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ORIGINAL ARTICLE

Growth hormone treatment in children with idiopathic short stature: correlation of growth response with peripheral thyroid hormone action

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Objective Idiopathic short stature (ISS) describes short children with normal GH secretion. Although GH treatment increases their heights, growth response to the therapy differs among patients. Thyroid hormones (TH) are essential for longitudinal growth acting mainly through TH receptors (TR) α and β. We have previously reported that GH treatment reduced peripheral TH action in Turner Syndrome by TR down-regulation. The aims of the study were to assess the effect of GH treatment to ISS on peripheral TH action and the correlation between thyroid status and growth response to the therapy.

Subjects, design and measurements Eighteen normal (control) and twenty-five ISS children were enrolled and evaluated before and after 12 months of life time (control) or 12 months of GH therapy (ISS). Fasting blood was used for serum biochemical evaluations, peripheral blood mononuclear cells for TR mRNA determination by QRT-PCR and growth parameters by standard methods.

Results GH treatment modified neither TR mRNA levels nor serum markers of TH action in ISS evaluated as a whole group. However, the individual change in TRβ mRNA levels correlated to the change in sex hormone–binding globulin (SHBG) levels after GH therapy. The growth response to GH correlated positively with the change in TRα mRNA level and negatively with that in TRβ mRNA, TSH and SHBG levels. The change in each TR mRNA isoform after GH treatment correlated negatively with its own basal level.

Conclusions GH therapy induced individual changes in TR expression in ISS that correlated with their growth response. The basal TR mRNA level could predetermine the change in TR expression and therefore the sensitivity to GH treatment.

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Introduction

Idiopathic short stature is a condition in which the height of an individual is more than 2 SD scores (SDS) below the corresponding mean height for a given age, sex and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities.[1] The exclusion of those specific causes leaves a large heterogeneous group of children consisting of many presently unidentified causes of short stature.[1,2] It has been proved that GH treatment increased the height of children with ISS[2] and the US Food and Drug Administration (FDA) approved its use for ISS in 2003. Even when there is an increase of the height associated with GH therapy, the growth rate differs from one child to another as a consequence of their individual variability in growth response.[1] A possible predictor of adult height gain is the growth velocity (GV) during the first year of treatment,[3] although the first-year growth response, as well as the adult height gain attained, is influenced by several factors including age and height deficit at the start of treatment, the underlying cause of short stature and the dose and duration of the therapy.[4] However, these factors account only for approximately 40% of the variance in the growth response.[1]

On the other hand, thyroid hormones (TH) are essential for skeletal development.[5] The classic genomic actions of TH are mediated by nuclear TH receptors (TR) that act as hormone-inducible transcription factors. Several TRα and TRβ isoforms are encoded by the TRA and TRB genes, respectively. The TRα1, TRα2, TRβ1 and TRβ3 isoforms are widely expressed, whereas TRβ2 is restricted to the hypothalamus–pituitary axis.[6] In spite of the involvement of indirect TH effects through the GH–IGF-I axis, growing evidence suggests that direct effects of TH are critical for bone development. It has been shown, for instance, that GH without triiodothyronine (T3) is unable to stimulate the maturation and organization of

growth plate chondrocytes and GH replacement does not rescue the ossification defect seen in TRα and TRβ-null mice.[7] Moreover, it was reported that TR are essential for the direct effects of T3 on bone formation and that normal endochondral ossification requires T3 actions mediated by both TRα1 and TRβ1.[8]

Previously, we reported data showing that IGF-I and GH reduced specific T3 responses in rat tissues by a down-regulation of TR in both rat cell culture and *in vivo*.[9–11] In consonance, we provided evidence for a reduced peripheral TH action induced by GH treatment to Turner Syndrome (TS) girls assessed by the measurement of TR mRNA expression in peripheral blood mononuclear cells (PBMC) and serum biochemical markers of TH action at tissue level.[12] However, these results could not be extrapolated to other patients receiving GH treatment, taking into consideration the different endogenous GH secretion as well as the different GH dosage administered to patients carrying different etiopathogenic growth deficiencies.[13] Considering this evidence as well as the widely documented individual variability in the growth response of children with ISS to GH treatment, a variable peripheral TH action may also be induced in ISS children. Consequently, an effect of GH treatment on TH action could be expected to be involved in the growth response obtained.

