selection on these genetic variants [29].

Several meta-analyses of GWA studies have mapped genetic variants contributing to height determination within (intergenic region) or in the vicinity (within 245 kb) of the h*GH/CSH* gene cluster. The studied *GH2* intronic variant rs2006123 was in strong LD ($r^2 \geq 0.87$) with three of the four reported top hits mapped to this region to be associated with height (rs2665838 [17]; rs7209435 [18]; rs11658329 [19]). Thus, the current paper is essentially providing important experimental support for an *in silico* imputation, an essential step in meta-analyses of GWAS datasets. This observation favors the scenario that the reported GWAS associations with height may be attributable to the functionality of the *GH2* rs2006123 alternative alleles, although it does not explicitly exclude that it acts as a proxy of the actual causal variant(s) within h*GH/CSH* cluster modulating the growth potential. Here, we speculate that alternative alleles of this SNP (c.171 + 50C > A) located in intron 2 may modulate splicing patterns and expression of *GH2*, encoding in total four splice variants [9]. Interestingly, one of the *GH2* gene alternative mRNA transcripts, *GH2-4* (20-kDa hGH-V) skips the regular acceptor site in intron 2 and uses alternative site 45 bp downstream within exon 3 [30]. Functional studies on human pituitary growth hormone (GH) encoding *GH1* (homologous to *GH2*) have indicated that a specific secondary structure within the native human GH transcript controls the relative utilization of the two competing splice-acceptor sites with the consequent generation of two functionally distinct hormone isoforms [31]. The deleted region (identical in *GH1* and *GH2*) involves receptor binding site 1 and secreted hormone has therefore lower activity for GH receptor and GH binding protein [32]. The profile of PGH isoforms during fetal period may not only contribute to the determination of intra-uterine growth and metabolism, but may also program the activity of the GH/IGF-1 axis for the post-natal period [33].

Increasing attention is drawn to the *in utero* programming of postnatal metabolism and risks for adult disease [34]. Although the majority of observations have been ascribed to maternal malnutrition, infection, exposure to environmental factors or placental pathology, there is growing evidence that developmental programming may be also modulated by genetic factors. A polymorphism in the *IGF2BP2* gene was shown to interact with fetal malnutrition to program postnatal glucose metabolism [35]. The carrier status of the T-allele of rs12979860 upstream of *IL28B* has been suggested to contribute to subsequent innate immune development in children who develop allergic disease, as it correlates with differences in the pro-inflammatory profile during the first five years of life [36]. A recent study showed that many genes with high expression in mid-gestation placenta have also been implicated in adult complex disease, promoting the discussion on the role of placenta in developmental programming [37].

In summary, the study reports the association between PGH encoding *GH2* polymorphism and adult height, and promotes discussions and further research on the role of placental genes in prenatal programming of human metabolism and growth potential.

Disclosure statement

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2013.08.012.

References

[1] Wallis M. The molecular evolution of vertebrate growth hormones: a pattern of near-stasis interrupted by sustained bursts of rapid change. J Mol Evol 1996;43:93–100.
[2] Chen EY, Liao YC, Smith DH, Barrera-Saldana HA, Gelinas RE, Seeburg PH. The human growth hormone locus: nucleotide sequence, biology, and evolution. Genomics 1989;4:479–97.
[3] Procter AM, Phillips 3rd JA, Cooper DN. The molecular genetics of growth hormone deficiency. Hum Genet 1998;103(3):255–72.
[4] Esteban C, Audí L, Carrascosa A, Fernández-Cancio M, Pérez-Arroyo A, Ulied A, et al. Human growth hormone (GH1) gene polymorphism map in a normal-statured adult population. Clin Endocrinol (Oxf) 2007;66(2):258–68.
[5] MacLeod JN, Lee AK, Liebhaber SA, Cooke NE. Developmental control and alternative splicing of the placentally expressed transcripts from the human growth hormone gene cluster. J Biol Chem 1992;267:14219–316.
[6] Lacroix MC, Guibourdenche J, Frendo JL, Muller F, Evain-Brion D. Human placental growth hormone–a review. Placenta 2002;23(Suppl. A):S87–94.
[7] Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. Curr Opin Endocrinol Diabetes Obes 2011;18(6):409–16.
[8] Männik J, Vaas P, Rull K, Teesalu P, Rebane T, Laan M. Differential expression profile of growth hormone/chorionic somatomammotropin genes in placenta of small- and large-for-gestational-age newborns. J Clin Endocrinol Metab 2010;95:2433–42.
[9] Männik J, Vaas P, Rull K, Teesalu P, Laan M. Differential placental expression profile of human growth hormone/chorionic somatomammotropin genes in pregnancies with pre-eclampsia and gestational diabetes mellitus. Mol Cell Endocrinol 2012;355(1):180–7.
[10] Frankenne F, Closset J, Gomez F, Scippo ML, Smal J, Hennen G. The physiology of growth hormones (GHs) in pregnant women and partial characterization of the placental GH variant. J Clin Endocrinol Metab 1988;66(6):1171–80.
[11] Fuglsang J, Lauszus F, Flyvbjerg A, Ovesen P. Human placental growth hormone, insulin-like growth factor I and -II, and insulin requirements during pregnancy in type 1 diabetes. J Clin Endocrinol Metab 2003;88:4355–61.
[12] McIntyre HD, Serek R, Crane DI, Veveris-Lowe T, Parry A, Johnson S, et al. Placental growth hormone (GH), GH-binding protein, and insulin-like growth factor axis in normal, growth-retarded, and diabetic pregnancies: correlations with fetal growth. J Clin Endocrinol Metab 2000;85:1143–50.

