Expression profile of active genes in the human pituitary gland

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Abstract

To characterize transcripts abundantly expressed in the human pituitary gland in general as well as to isolate novel transcripts expressed specifically in this gland, we generated an expression profile of the active genes transcribed in it. A total of 1015 randomly collected 3' expressed sequence tags (ESTs) (gene signatures, GSs) were grouped into 527 GS species. The results showed the relative expression levels of genes in the pituitary gland. The genes comprising more than 1% of total mRNA were prolactin, growth hormone and chromogranin B genes. When known genes were categorized, the genes for pituitary hormones were the most actively transcribed, followed by the genes for ribosomal proteins, nuclear proteins and secretory granule proteins. Through comparison of this gene expression profile with the BodyMap database containing profiles generated from 63 other human tissues, we obtained 11 genes which appeared to be specifically expressed in the pituitary gland. In addition to the eight known genes, we identified three novel pituitary-specific transcripts which encode putative proteins: pituitary gland specific factor 1a (PGSF1a), PGSF1b and PGSF2. This expression profile method is a novel approach to the isolation of pituitary-specific genes that may have important functions.

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Introduction

The pituitary gland is composed mainly of endocrine cells producing pituitary hormones. These hormones are stored in the secretory granules and are released by regulated exocytosis. The secretory granules also contain processing enzymes and chromogranins, and fuse with the plasma membrane to release hormones and other contents (Arvan & Castle 1998).

The unique functions of the pituitary gland are maintained by the pituitary-specific proteins, such as pituitary hormones and transcription factors. Abnormalities in these genes cause both isolated anterior pituitary hormone deficiencies and combined pituitary hormone deficiencies (Phillips *et al.* 1981, Tatsumi *et al.* 1992, Cushman & Camper 2001). Some modification enzymes, such as GalNAc-4-sulfotransferase, are expressed specifically in the pituitary gland (Okuda *et al.* 2000),

while others, such as prohormone convertase (PC) 1/3, PC2, carboxypeptidase E (CPE) and neuroendocrine protein 7B2 (Iguchi *et al.* 1984) are also expressed in the pancreas and neural tissues (Gorr *et al.* 2001). Abnormalities in CPE and 7B2 cause adrenocorticotropin (ACTH) to be secreted in an unregulated manner (Gorr *et al.* 2001). Autoantibodies against tissue-specific proteins have proven to be useful markers for autoimmune diseases. Although pituitary-specific autoantibodies for lymphocytic hypophysitis have been reported, the corresponding autoantigens have not been identified (Crock 1998, Nishiki *et al.* 2001).

In order to clarify the molecular basis of the tissue specificity of the pituitary gland and to resolve the mechanism of pituitary diseases, isolation of pituitary-specific transcripts is important. In an attempt to identify the molecules expressed specifically in the human pituitary gland, we used a method called gene expression profiling

(Okubo *et al.* 1992). This method involves the construction of a 3'-directed cDNA library that faithfully represents the original composition of the mRNA species, and random sequencing of the cDNA clones to obtain 3' expressed sequence tags (ESTs), each consisting of short nucleotide sequences just upstream of the poly(A) tail to the adjacent GATC (*MboI* site). These 3' ESTs are called gene signatures (GSs), since they are unique to the individual genes (Okubo *et al.* 1992). Expressed genes are identified by their GS species, and the relative abundance of each transcript can be estimated by the frequency of the corresponding GS species in the library. The resulting gene expression profile thus describes quantitative aspects of the genes active in the human pituitary gland. Similar gene expression profiles based on 64 human tissues make up the molecular anatomy database known as the BodyMap database (Hishiki *et al.* 2000, Kawamoto *et al.* 2000). By comparing the expression profiles obtained from various tissues in the BodyMap database, many tissue-specific genes and a disease causing gene have been identified (Okubo *et al.* 1995, Kita *et al.* 1996, Nishida *et al.* 1996, Yokoyama *et al.* 1996, Maeda *et al.* 1997, Nishida *et al.* 1997, Shimizu-Matsumoto *et al.* 1997, Itoh *et al.* 1998). Thus, this method is an effective alternative method for identifying novel genes in addition to other methods, such as functional cloning, similarity cloning, positional cloning and traditional subtraction cloning.