TR are expressed in PBMC, and several reports paralleled TR mRNA expression from PBMC with peripheral tissue responses to TH.[14,15] In turn, the determination of serum biochemical markers of TH action at tissue level has been extensively used.[12,16] Serum thyrotrophin (TSH), sex hormone binding globulin (SHBG), osteocalcin (OC) and β–cross laps (β-CL) are measured as serum biochemical markers reflecting local TH action at the tissue sites:[17] TSH for pituitary, SHBG for liver, and OC and β-CL for bone.

The aims of this study were to assess the effect of GH treatment on peripheral TH action in ISS and the correlation between thyroid status and GV in response to 1-year GH treatment. For this purpose, TRα1, TRα2 and TRβ mRNA levels from PBMC and GV were determined before and after GH therapy. Serum IGF-I, IGFBP3, tetraiodothyronine (T4), free-tetraiodothyronine (FT4), T3 and biochemical serum markers of TH-tissue action: SHBG, TSH, OC and β-CL were also determined.

Subjects and methods

Patients

Ninety-eight children, 38 normal and 60 ISS, were recruited from the Santisima Trinidad Children's Hospital of Cordoba, Argentina; and analysed according to the following inclusion criteria at study start: chronological age: control, 11 ± 1·5 year; ISS, 10·8 ± 1·1 year; bone age <12 year (ISS); normal thyroid function according to clinical and biochemical evaluation; height less than the 5th percentile for chronological age on national growth chart (ISS), GV <−2 SDS during a 6-month prerandomized period (ISS) and normal IGF-I levels before starting the GH therapy. All subjects included were prepubertal and none of them changed this condition during the examination period. Exclusion criteria were chronic illness or a clinical syndrome, disproportion at body measurements, current or prior GH treatment, organic cause of growth failure, primary bone disease, endocrine or metabolic disorder, dysmorphic syndrome. Children born small for gestational age were not included. GH deficiency was ruled out by spontaneous or stimulated serum GH level of 8·0 μg/l or greater if this was suspected on the basis of slow GV or subnormal serum IGF-I or IGFBP3.

Study design

Twenty-five eligible ISS patients were studied at baseline and after 12 months of continuous GH treatment (0·35 mg/kg/week) for TR mRNA measurement in PBMC, biochemical determinations and GV calculated in cm per year and expressed as SDS for prepubertal children.[18] Eighteen eligible normal children (Control) were evaluated at baseline and after 12 months of life time for TR mRNA and only at basal level for biochemical determinations. Fasting blood was obtained for determination of plasma T4, FT4, T3, TSH, IGF-I, IGFBP-3, SHBG, OC and β-CL as well as for PBMC isolation and also for total RNA extraction for TR mRNA determination by RT-QPCR. The clinical protocol was approved by the Ethics Committee of Santisima Trinidad Children's Hospital of Cordoba, Argentina. Patients gave informed consent in accordance with the Santisima Trinidad Children's Hospital Institutional Review Board using the Declaration of Helsinki guidelines.

Peripheral blood mononuclear cells (PBMC) isolation

Peripheral blood mononuclear cells were isolated by Ficoll density gradient as previously described.[12]

Biochemical determination of TSH, SHBG, T4, FT4, T3, OC, β-CL, IGF-I and IGFBP3. Except for IGF-I and IGFBP3, all analytes were measured by electrochemiluminescence immunoassay (EQLIA; Roche Diagnostics GmBH, Mannheim, Germany), using commercial Elecsys System 2010 (Elecsys Corporation, Lenexa, KS, USA). IGF-I and IGFBP3 were determined by immunoradiometric assay (IRMA) with extraction (Diagnostic Systems Laboratories, Webster, TX).

Determination of TR mRNA by QRT-PCR. Total RNA was prepared from PBMC using Trizol according to the manufacturer's protocol (Invitrogen, Grand Island, NY, USA). Real-time quantitative PCR (QRT-PCR) was performed on the Real-Time PCR System Mx3005P (Stratagene, La Jolla, CA, USA), monitoring the increase of fluorescence because of the binding of SYBR Green to double-stranded DNA. Dissociation analysis was performed at the end of each PCR reaction to ensure that only the specific product was amplified. The first strand cDNA template was synthesized from 1 μg total RNA using oligo(dT) in 20 μl of final volume, following the instructions of the SuperScriptTM First Strand Synthesis System (Invitrogen). For a 25-μl PCR, 2 μl of cDNA template were mixed with forward (F) and reverse (R) primers (150 nM final concentration) and 2 × Brilliant SYBR Green QPCR Master Mix (Stratagene). The conditions of reaction were as follows: 95°C 30 s, 60°C 1 min, 72°C 1 min, 40 cycles. To quantify changes in gene

