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Both pituitary and placental growth hormone transcripts are expressed in human peripheral blood mononuclear cells (PBMC)

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SUMMARY

The hGH-V gene codes for a variant of human pituitary growth hormone (hGH-N) named placental growth hormone (hPGH). hPGH shares 93% amino acid identity with hGH-N. Until now the hGH-V gene was considered to be exclusively expressed in human placenta, where it replaces maternal circulating hGH-N at the end of pregnancy. In this study we investigated by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis hGH-N, and hGH-V, gene expression in PBMC in men, women and pregnant women. We have demonstrated that hGH-N and hGH-V transcripts are simultaneously produced by PBMC in both men and women as well as pregnant women. The PBMC of a *PIT-1*-negative woman expressed only the hGH-V transcript, but not the hGH-N one as expected. In conclusion, hGH-V mRNA is expressed by cells other than the syncytiotrophoblast, is not regulated by *PIT-1*, and may be involved in immune regulation, as is pituitary GH.

Keywords human pituitary growth hormone hGH-V peripheral blood mononuclear cells pregnancy

INTRODUCTION

The human growth hormone (hGH) gene locus consists of five closely related genes located on chromosome 17, in the order: 5′-hGH-N/hCS-L/hCS-A/hGH-V/hCS-B-3′ [1,2]. The hGH-N gene is expressed in the pituitary, although the hGH-V, hCS-A, hCS-B and hCS-L genes are expressed in the placental syncytiotrophoblastic cells [3].

Recently, hGH-N transcripts were detected in human lymphoid tissues by reverse transcriptase-polymerase chain reaction (RT-PCR) and *in situ* mRNA hybridization [4].

Pituitary GH and its receptor transcripts have also been shown to be synthesized in lymphoid cells [5,6], but transcripts of the other members of the GH/CS locus were undetectable in human myeloid and lymphoid cells [7].

PIT-1 is a pituitary-specific transcription factor which allows the transcription of hGH-N and hPRL genes in the anterior pituitary [8,9]. Moreover, *PIT-1* mRNAs and proteins have been detected by RT-PCR, *in situ* hybridization (ISH) and immunohistochemistry (IHC) in human lymphoid cells [10] and in 1st and 3rd trimester human syncytiotrophoblast cells [11].

Other studies have identified highly conserved sequences (named PSF-A and PSF-B) in the distal 5′ flanking regions of the placental members of the GH/CS gene locus. These elements were able to bind the *PIT-1* factor *in vitro* [12].

These results indicate a possible role for *PIT-1* in the regulation of hCS and hGH-V gene expression in the placenta. Moreover, *PIT-1* might control the expression of hGH-N, possibly in combination with other transcription factors, in lymphoid cells.

During human pregnancy, the production of maternal pituitary human pituitary growth hormone (hGH-N) is progressively suppressed, while placental GH (hGH-V/hPGH) is synthesized in the placenta [13,14] and secreted in increasing amounts into the maternal blood [15]. The hGH-V and hGH-N transcripts share 94% sequence identity [16].

Little is known about the effect of pregnancy on hGH-V and hGH-N mRNA expression in human lymphoid cells. The hGH-V mRNA was localized in our laboratory [17] by ISH in the placental syncytiotrophoblast, and seemed to be expressed not uniformly, but by a few cells only. Since the placenta contains a large number of cells of haematopoietic origin, it is conceivable that these cells in the syncytiotrophoblast constitute the GH-V-positive cell population. The present study was therefore conducted to examine whether transcripts are present in PBMC, not only from healthy pregnant women, but also from men and non-pregnant women, in order to determine if hGH-V is expressed or not in other physiological circumstances. Furthermore, we were able to evaluate the

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expression of hGH-V and hGH-N mRNAs in a patient with congenital GH, thyroid-stimulating hormone (TSH) and prolactin deficiencies due to an homozygous mutation Ala-Pro (A158P) in the POU-specific domain of the *PIT-1* gene.