[13] Mittal P, Espinoza J, Hassan S, Kusanovic JP, Edwin SS, Nien JK, et al. Placental growth hormone is increased in the maternal and fetal serum of patients with preeclampsia. J Matern Fetal Neonatal Med 2007;20:651–9.
[14] Chellakooty M, Vangsgaard K, Larsen T, Scheike T, Falck-Larsen J, Legarth J, et al. A longitudinal study of intrauterine growth and the placental growth hormone (GH)-insulin-like growth factor I axis in maternal circulation: association between placental GH and fetal growth. J Clin Endocrinol Metab 2004;89:384–91.
[15] Day IN, Chen XH, Gaunt TR, King TH, Voropanov A, Ye S, et al. Late life metabolic syndrome, early growth, and common polymorphism in the growth hormone and placental lactogen gene cluster. J Clin Endocrinol Metab 2004;89(11):5569–76.
[16] Sedman L, Padhukasahasram B, Kelgo P, Laan M. Complex signatures of locus-specific selective pressures and gene conversion on human growth hormone/chorionic somatomammotropin genes. Hum Mutat 2008;29(10):1181–93.
[17] Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature 2010;467(7317):832–8.
[18] Gudbjartsson DF, Walters GB, Thorleifsson G, Stefansson H, Halldorsson BV, Zusmanovich P, et al. Many sequence variants affecting diversity of adult human height. Nat Genet 2008;40(5):609–15.
[19] Carty CL, Johnson NA, Hutter CM, Reiner AP, Peters U, Tang H, et al. Genome-wide association study of body height in African Americans: the Women's Health Initiative SNP Health Association Resource (SHARe). Hum Mol Genet 2012;21(3):711–20.
[20] Org E, Veldre G, Viigimaa M, Juhanson P, Putku M, Rosenberg M, et al. HYPEST study: profile of hypertensive patients in Estonia. BMC Cardiovasc Disord 2011;11:55.
[21] Janosíková B, Pavlíková M, Kocmanová D, Vítová A, Veselá K, Krupková L, et al. Genetic variants of homocysteine metabolizing enzymes and the risk of coronary artery disease. Mol Genet Metab 2003;79(3):167–75.
[22] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(3):559–75.
[23] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327(7414):557–60.
[24] N'Diaye A, Chen GK, Palmer CD, Ge B, Tayo B, Mathias RA, et al. Identification, replication, and fine-mapping of Loci associated with adult height in individuals of African ancestry. PLoS Genet 2011;7(10):e1002298.
[25] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21(2):263–5.
[26] Pérez-Maya AA, Rodríguez-Sánchez IP, de Jong P, Wallis M, Barrera-Saldaña HA. The chimpanzee GH locus: composition, organization, and evolution. Mamm Genome 2012;23(5–6):387–98.
[27] Barbour LA, Shao J, Qiao L, Pulawa LK, Jensen DR, Bartke A, et al. Human placental growth hormone causes severe insulin resistance in transgenic mice. Am J Obstet Gynecol 2002;186:512–7.
[28] Alsat E, Guibourdenche J, Luton D, Frankenne F, Evain-Brion D. Human placental growth hormone. Am J Obstet Gynecol 1997;177:1526–34.
[29] Turchin MC, Chiang CW, Palmer CD, Sankararaman S, Reich D, Genetic Investigation of ANthropometric Traits (GIANT) Consortium, Hirchhorn JN. Evidence of widespread selection on standing variation in Europe at height-associated SNPs. Nat Genet 2012;44(9):1015–9.
[30] Boguszewski CL, Svensson PA, Jansson T, Clark R, Carlsson LM, Carlsson B. Cloning of two novel growth hormone transcripts expressed in human placenta. J Clin Endocrinol Metab 1998;83(8):2878–85.
[31] Estes PA, Cooke NE, Leibhaber SA. A native RNA secondary structure controls alternative splice-site selection and generates two human growth hormone isoforms. J Biol Chem 1992;267(21):14902–8.
[32] Baumann GP. Growth hormone isoforms. Growth Horm IGF Res 2009;19(4):333–40.
[33] Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. Endocr Rev 2006;27:141–69.
[34] Barker DJ, Eriksson JG, Forsén T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol 2002;31(6):1235–9.
[35] van Hoek M, Langendonk JG, de Rooij SR, Sijbrands EJ, Roseboom TJ. Genetic variant in the IGF2BP2 gene may interact with fetal malnutrition to affect glucose metabolism. Diabetes 2009;58(6):1440–4.
[36] Gaudieri S, Lucas M, Lucas A, McKinnon E, Albloushi H, Rauch A, et al. Genetic variations in IL28B and allergic disease in children. PLoS ONE 2012;7(1):e30607.
[37] Uusküla L, Männik J, Rull K, Minajeva A, Kõks S, Vaas P, et al. Mid-Gestational gene expression profile in placenta and link to pregnancy complications. PLoS ONE 2012;7(11):e49248.