expression, the $2^{-\Delta\Delta ct}$ method was used to calculate relative changes normalized against the L19 mRNA. The sequences of the primers were the following:
TRβ-F 5′-CTGCAGAAGTCCATCGGGCACAAG-'3;
TRβ-R 5′-ACTCTGGTAATTGCTGGTGTGATGAT-3′.
TRα1-F 5′-ACAAGATCGAGAAGAGTC-3′;
TRα1-R 5′-TGGGGCACTCGACTTTCAT-3′.
TRα2-F:5′-ACAAGATCGAGAAGAGTC-3′;
TRα2-R 5′-GGACCCTGAACAACATGCAT-3′.
L19-F -5′-GCGGAAGGGTACAGCCAAT-3′;
L19-R 5′-GCAGCCGGCGCAAA-3′.

Statistical analysis

Statistical analysis was performed using the two-tailed unpaired Student's *t* test (Table 1: Control *vs* ISS Basal and Fig. 1c: Control Basal *vs* ISS Basal, and Control Post-12 months *vs* ISS Post-GH) and the two-tailed paired Student's *t* test (Table 1: ISS Post-GH *vs* ISS Basal; Fig. 1b; and Fig. 1c: Control Basal *vs* Control Post-12 months, and ISS Basal *vs* ISS Post-GH). Pearson's correlation analysis was used to examine bivariate relationships (Figs 2–5). *P* values <0·05 were considered statistically significant. The statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

Results

IGF-I, IGF-I/IGFBP3 as well as OC and β-CL serum levels were increased by GH treatment in children with ISS

In agreement with previous reports,[19,20] serum IGF-I, the relationship IGF-I/IGF-BP3 as well as OC and β-CL serum levels were increased by GH treatment in ISS patients (ISS, Post-GH *vs* ISS, Basal; Table 1). No statistical differences in these parameters were recorded between Control group and children with ISS at basal level (ISS, Basal *vs* Control, Basal; Table 1).

GH treatment to children with ISS did not modify TH serum levels and serum markers of peripheral TH action

Levels of circulating TH and serum markers of TH action from children with ISS are depicted in Table 1. Non significant differences in T4, FT4 and T3 serum levels, as well as in SHBG and TSH were recorded in children with ISS after GH therapy (Post-GH *vs* Basal), in agreement with previous reports.[21,22] Besides, no statistical differences were found in basal levels between Control group and ISS children (ISS, Basal *vs* Control, Basal; Table 1).

GH treatment to children with ISS did not modify TR mRNA levels from PBMC

We firstly determined the relative expression of the different TR mRNA isoforms in PBMC from control children. As shown in Fig. 1a, the profile recorded was similar to that extensively reported for rat bone cells,[23] where TRα1 was almost 10 times higher than TRβ and 20 times higher than TRα2. The same results were obtained in PBMC from ISS (data not shown).

The mRNA expression of α1, α2 and β1 TR of each ISS patient were computed as the relative change in the levels recorded after GH treatment (Post-GH) compared to those at basal level, which were assigned a value of 1. Results show that GH treatment tended to increase TRα1 and β mRNA levels, but these failed to reach statistical significance (Fig. 1b). However, the absolute levels of all TR mRNA isoforms from ISS patients (expressed as the relative expression to that of L19 mRNA) exhibited a broad range in averages and variances and were statistically different from those of control children, both at basal and after 12 months of GH treatment (Fig. 1c, *Test F*, $P < 0{\cdot}01$).

Table 1. Effect of growth hormone (GH) therapy on serum biochemical markers of growth response, thyroid function and peripheral thyroid hormone action in ISS children and in control group

	Control	ISS			
	Basal	Basal	Post-GH	*P* (ISS, basal *vs* control, basal)	*P* (ISS, post-GH *vs* ISS, basal)
T4 (nM)	106 ± 12	109 ± 33	98 ± 21	0·42	0·14
FT4 (pM)	16·7 ± 2·6	17·2 ± 2·1	15·5 ± 1·3	0·47	0·09
T3 (nM)	2·6 ± 0·4	2·4 ± 0·3	2·7 ± 0·6	0·13	0·11
OC (μg/l)	93 ± 22	105 ± 27	152 ± 47	0·13	0·0001*
β-CL (ng/l)	1670 ± 390	1760 ± 460	2400 ± 400	0·55	0·0001*
IGF-I (μg/l)	227 ± 96	285 ± 111	429 ± 159	0·08	0·0002*
IGFBP3 (mg/l)	4·6 ± 1·3	5·5 ± 1·2	6·3 ± 1·4	0·02*	0·12
IGF-I/IGFBP3	5·8 ± 2·2	5·3 ± 1·4	7·1 ± 1·7	0·37	0·003*
SHBG (nM)	59·2 ± 29·5	69·5 ± 29·3	57·6 ± 18·0	0·26	0·11
TSH (mU/l)	2·80 ± 1·00	2·35 ± 0·85	2·28 ± 0·95	0·12	0·78

