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

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\textbf{A B S T R A C T}

\textit{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.

\textit{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.

\textit{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).

\textit{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), \(P = 5.90 \times 10^{-5}\); additive model: A-allele effect: beta (SE) \(= 0.45\) (0.18), \(P = 1.40 \times 10^{-3}\)). 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.

\textit{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.

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1. Introduction

The human \textit{Growth hormone/Chorionic somatomammotropin} (hGH/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...
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 choricion somatomammotropin (CSH, also known as placental lactogen, PL). CSH1 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 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 in-utero 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 hGH/CSH genes on intrauterine growth and programming of the postnatal metabolism [15]. Detailed research on hGH/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 hGH/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 intron 2 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 hGH/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 hGH/CSH gene cluster

\[ rs2006123 \] and 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 (permissons 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, 25.07.2005).

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 48.1 ± 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 Kralovske 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 = 404 Tatars, n = 490 Bashkins) 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 $(\text{m}^2)$.

2.2. Genotyping

In order to exclude amplification of other highly homologous hHG/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'–AGCTTGGAAAGGAGCAGAAAG–3', reverse primer: 5'TGATTAAAGCTGTTGATCTCTCCAGA–3', allele-specific restriction fragment length polymorphism (RFLP) analysis (Supplemental Fig. 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’–GCTGTGGTGTCTGCTGTTCC–3’, reverse primer: 5’–GTTGGCCATGCAGCTGTCAGT–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 Xap 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 0.5xTrit/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 Table S1).

2.3. Data analysis

Estimation of allele frequencies, conformity to Hardy–Weinberg Equilibrium (HWE; \( \chi^2, P > 0.05 \)) and association testing of the \( \text{GH} \) 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 \( \alpha = 0.05/[2(\text{parameters}) \times 2(\text{gender})] = 6.25 \times 10^{-3} \), and for the meta-analysis \( \alpha = 0.05/[3(\text{studies}) \times 2(\text{models}) \times 2(\text{parameters: height, BMI}) \times 2(\text{gender})] = 2.08 \times 10^{-4} \). 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 (power package). Height distribution data was used to estimate \( \beta^2 \) values assuming additive genotype effects.

Linkage disequilibrium (LD; \( r^2 \)) in the \( \text{GH} \) and \( \text{CSH} \) 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 the \( \text{GH} \) region in the published meta-analysis of GWAS studies (rs2665838 [17]; rs2006123 [18]; rs11658329 [19]; rs2854160 [20]). Additionally, LD between \( \text{GH2} \) rs2006123 and the GH1 and \( \text{GH2} \) SNPs was estimated using published resequencing dataset from Ref. [16]. All LD plots were composed using the Haploview 4.2 program (http://www.broadinstitute.org/haplovew/haplovew.html) [24]. Sequence alignments were performed using the Web-based ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) [25]. Statistical difference between men and women in the distribution of rs2006123 genotype frequencies was assessed using \( \chi^2 \)-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 = 10,000, iterations = 10,000.

3. Results

3.1. \( \text{GH2} \) rs2006123 genotype and allele frequencies

The HYPEST (\( n = 1464 \)), CADCZ (\( n = 871 \)), and UFA (\( n = 954 \)) samples were genotyped for the \( \text{GH2} \) rs2006123 polymorphism (intron 2; c171 + 50C > A; original nomenclature g.943C > A [18]) using the combination of gene-specific long-range and nested PCR followed by allele-specific RFLP. In all sample sets, the distribution of rs2006123 genotype frequencies was in accordance with HWE (\( P > 0.05 \); Table 2). Minor A-allele frequency of the \( \text{GH2} \) 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 (\( \chi^2 \)-test, \( P > 0.27 \); Supplemental Table S1).

3.2. \( \text{GH2} \) rs2006123 is associated with adult height

In the discovery analysis of HYPEST samples (Estonians), \( \text{GH2} \) 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 \( \text{GH2} \) 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-homoygote 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 \( \text{GH2} \) rs2006123 and adult height (Table 5).