In the present study, we report a list of genes actively expressed in the human pituitary gland; prolactin (PRL) was found to be the most abundantly expressed gene in this tissue sample. By comparing the gene expression profile of the human pituitary gland with the BodyMap database, we identified three novel transcripts that are expressed specifically in the human pituitary gland.

Materials and methods

Construction of the 3'-directed library and sequencing analysis

Poly(A) RNA from the human pituitary gland, obtained from 21 individuals aged 16 to 70 years, was purchased from Clontech (Palo Alto, CA, USA). Construction of the 3'-directed cDNA libraries and transformation into *E. coli* were performed as previously described (Okubo *et al.*

1992). Briefly, cDNA was synthesized using a pUC19 based vector-primer, which was digested by *MboI*, and circularized and transformed into *E. coli*. The insert cDNAs of the randomly isolated transformants were prepared by PCR amplification for the sequence templates. The amplified products were sequenced directly using a dye primer cycle sequencing kit (Perkin-Elmer, Norwalk, CT, USA) and an ABI PRISM 373 Genetic Analyzer (Perkin-Elmer).

The resulting sequences, referred to as GSs, were compared with each other using BLAST (Altschul *et al.* 1994), and clustered. From each cluster, one representative GS was selected and compared with representative sequences from the previously generated clusters. A GS number was assigned to each independent cluster. The representative sequences for the GS clusters (GS species) were searched against the DDBJ/GenBank/EMBL database using BLAST (Altschul *et al.* 1994).

In silico RNA experiments

Frequencies of each GS were compared with those in the BodyMap database. Specific expression of each GS species in the human pituitary gland was described as a pituitary TS ratio according to the following formula:

pituitary TS ratio

$$= \frac{\frac{(\text{frequency of a GS species in the pituitary gland})}{(\text{total number of GSs in the pituitary gland})}}{\frac{(\text{frequency of a GS species in the whole BodyMap database})}{(\text{total number of GSs in the whole BodyMap database})}}$$

Screening of the cDNA library

The human pituitary gland cDNA library, which was constructed with a ZAP-cDNA Synthesis kit (Stratagene, La Jolla, CA, USA) using a Uni-ZAP XR vector, was screened using standard techniques. The alkaline phosphatase-labeled probes for the GS sequences were synthesized by an Alphos direct system (Amersham Pharmacia, Arlington Heights, IL, USA). After the phage plaques were transferred to a nylon membrane (Hybond N+, Amersham Pharmacia) according to the standard alkali transfer method, the membranes were baked at 80 °C for 2 h. Then the membranes were hybridized with each probe at

Table 1 Nucleotide sequence of primers used for expression analysis

	Sense primer	Antisense primer
GS		
GS9544 (PGSF1)	GACAGGACGGCGAAGTTTGA	TCCTTATTGCCTCACCATTTC
GS9589 (PGSF2) 3'	AGGACTTATATGCCAGGCAC	TGTTATTTTATGTGTGGAA
GS9589 (PGSF2) 5'	CGCTCTAGAACTAGTGGATC	TTTGTGTCTTATCCTTCAGCAGCAG
GS9573 (KIAA0512)	TGCTGAGTTCAGGTTAGAGGCC	GTTACTGCTTTTCCACTTGCTC

PGSF, pituitary gland specific factor.