Data of TSH, T4, FT4, T3 IGF-I, IGFBP3, OC, B-CL and SHBG (mean ± SD) of three independent serum determinations from 25 ISS patients at basal and after GH treatment (Post-GH) and 18 normal children (Control) at basal level are presented. Statistical analysis was performed using the two-tailed unpaired Student's *t* test (ISS, Basal *vs* Control, Basal) and the two-tailed paired Student's *t* test (ISS Post-GH *vs* ISS, Basal). The *P* value <0·05 was considered statistically significant and is depicted in the table as (*).

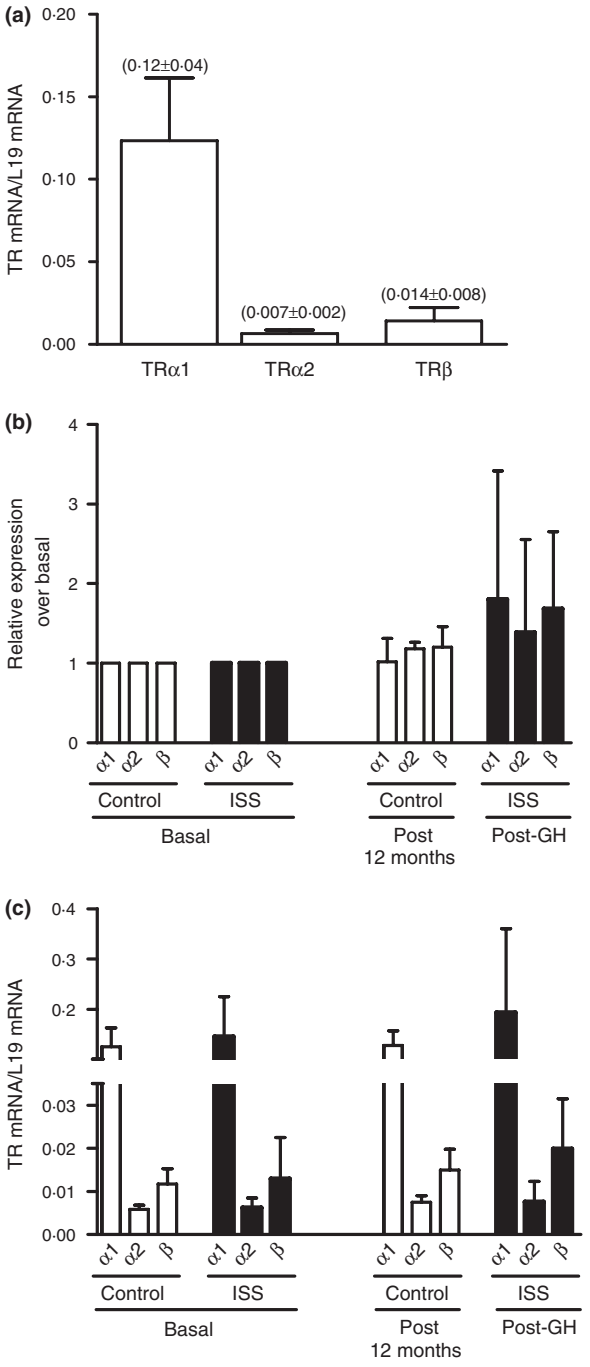

Fig. 1 Effect of GH treatment to Idiopathic Short Stature (ISS) patients on thyroid hormone receptor (TR) mRNA level in peripheral blood mononuclear cells (PBMC). (a) Relative expression of TR mRNA levels in PBMC from Control group. The data (mean ± SD) from three independent determinations of TR mRNA α1, α2 and β in PBMC from 18 normal children were presented as the expression of TR mRNA relative to that of ribosomal protein L19 mRNA. (b) First-year change in mRNA level of TRα1, TRα2 and TRβ in PBMC from 25 ISS and 18 control patients were analysed before and after 12 months of GH treatment for ISS patients or after 12 months of life time for control group. Samples were matched up and analysed as the relative expression of TR mRNA (ΔTR) after 12 months compared with that at basal level, which was assigned a value of 1. (c) Data from (b) is presented as the relative expression of TR mRNA to L19 mRNA.