MATERIALS AND METHODS

Cell preparation and RNA isolation

Whole blood samples (50 ml) from pregnant women were obtained from the hospital of the Bois de l'Abbaye and the hospital of the Citadelle (Liège, Belgium). Blood was also obtained from healthy volunteers in our institute. The *PIT-1* GH-deficient patient has been described previously [18]. When whole blood was obtained from this patient, she was clinically and biochemically euthyroid while using levothyroxine. She had been on GH substitution treatment, and this medication was stopped 7 months before blood sampling. PBMC were isolated by the Ficoll–Paque method (Pharmacia Biotech, Uppsala, Sweden). Cells were counted, pelleted by centrifugation, and frozen at −70°C. Total cell RNA was isolated using the guanidinium thiocyanate method (RNA Instapure, Eurogentec, Liège, Belgium), followed by phenol-chloroform extraction and precipitation with isopropanol. The RNA samples were analysed by RT-PCR. Total RNAs from mammary gland fibroblasts and trophoblastic cells were obtained from the Service of Histology (Liège, Belgium). Total RNA from *Chlamydomonas* was obtained from the Botanic Institute (Liège, Belgium). Isolation of a T lymphocyte population was realized in the Service of Histology (Liège, Belgium).

RT-PCR with GH-specific primers

Total RNA (1 μg) was added to a 20-μl (final volume) reaction mixture containing 1×AMV buffer (Boehringer-Mannheim, Mannheim, Germany), 5 mM $MgCl_2$, 1 mM of each deoxynucleotide triphosphate, 50 U RNase inhibitor (Boehringer-Mannheim), 20 U AMV reverse transcriptase (Boehringer-Mannheim), and 1·6 μg oligo $d(T)_{15}$ primer. The reaction mixture was incubated at 25°C for 10 min, then 42°C for 60 min, followed by heat denaturation at 99°C for 5 min and cooling at 4°C for 5 min. Five microlitres of the RT reactions were then submitted to 35 cycles PCR (denaturation 1·5 min at 94°C, annealing 1·5 min at 72°C, elongation 2 min at 72°C, with an additional incubation step of 5 min at 72°C). To amplify a 392-bp cDNA fragment, we had chosen primers for hGH-N and hGH-V in exons 2 and 5. The upstream primers for hGH-N and hGH-V were, respectively: OL2N, GTCTGCACCAGCTGGCCTTTGACACC; OL2V, GCCTGTACCAGCTGGCATATGACACC. The downstream primers for hGH-N and hGH-V were, respectively: OL5N, GTTTGTGTCGAACTTGCTGTAGGTCTGC; OL5V, TTTTGTGTCAAACTTGCTGTAGGACTGATT.

The upstream and downstream primers for hGH-N and hGH-V contained four nucleotide mismatches, as indicated by bold characters. The PCR products were generated in a total volume of 100 μl containing 1×Goldstar buffer (Eurogentec), 1·5 mM $MgCl_2$, 0·2 μM of each primer, 0·2 mM of each dNTP, 5 μl of cDNA template, and 2·5 U of Goldstar DNA polymerase (Eurogentec). The RT-PCR products were separated on a 1% agarose gel, and then submitted to restriction enzyme digestion.