Table 2 Cellular localization of GSs in the expression profile of the pituitary gland

	GS species	GSs	Frequency (%)
Localization			
I. Known genes	263	658	64.8
Extracellular proteins	16	238	23.4
Secretory proteins	12	234	23.1
Pituitary hormones	5	219	21.6
Other secretory proteins	7	14	1.4
Matrix proteins	4	5	0.5
Plasma membrane proteins	12	20	2.0
Intracellular proteins	172	301	29.8
Secretory granule proteins	(9–10) ^a	(25–34) ^a	(2.5–3.4) ^a
ER-Golgi proteins	18	21	2.1
Nuclear proteins	32	49	4.8
Mitochondrial proteins	17	23	2.3
Lysosomal proteins	15	17	1.7
Cytoplasmic proteins	80	158	15.6
Ribosomal proteins	50	100	9.9
Signal transducers	(15–16) ^a	(17–26) ^a	(1.7–2.6) ^a
Cytoskeletal proteins	8	17	1.7
Others	6	15	1.5
Uncharacterized	63	98	9.7
II. Novel genes	264	357	35.2
Total	527	1015	100.0

Sequences of the 1015 independent clones from the 3'-directed library were grouped into 527 GS species according to the sequence identity. These GS species were divided into those that matched known genes and those that did not. Species in the former group were categorized according to the cellular localization of their products and then subcategorized. The numbers in parenthesis show the numbers of GS species and the number of GSs in subcategories. ^aVariation caused by GNAS1 gene corresponding to GS1007 (see Table 3). ER, endoplasmic reticulum.

55 °C overnight. After hybridization, the membranes were washed with a primary wash buffer (2 M urea, 0.1% SDS, 50 mM Na phosphate pH 7.0, 150 mM NaCl, 1 mM MgCl₂, with 0.2% blocking reagent) at 55 °C for 10 min twice and then washed with a secondary wash buffer (50 mM Tris base, 100 mM NaCl, 2 mM MgCl₂) at room temperature for 5 min twice. The positive signals were detected with CDP-Star detection reagent (Amersham Pharmacia) by exposure to diagnostic film (RX-U, Fuji Film, Tokyo, Japan) with an

intensifier screen at room temperature for 1 h or more. Double-strand plasmid DNAs of the positive clones were rescued by following the manufacturer's procedure. DNA sequencing was performed with an ABI PRISM 373 Genetic Analyzer (Perkin-Elmer).

Rapid amplification of 5'-cDNA ends (5'-RACE)

A Marathon cDNA Amplification kit (Clontech) was used to determine the 5' ends of GS9544,

Table 3 Recurrently appearing known genes and expression specificity in the pituitary gland. Abundance of the GS species in other tissues is also shown

Localization	Definition	Pituitary							Acc. no.
		Appearance	TS ratio	All	Neural	Blood	Connective	Epithelial	
Extracellular proteins Secretory proteins Pituitary hormones	PRL	181	85.53	185	4	0	0	0	NM_000948
	GH	20	87.42	20	0	0	0	0	NM_000515
	LH beta	7	87.42	7	0	0	0	0	NM_000737
	glycoprotein alpha	6	87.42	6	0	0	0	0	NM_000735
	FSH beta	5	87.42	5	0	0	0	0	NM_000510
Other secretory proteins	B2M	4	0.74	471	20	151	33	218	NM_004048
	SEPP1	4	6.60	53	6	0	7	29	NM_005410
Plasma membrane proteins	HLA-C	4	2.59	135	7	41	12	55	NM_002117
	TNFRSF12	3	23.84	11	3	2	0	2	NM_003790
Intracellular proteins Secretory granule proteins	CHGB	12	61.71	17	4	0	1	0	NM_001819
	(NESP55)	(9)	(6.15)	(128)	(32)	(8)	(36)	(29)	(NM_016592)
	CPE	3	5.96	44	30	0	8	0	NM_001873
	7B2	3	26.23	10	7	0	0	0	NM_003020
Nuclear proteins	RB1	5	33.62	13	1	3	3	1	NM_000321
	CDC2L1	3	0.66	399	8	27	70	223	NM_001787
	TB4X	3	0.81	322	72	106	40	64	NM_021109
	DOC-1	3	4.86	54	21	3	4	13	NM_00464
Mitochondrial proteins	ATP5	3	2.62	100	19	11	39	20	NM_001685

Continued opposite.