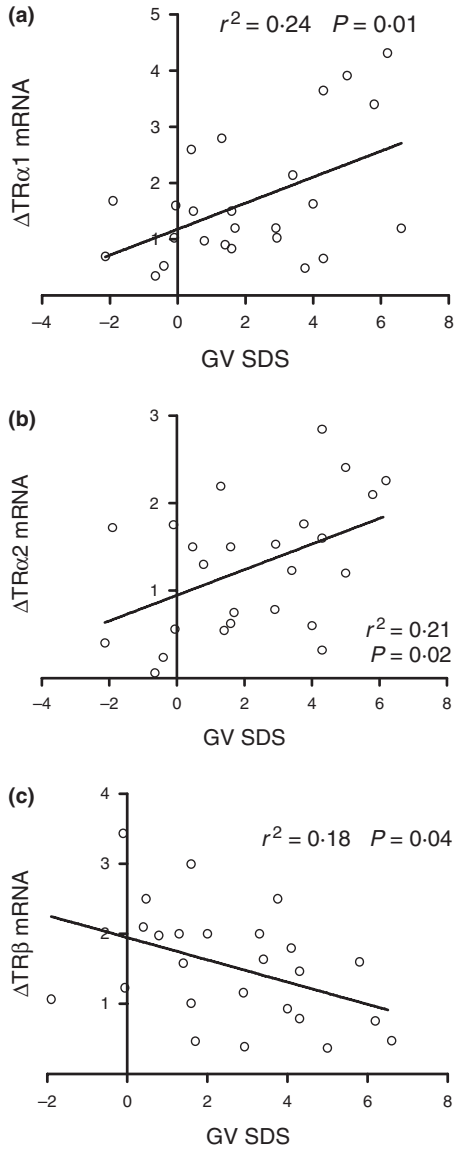

Fig. 2 Correlation between the changes in thyroid hormone receptor (ΔTR) mRNAs and the growth velocity (GV SDS) attained by Idiopathic Short Stature (ISS) children after growth hormone (GH) therapy. First-year change in TRα1 (a), TRα2 (b) TRβ (c) mRNA levels achieved in 25 ISS children are plotted against the first-year GV SDS. The data of ΔTRα1, ΔTRα2 and ΔTRβ were matched up and presented as the relative expression after GH treatment (Post-GH) compared with that at basal level, which was assigned a value of 1.

The growth response to GH therapy of ISS children correlated with the changes in TR mRNA levels from PBMC but not with TH serum levels

The GV of ISS children recorded after GH therapy was highly variable (GV SDS: −2 to +6). In view of this variable growth response and the heterogeneous individual changes recorded in TR mRNA levels in ISS children after GH therapy, we assessed the relationship between these parameters. The relative changes in TRα1 and TRα2 mRNAs showed a positive correlation with GV SDS (Fig. 2a and b

respectively), whereas a negative correlation with TRβ mRNA was recorded (Fig. 2c). All correlations were significant and showed an r^2 of 0·24, 0·21 y 0·18 for TRα1, α2 and β, respectively. On the other hand, nonsignificant correlations were registered between changes in serum levels of T4, FT4 and T3, and GV SDS (data not shown).

The growth response to GH therapy of ISS children negatively correlated with the changes in serum levels of TSH and SHBG

Serum SHBG and TSH levels were not modified by GH treatment in ISS patients (Table 1). However, considering the negative correlation between GV SDS and the relative changes in TRβ mRNA levels (Fig. 2c), we evaluated the correlation between GV SDS and the individual changes recorded in TSH and SHBG (serum markers of TH action at pituitary and liver level respectively, where TRβ is the main TR isoform expressed) after GH treatment to ISS. Both, serum SHBG and TSH relative changes correlated negatively with the GV SDS attained after GH treatment (Fig. 3a and b, respectively). These correlations were statistically significant with r^2 of 0·29 and 0·34 respectively.