RT-PCR with the other primers

As described in [19], the 5′ and 3′ primers for the specific T cell surface markers CD2 and CD3 were, respectively: for CD2, GGTCATCGTTCCCAGGCACCTAGT and TGGTGTGATGGAGCTCTCTGAGGA; for CD3, GGCTGTCCTCATCCTGGCTATCAT and ACTGGTTTCCTTGAAGGTGGCTGT. The 5′ and 3′ primers for the β-actin sequence were: GTGATGGTGGGCATGGGTCA and TTAATGTCACGCACGATTTCCC. Total RNA (5 μg) was reverse transcribed as with the GH-specific primers. cDNA (5 μl) was submitted to 35 PCR cycles (1 min at 95°C, 1 min at 56°C, then 2 min at 72°C). The PCR reaction mixture was the same, except for 5 μM of each specific primer. The expected CD2, CD3 and β-actin RT-PCR product sizes were 405, 514 and 510 bp, respectively. In order to amplify the *PIT-1* transcript in the T cells, we made a nested RT-PCR, as described [11]. The RT reaction was as described previously (5 μg total RNA). The outer 5′ and 3′ primers used were: GCTTTTACTTCGGCTGATACCTTTA and CAGACTTGTTTTCACCCGTTTTTCT. After 35 PCR cycles (1·5 min at 94°C, 1·5 min at 52°C, then 2 min at 72°C) in the same conditions as described previously, except for 1 μM of each outer primer, 10 μl of the outer PCR product were resubmitted to the same 35 cycles with the inner 5′ and 3′ primers: ACAGGACTTCATTATTCTGT and CTCAGCTTCCTCCAGCCA, respectively. The expected product was 450 bp. To check the specificity of this product, a Southern blot hybridization was made in the presence of two internal specific probes (AAGAGCCAATAGACATGGA and AACTTGAAAAGTTTGCCAA) (data not shown).

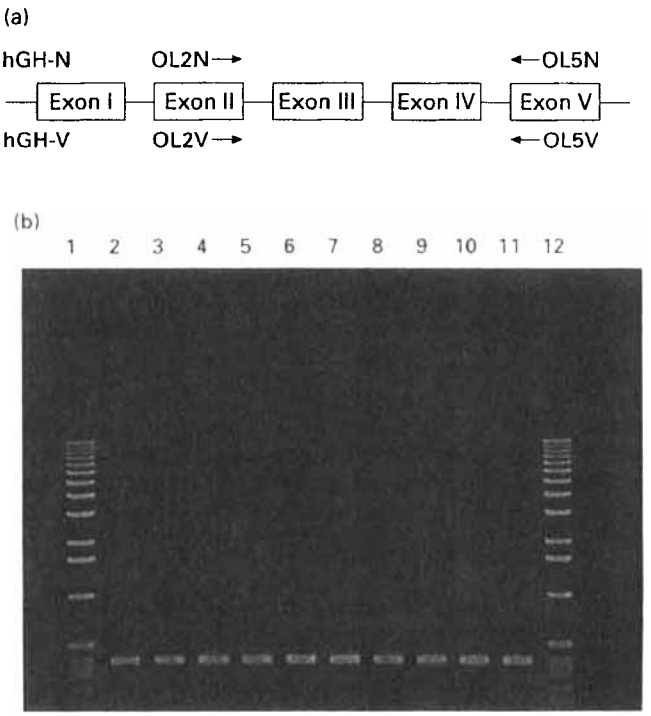

Fig. 1. The hGH-N and hGH-V genes are organized in five exons and four introns. As shown in (a), the primers for each transcript were chosen in exons II and V. The products of reverse transcriptase-polymerase chain reaction (RT-PCR) for hGH-V (2,3,4,5,6) and hGH-N transcripts (7,8,9,10,11) were analysed by 1% agarose gel electrophoresis (b). Tracks 1 and 12: 1-kb DNA ladder molecular weight marker. Results for five of the 15 pregnant women are shown.