3.3. No association of \( \text{GH2} \) rs2006123 with BMI

The \( \text{GH2} \) 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. \( \text{GH2} \) rs2006123 is in strong LD with top hits from meta-analysis of GWA studies for height within or nearly the \( \text{hGH}/\text{CSH} \) region

Intergenic SNPs within the \( \text{hGH}/\text{CSH} \) cluster or in the vicinity have been associated with adult height in several independent meta-analysis of conducted genome-wide association studies
GWAS) among Europeans and non-Europeans (rs2665838 [17]; rs7209435 [18]; rs11658329 [19]; rs2854160 [24]). We estimated the strength of LD between the GH2 rs2060123 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 hGH/CSH gene region was characterized by weak LD (Supplemental Fig. S3). Notably, the rs2006123 showed strong LD with three previously identified SNPs associated with adult height within or near the hGH/CSH cluster: rs2665838 (r² = 0.87, ~7.2 kb upstream from GH2), and rs7209435, rs11658329 (r² = 0.92, within the MAP3K3 gene ~245 kb and ~195 kb upstream from rs2060123, respectively) (Fig. 1). The GH2 rs2060123 was not in LD with the hGH/CSH cluster SNP rs2854160 detected in combined GWA results for 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 rs2060123 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 × 10⁻²) and 1.26 cm for the AA-genotype (P = 5.90 × 10⁻³); whereas among men (n = 1679) the A-allele carrier effect was 0.86 cm per allele (P = 1.83 × 10⁻²) and 1.47 cm for AA-homozygotes (P = 3.93 × 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) = 0.107 (0.58); P = 6.37 × 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 rs2060123 (CA; 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=2060123, 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

| Study    | N   | Beta (SE) | 95% CI   | P-value |
|----------|-----|-----------|----------|---------|
| HYPEST   |     | Additive model |         |         |
| All      | 1464 | 0.43 (0.26) | [0.07, 0.94] | 9.09 × 10⁻² | 1.41 (0.67) | [0.10, 2.73] | 3.52 × 10⁻² |
| Men      | 506  | 1.52 (0.50) | [0.54, 2.50] | 2.54 × 10⁻³ | 2.07 (1.51) | [0.80, 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 × 10⁻¹ |
| CADCZ    | All  | 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  | 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  | 0.45 (0.18) | [0.09, 0.81] | 1.40 × 10⁻² | 1.26 (0.46) | [0.36, 2.15] | 5.90 × 10⁻³ |
| Men      | 1679 | 0.86 (0.28) | [0.32, 1.41] | 1.83 × 10⁻³ | 1.47 (0.71) | [0.07, 2.87] | 3.93 × 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 (n = 6.25 × 10⁻³) and in the meta-analysis (n = 2.08 × 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.

4. Discussion

The current study reports the significant effect of the genetic variant (rs2060123; 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

| Genotype | Men | Women |
|----------|-----|-------|
| HYPEST   |     |       |
| N: CC, CA, AA | 281, 206, 19 | 523, 371, 64 |
| CC       | 178.1 ± 6.8 | 164.7 ± 5.8 |
| CA       | 179.8 ± 7.0 | 164.2 ± 6.3 |
| AA       | 180.8 ± 5.0 | 166.3 ± 6.3 |
| CADCZ    |     |       |
| N: CC, CA, AA | 281, 206, 19 | 523, 371, 64 |
| CC       | 178.1 ± 6.8 | 164.7 ± 5.8 |
| CA       | 179.8 ± 7.0 | 164.2 ± 6.3 |
| AA       | 180.8 ± 5.0 | 166.3 ± 6.3 |
| UFA      |     |       |
| N: CC, CA, AA | 281, 206, 19 | 523, 371, 64 |
| CC       | 178.1 ± 6.8 | 164.7 ± 5.8 |
| CA       | 179.8 ± 7.0 | 164.2 ± 6.3 |
| AA       | 180.8 ± 5.0 | 166.3 ± 6.3 |

* Data is provided as mean ± SD of height in centimeters (cm); N, number of subjects in each genotype group (CC, CA, AA).
the hGH/CSH cluster (GH1, CSH1, CSH2, CSLH1) 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 stature, 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].

Table 5

| Study  | N     | Additive model | Recessive model |
|--------|-------|----------------|-----------------|
|        |       | Beta (SE) 95% CI | Beta (SE) 95% CI |
|        |       | P-value                              | P-value |
| HYPEST | 1464  | 0.066 (0.040) [0.012, 0.145] 1.01 × 10⁻¹ | 0.238 (0.105) [0.032, 0.444] 2.36 × 10⁻² |
| CADCZ  | 871   | 0.038 (0.050) [0.001, 0.186] 4.56 × 10⁻¹ | 0.113 (0.115) [0.011, 0.338] 1.27 × 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] 1.44 × 10⁻² | 0.199 (0.069) [0.005, 0.333] 3.64 × 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.

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 hGH/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 (r² > 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 hGH/CSH gene cluster is shown with dashed arrows. LD (r²) 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 r² (%) values between SNP pairs, scaling from no LD (white squares, r² = 0) to complete allelic association (black squares, r² = 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 expression 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 hGH/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 \((rs2665985) [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 GWA datasets. This observation favors the scenario that the reported GWA 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 hGH/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, GHZ-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 PGF 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 placentation 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 IGF2B8 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.

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