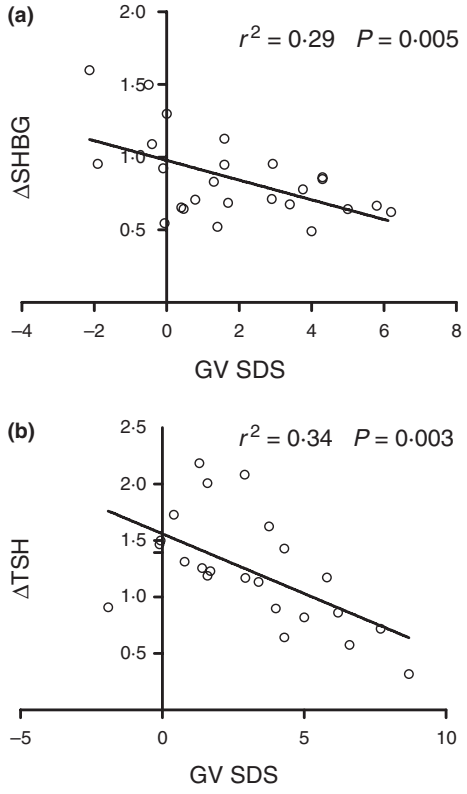

Fig. 3 Correlation between changes in sex hormone–binding globulin (SHBG) and thyrotrophin (TSH) with the growth velocity (GV SDS) attained by Idiopathic Short Stature (ISS) children after growth hormone (GH) therapy. First-year change in SHBG (A) and TSH (B) serum levels achieved in 25 ISS children are plotted against the first-year GV SDS. The data of ΔSHBG and ΔTSH were matched up and presented as the relative expression after GH treatment (Post-GH) compared with that at basal level, which was assigned a value of 1.

The change in TRβ mRNA level of children with ISS after GH therapy was related to the change in SHBG serum levels

We next correlated the relative change in TRβ mRNA level from each ISS patient after GH treatment with the individual relative change in SHBG and TSH serum levels attained (Fig. 4a and b respectively). There was a positive and statistically significant correlation between the change in TRβ mRNA and the change in SHBG (r^2 = 0·2, P = 0·02). However, no significant correlation was registered between changes in TRβ mRNA and TSH.

The relative change in each TR mRNA isoform after GH treatment to ISS patients was correlated with its own basal level

Considering that the expression of TR mRNA from ISS patients showed a significant variability, both at basal and post-GH levels (Fig. 1c), we aimed at evaluating whether the relative change in each TR mRNA achieved after GH therapy, which correlated with the growth response (Fig. 2a–c), was related to the basal expression level of each TR mRNA. A significant negative correlation between the relative changes of each TR mRNA achieved after

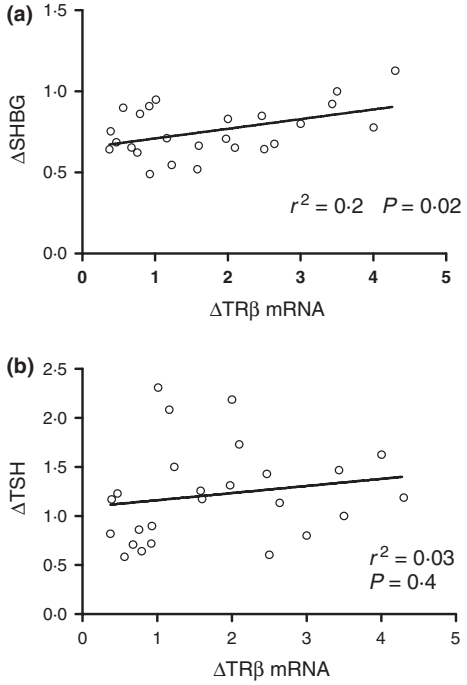

Fig. 4 Correlation between changes in sex hormone–binding globulin (SHBG) and thyrotrophin (TSH) with those in thyroid hormone receptor (TR) β mRNA in Idiopathic Short Stature (ISS) children after growth hormone (GH) therapy. First-year change in TRβ mRNA level in peripheral blood mononuclear cells from 25 ISS children is plotted against the first-year change in the serum markers of liver and pituitary thyroid hormone action: SHBG (a) and TSH (b). The data of ΔSHBG and ΔTSH were matched up and presented as the relative expression after GH treatment (Post-GH) compared with that at basal level, which was assigned a value of 1.

GH therapy compared to those at basal level was observed. The correlations were statistically significant, with r^2 values of 0·46, 0·19 and 0·28 for TRα1, TRα2 and TRβ mRNAs respectively (Fig. 5a–c).