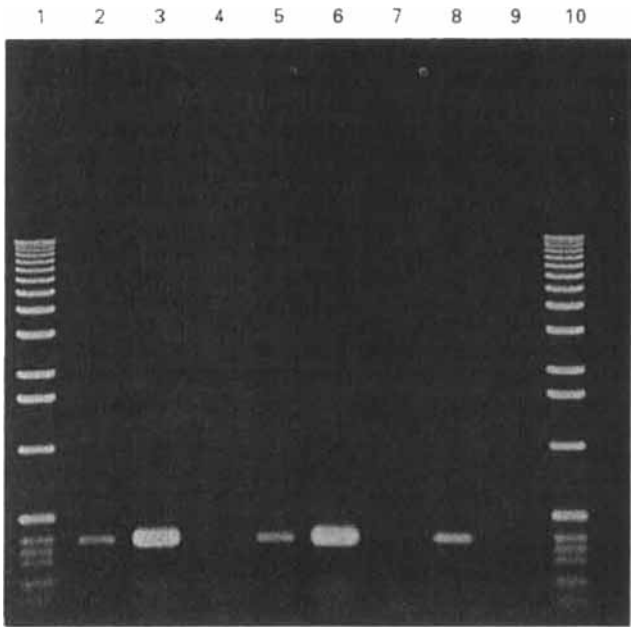

Fig. 2. The reverse transcriptase-polymerase chain reaction (RT-PCR) product of hGH-V (2) was amplified with hGH-V (3) and hGH-N (4) primers. Also the RT-PCR product of hGH-N (5) was amplified with hGH-N (6) and hGH-V (7) primers. The *PIT-1*-negative patient RT-PCR products are shown in tracks 8 (for hGH-V) and 9 (for hGH-N). Tracks 1 and 10: 1-kb DNA ladder molecular weight marker.

Restriction enzyme digestions and nucleotide sequencing

Restriction enzyme digestions (*PstI* and *BglII*) were carried out to check the specificity of the PCR products. Twenty microlitres of each PCR product were incubated at 37°C for 2 h with 30 U of *PstI* or *BglII*. After electrophoresis on a 1% agarose gel, the products were stained with ethidium bromide. The RT-PCR products for each transcript were analysed by sequencing [18].

RESULTS

Both hGH-V and hGH-N mRNAs were detected in PBMC from all 15 pregnant women. The predicted PCR products of 392 bp were obtained with each set of primers specific for hGH-N or hGH-V genes (Fig. 1). As negative control, total RNA from *Chlamydomonas* was also reverse transcribed and submitted to 35 PCR cycles with the hGH-N- and hGH-V-specific primers; no PCR products were obtained with this negative control (data not shown). Two fragments for *PstI* (312 and 80 bp) and *BglII* (356 and 36 bp) enzyme digestions were also consistent with the expected sizes (data not shown).

The primer set specificity was also confirmed by crossed PCR reactions. Each primer set was used in order to amplify the product of RT-PCR with the other primers. We found no contaminating product resulting from non-specific amplification (Fig. 2).

The 392-bp RT-PCR products were sequenced according to the Sanger *et al.* method [20]. Our results showed that the products amplified with the hGH-V- and hGH-N-specific primers were identical to the hGH-V and hGH-N mRNAs, respectively.

The presence of hGH-N and hGH-V mRNAs was then investigated by RT-PCR amplification of RNA isolated from men and non-pregnant women PBMC. The hGH-N mRNAs were present in all samples examined. Surprisingly, hGH-V mRNAs were also detected in all samples. By nucleotide sequencing, the identity between PCR products and hGH-N and hGH-V transcripts was confirmed.

We also found hGH-V mRNAs by RT-PCR amplification in PBMC from the GH-deficient woman with the homozygous *PIT-1* mutation (this mutation generates a protein capable of binding to DNA but unable to activate the GH gene) (Fig. 2). In contrast, no hGH-N mRNA product was detected by the same method, which suggests that *PIT-1* is involved in hGH-N gene transcription in human mononuclear cells (Table 1).

We also examined the presence of hGH-V and hGH-N transcripts in purified T cells from several non-pregnant women. Positive controls (surface markers CD2 and CD3, β-actin and *PIT-1*-specific primers) were included to check T cell line purity. Both hGH-V and hGH-N mRNAs were present in these cells (Fig. 3).

We compared hGH-V transcript expression in immune (T lymphocyte) and placental (trophoblastic) cells. The hGH-V expression level was much higher in trophoblastic than in T cells (Fig. 4). As negative control, fibroblasts from human mammary gland were also examined. We did not investigate the hGH-N transcript expression due to their extensive study by other authors [5,6].

DISCUSSION

The present study demonstrates that both hGH-N and hGH-V transcripts are simultaneously and normally expressed in human PBMC.