Discussion

The results presented in this study provide evidence for nonhomogeneous changes in peripheral TH action induced by GH therapy to ISS, evaluated through the expression of TR mRNA in PBMC and serum markers of TH action in peripheral tissues. ISS represents a heterogeneous group of patients with different and mostly unknown alterations that share growth deficiency. Many genetic alterations in different proteins involved in the growth deficiency of ISS have been described.[24–26] Because of this heterogeneous etiopathogenic profile, the variable growth response to GH treatment is not surprising. Given the main role of TH action on skeletal development,[5] the knowledge of the impact of GH treatment to ISS on peripheral TH action may also contribute to the success of growth improvement.

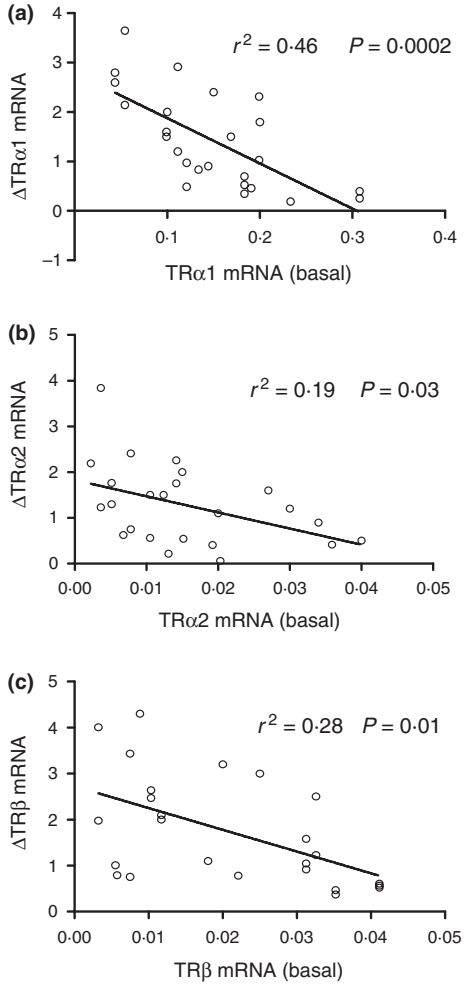

Fig. 5 Correlation between basal thyroid hormone receptor (TR) mRNA levels and changes in TR mRNA (ΔTR) levels achieved at the end of the first year of growth hormone (GH) treatment to Idiopathic Short Stature (ISS) children. First-year changes in TRα1 (a), TRα2 (b) and TRβ (c) mRNA levels in 25 ISS children are plotted against the corresponding basal TR mRNA. The data of ΔTR were presented as the relative expression after GH treatment (Post-GH) compared with that at basal level, which was assigned a value of 1.

GH therapy to ISS increased IGF-I, IGF-I/IGFBP3 as well as OC and β-CL serum levels, as previously reported.[19,20] The registered increase in IGF-I and in the ratio IGF-I/IGF-BP3 after GH therapy indicated that participants were compliant with therapy and GH sensitive, and revealed an increase in IGF-I bio-availability, foretelling success in GH treatment. Regarding OC and β-CL, the increase in these levels achieved by GH treatment showed that metabolism at bone level was improved. In turn, GH therapy did not induce modifications in serum thyroid function profile, determined by the levels of TH as well as TSH in serum. However, the results obtained show that peripheral TH action is modified by GH treatment, that different ISS patients exhibited a particular TR expression, at least in PBMC, and that the basal TR level is associated with the modification induced by therapy.

The magnitude of the cellular response to TH depends on the abundance of different TR isoforms. It is therefore possible that physiological or pharmacological alterations in the number of receptors may be able to modify the tissue response to TH,[27] as we previously reported.[9,10] Unlike the decreased expression of TRα1 and TRβ1 that we have recorded in TS under GH therapy,[12] the group of ISS, considered as a whole, did not reveal significant modifications in TR mRNA levels after 12 months of GH treatment. However, the broad range of individual levels registered both in basal TR mRNA expressions as well as after GH therapy may be responsible, at least in part, of the lack of significance in the changes induced by treatment. Perhaps constituting groups of ISS on the basis of similar molecular alterations in the future could yield more homogeneous changes in TR mRNA levels than the ones exhibited by our cohort.

Nevertheless, the relative change in the TRβ mRNA level after 12 months of GH therapy to each ISS subject was higher in patients who exhibited a lower change in GV. On the contrary, the higher increase in TRα1 and TRα2 mRNAs levels after GH treatment was positively correlated to the gain in GV. These data are coincident with previous reports indicating that TRα1 is the major isoform expressed at rat bone level[23] and that their disturbance produces growth deficiency.[28] Overall, the data suggest that the lower gain in GV achieved in some patients under GH treatment may involve a reduced or small increase of TRα1 expression, even when the parallel increase in TRβ level should have partially compensated for the lack of TRα.[29] Moreover, results reveal that the larger gain in GV after GH therapy was achieved by those who registered the higher increase of TRα1 mRNA level, in spite of the concomitant TRβ decrease. This finding is in agreement with the main role of TRα1 in bone longitudinal growth.[29]

Although it has been described that TR mRNA expression level in PBMC diminishes with age,[15] it seems unlikely that the dispersion of molecular and biochemical data registered in ISS were because of an age effect, as these were not evidenced in the Control group of the same age range.

Reports of the impact of GH administration on thyroid function have provided no conclusive data. Decreased TSH and T4, and increased T3 after GH administration to GH-deficient patients has been reported,[30] while IGF-I administration to Laron syndrome patients has been reported to decrease TSH and FT4 without changes in T3.[31] Besides, GH administration to TS girls increased TSH and T3, although they did not exceed normal values.[12] In turn, thyroid function of the group of ISS enrolled in this study did not achieve significant modifications when compared to controls, neither in basal nor in GH-treated conditions.

Regarding SHBG, although it was not modified by GH treatment in the group of ISS analysed, the modification in individual levels after GH treatment correlated positively with the change in TRβ mRNA achieved, in consonance with the large dispersion of individual SHBG levels as well as of TRβ mRNA levels and considering that TRβ is the main TR isoform expressed in liver.[6] On the contrary, the individual changes in TSH induced by GH treatment to ISS were not correlated to the change in TRβ mRNA. While other authors have previously associated a reduction in TRβ mRNA levels in PBMC with an increase in serum TSH,[15] the lack of association registered in our study may be related to the scarce PBMC expression of TRβ2, the main functional TR isoform expressed in pituitary.[6] Possibly in this group of ISS patients, or a subset of them, GH therapy does not affect TRβ mRNA expression in pituitary and in PBMC tissues in the same way.

Unlike the observed association between GV SDS and ΔTR mRNA levels, there was no correlation between GV SDS and relative changes in T3, T4, FT4 serum levels induced by GH in ISS. These findings are also in favour of the importance of TR expression measurement over serum thyroid function evaluation for the follow-up of GH therapy. In turn, both SHBG and TSH individual changes induced by GH therapy correlated negatively with GV SDS. The correlation of higher gains in GV SDS with lower changes in SHBG serum level is in accordance with the concomitant decrease in TRβ level, which in turn, correlates positively with SHBG. Regarding TSH and its negative correlation with GV SDS, it is in agreement with the lower normal TSH levels that parallel normal high TH levels that ensure a normal longitudinal growth. These data corroborate the already described best sensitivity of TSH as serum marker of thyroid function[32] and allow us to hypothesize that increased levels of TSH may have adverse affects on growth. As a consequence, small reductions in TSH level may be an adaptive mechanism favouring growth, in agreement with the proposed function of TSH as a negative regulator of bone turnover.[33]

Finally, we attempted to find out whether TR mRNA basal levels may influence the relative change in TR mRNA induced by GH and as a consequence the peripheral thyroid action that impacts the growth response to GH therapy. The basal expression of all TR mRNA seems to predetermine the relative change in TR mRNA achieved 12 months after GH therapy. Thus, it is possible that the potential growth response of each ISS individual is, at least in part, predetermined by the aetiology underling each ISS patient. Although the increased peripheral TH action achieved as a consequence of increased TR mRNA expression supports growth in the short term (12 months), we cannot predict if this effect will be held at longer times, considering reports showing that excess of thyroid function is also able to induce short stature through the early closure of the bony growth plates.[34]

A relevant point is the weak association in all the correlations analysed evidenced by r^2 values between 0·18 and 0·46, even though when they were statistically significant. This is consistent with the fact that TH are just one of the many hormonal systems that influence skeleton longitudinal growth and are a part of a complicated network of interacting intracellular signalling pathways which involve also GH, IGFs, oestrogen, androgen, the peptide related to parathyroid hormone, glucocorticoids, the indian hedgehog, and members of the Wnt family among others.[35]

In conclusion, the results from this work show that GH therapy to ISS children induced changes in TR expression in some patients but not in others, that these changes correlate with growth rate, and also that TR mRNA expression at basal levels may predetermine the change in TR attained after GH treatment, and therefore growth response, at least in a 12-month period. At the same time, no changes in serum thyroid state of ISS patients were recorded, considering that the most important parameters that define it, TSH and TH, exhibited no differences with the basal state.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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