The hGH-N transcripts had previously been detected by ISH in human lymphocytes. GH mRNA levels were high in the B cell population [4]. Maggiano *et al.* [21] demonstrated that human T lymphocytes were negative for GH mRNA by ISH. However, our data demonstrate by RT-PCR, which is a more sensitive technique, that both hGH-V and hGH-N transcripts are expressed in the T cells.

Concerning the hGH-V transcript, our results are in disagreement with those of Palmetshofer *et al.* [7], who were unable to detect any hGH-V mRNA in lymphoid cells by RT-PCR. The primers used in their study were selected to amplify the closely related hGH-V, hGH-N, hCS-A and hCS-B transcripts. Perhaps

Table 1. Recapitulative results and numbers of individuals analysed

mRNA	Pregnant women, $n = 15$	Non pregnant women, $n = 9$	Men, $n = 8$	*PIT-1*-deficient woman, $n = 1$
hGH-N	+	+	+	−
hGH-V	+	+	+	+

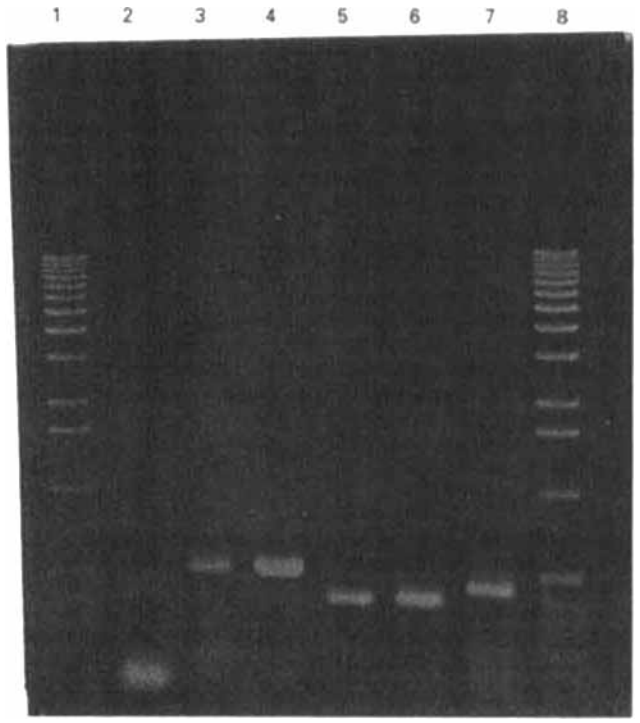

Fig. 3. Reverse transcriptase-polymerase chain reaction (RT-PCR) products obtained in T cells with specific primers for CD2 (2), CD3 (3), β-actin (4), hGH-V (5), hGH-N (6) and *PIT-1* (7) transcripts. Tracks 1 and 8: 1-kb DNA ladder molecular weight marker.

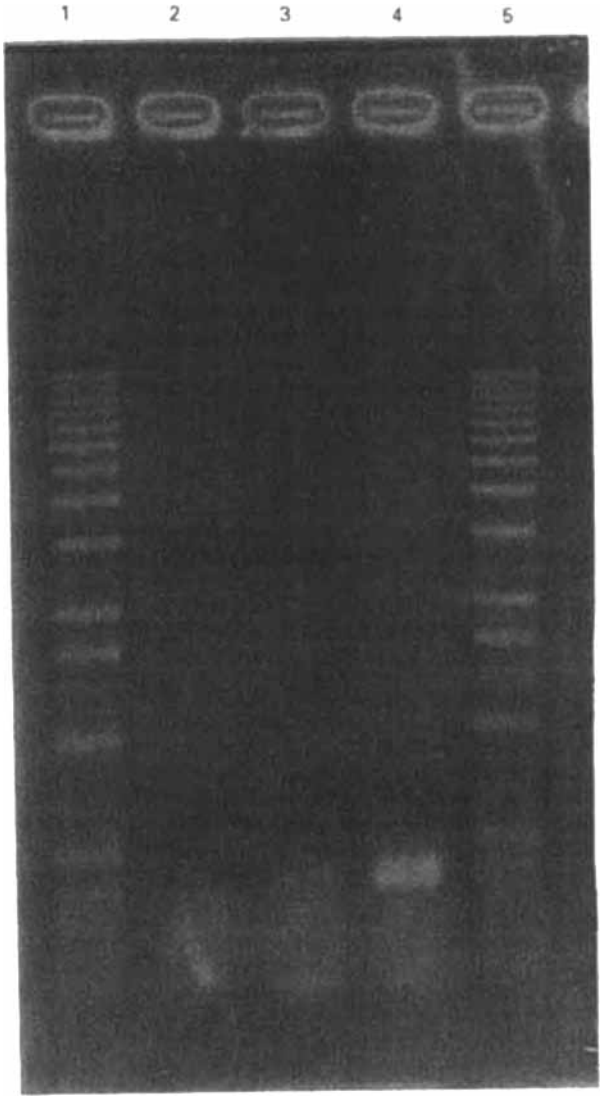

Fig. 4. Reverse transcriptase-polymerase chain reaction (RT-PCR) products amplified with the hGH-V-specific primers in mammary gland fibroblasts (2), T lymphocytes (3) and trophoblastic cells (4). Tracks 1 and 5: 1-kb DNA ladder molecular weight marker.

only the more abundant hGH-N transcript is amplified in these low specificity conditions.

During human pregnancy, pituitary hGH-N is suppressed, while hGH-V is synthesized in the placenta [13,14]. We have demonstrated that the hGH-V transcript is also expressed in mononuclear cells during this period. So, human lymphoid cells represent an extra-placentary site for expressing hGH-V during pregnancy.

Surprisingly, hGH-N transcript is also expressed during pregnancy, although the hGH-N levels are undetectable in maternal blood. Perhaps growth hormone is still synthesized in human mononuclear cells during pregnancy, and binds to the GH receptor in the same cells, thus acting in an autocrine manner, as we demonstrated recently [22].

The transcription factor *PIT-1* has recently been detected by ISH and IHC in human lymphoid [10] and placental [11] cells. *PIT-1* has been shown to bind to specific sequences at the 5′ flanking regions of the GH/CS placental genes. In addition, the hGH-V promoter can be activated *in vitro* by *PIT-1* [23]. These results raise the possibility that this factor may play a role in the regulation of the hGH-V gene in both lymphoid and placental cells. However, because hGH-V transcript is also expressed in PBMC from a woman who lacks normally processed *PIT-1*, this factor may thus not be involved in hGH-V gene regulation in human mononuclear cells. In contrast, the hypothesis that *PIT-1* may control hGH-N expression in human lymphoid cells [10] was confirmed by our results, since hGH-N transcripts were not expressed in lymphocytes of the *PIT-1*-deficient woman.

Our data demonstrate, finally, that the hGH-V transcript is not only produced during pregnancy, but also in lymphocytes from men and non-pregnant women. As the placental growth hormone is synthesized in cells of the immune system, it is conceivable that this hormone plays a role in its regulation.

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REFERENCES

1 Barsh GS, Seeburg PH, Gelinas RE The human growth hormone gene family: structure and evolution of the chromosomal locus. Nucl Ac Res 1983; **11**:3939–58.

2 Hirt H, Kimelman J, Birnbaum *et al.* The human growth hormone gene locus: structure, evolution, and allelic variations. DNA 1987; **6**:59–70.

3 Chen EY, Liao YC, Smith DH, Barrera-Saldana HA, Gelinas RE, Seeburg PH. The human growth hormone locus nucleotidic sequence, biology, and evolution. Genomics 1989; **4**:479–97.
4 Wu H, Devi R, Malarkey WB. Localization of growth hormone messenger ribonucleic acid in the human immune system—a clinical research center study. J Clin Endocrinol Metab 1996; **81**:1278–82.
5 Weigent DA, Baxter JB, Wear WE, Smith LR, Bost KL, Blalock JE. Production of immunoreactive growth hormone by mononuclear leukocytes. FASEB J 1988; **2**:2812–8.
6 Kelley KW. Growth hormone, lymphocytes and macrophages. Biochem Pharmacol 1989; **38**:705–13.
7 Palmetshofer A, Zechner D, Luger TA, Barta A. Splicing variants of the human growth hormone mRNA: detection in pituitary, mononuclear cells and dermal fibroblasts. Mol Cell Endocrinol 1995; **113**:225–34.
8 Bodner M, Castrillo JL, Theill LE, Deerinck T, Ellisman M, Karin M. The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. Cell 1988; **55**:505–18.
9 Ingraham HA, Chen R, Mangalam HJ *et al.* A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell 1988; **55**:519–29.
10 Delhase M, Vergani P, Malur A, Hooghe-Peters EL, Hooghe RJ. The transcription factor Pit-1/GHF-1 is expressed in hemopoietic and lymphoid tissues. Eur J Immunol 1993; **23**:951–5.
11 Bamberger AM, Bamberger CM, Pu LP, Puy LA, Peng Loh Y, Asa SL. Expression of PIT-1 messenger ribonucleic acid and protein in the human placenta. J Clin Endocrinol Metab 1995; **80**:2021–6.
12 Nachtigal MW, Nickel BE, Cattini PA. Pituitary-specific repression of placental members of the human growth hormone gene family. J Biol Chem 1993; **268**:8473–9.
13 Hennen G, Frankenne F, Pirens *et al.* New chorionic GH-like antigen revealed by monoclonal antibody radioimmunoassay (letter). Lancet 1985; **1**:399.
14 Hennen G, Frankenne F, Closset J, Gomez F, Smal J, El Khayat N. Physiology of the growth hormone in pregnancy and discovery of a human placental growth hormone (French). Bull Acad R Belgique 1986; **141**:111–9.
15 Frankenne F, Scippo ML, Van Beeumen J, Igout A, Hennen G. Identification of placental human growth hormone as the growth hormone-V gene expression product. J Clin Endocrinol Metab 1990; **71**:15–18.
16 Seeburg PH. The human growth hormone gene family: nucleotide sequences show recent divergence and predict a new polypeptide hormone. DNA 1982; **1**:239–49.
17 Scippo ML, Frankenne F, Hooghe-Peters EL, Igout A, Velkeniers B, Hennen G. Syncytiotrophoblastic localization of the human growth hormone variant mRNA in the placenta. Mol Cell Endocrinol 1993; **92**:R7–R13.
18 Pfaffle RW, DiMattia GE, Parks JS *et al.* Mutation of the POU-specific domain of Pit-1 hypopituitarism without pituitary hypoplasia. Science 1992; **257**:1118–21.
19 James-Yarish M, Bradley WG, Emmanuel PJ, Good RA, Noorbibi KD. Detection of cell specific cluster determinant expression by reverse transcriptase polymerase chain reaction. J Immunol Methods 1994; **169**:73–82.
20 Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977; **74**:5463–7.
21 Maggiano N, Piantelli M, Ricci R, Larocca LM, Capelli A, Ranelletti FO. Detection of growth hormone-producing cells in human thymus by immunohistochemistry and non-radioactive *in situ* hybridization. J Histochem Cytochem 1994; **42**:1349–54.
22 Thellin O, Coumans B, Zorzi W *et al.* Expression of growth hormone receptors by human lymphoid cells in the tonsilar lymph follicle. 12th International Conference on Lymphoid Tissues and Germinal Centers in Immune Reactions. Graz, Austria: Poster and Abstract Book, 1996.
23 Cattini PA, Eberhardt NL. Regulated expression of chimaeric genes containing the 5′-flanking regions of human growth hormone-related genes in transiently transfected rat anterior pituitary tumor cells. Nucl Acid Res 1987; **15**:1297–309.