Table 3 Continued

Localization	Definition	Pituitary		All	Neural	Blood	Connective	Epithelial	Complex	Acc. no.
		Appearance	TS ratio							
Intracellular proteins cont. Cytoplasmic proteins Ribosomal proteins Component proteins	RPS8	6	1-10	477	60	85	78	181	67	NM_001012
	RPS24	5	2-01	218	45	36	39	73	20	NM_001026
	RPS20	5	2-99	146	17	32	27	49	16	NM_001023
	RSP27A	4	2-13	164	16	43	21	56	24	NM_002954
	RPL34	3	1-33	197	39	22	39	73	21	NM_000995
Proteins related to peptide synthesis	TPT1	6	0-91	577	72	79	113	232	75	NM_003295
	NACA	6	2-19	239	58	44	43	63	25	NM_005594
	EEF1A1	5	0-67	652	151	145	76	208	67	NM_001402
	MAT2A	4	4-32	81	20	16	12	18	11	NM_005911
	EIF4G2	3	3-80	69	3	22	14	27	3	NM_001418
Signal transducers	(Gs-alpha)	(9)	(6-15)	(128)	(32)	(8)	(36)	(29)	(14)	(NM_000516)
	(XL-alpha-s)	(9)	(6-15)	(128)	(32)	(8)	(36)	(29)	(14)	(AJ251790)
	RALBP1	4	26-90	13	0	2	4	3	0	NM_006788
	PBP	3	3-32	79	41	3	8	17	7	NM_002567
	ANXA6	3	3-50	75	37	4	24	2	5	NM_001155
Cytoskeletal proteins	PRKACA	3	87-42	3	0	0	0	0	0	NM_002730
	PIN	6	6-81	77	29	4	8	29	1	NM_003746
	ACTG1	3	2-02	130	32	12	30	30	23	NM_001614
	ubiquitin C	4	1-75	200	28	37	49	58	24	M26880
	PSMB4	3	5-96	44	10	5	9	13	4	NM_002796
Others	PSMB5	3	9-71	27	5	2	7	5	5	NM_002797
	PUM1	3	9-37	28	3	1	5	10	6	NM_014676
	MLN51	3	12-49	21	4	4	8	0	2	NM_007359
	BM-002	3	14-57	18	5	2	4	2	2	NM_016617
	galanin-related peptide	3	23-84	11	0	1	0	7	0	NM_015973
Uncharacterized	KIAA0343	3	29-14	9	6	0	0	0	0	AB002341
	KIAA0512	3	65-57	4	1	0	0	0	0	NM_014782
Total appearance of each profile in the BodyMap database		1015		88732	22537	7112	19113	28917	10299	

Known genes appearing three times or more are listed according to the cellular localizations of their products with their frequencies in other tissues. Accession numbers (Acc. no.) are shown when matched to DDBJ/GenBank/EMBL databases. Numbers represent the frequency of the GS species analyzed. The abundance of the GS species in other tissues (Kawamoto *et al.*, 2000) is also shown for comparison. Total numbers of analyzed clones are indicated at the bottom. The proteins and numbers in parentheses show variations caused by the GNAS1 gene. The GNAS1 gene has three promoters, each encoding different proteins: one secretory granule protein (NESP55) and two signal transducers (Gs-alpha, XL-alpha-s).

Table 4 GS species expressed uniquely in the pituitary gland

GS species	Definition	Appearance in the pituitary	Expression analysis
9703	PRL	181	
9496	GH	20	
7687	CHGB	12	
9512	glycoprotein alpha	7	
9535	LH beta	6	
9544	PGSF1a ^a	6	ps
	PGSF1b ^a		ps
9504	FSH beta	5	
9651		4	
9589	PGSF2 ^a	3	ps
9606	PRKACA	3	
9573	KIAA0512	3	ppd

The GS species listed appeared frequently and specifically in the pituitary gland. Numbers represent the frequencies of the GS species analyzed. ^aDefinitions identified in this study; ps: pituitary-specific; ppd: pituitary-predominant.

GS9589 and GS9573 according to the manufacturer’s protocol. One microgram of poly(A) RNA from the human pituitary gland was reverse transcribed using a ReverTraAce (Toyobo, Osaka, Japan) with random hexamers. Double strand DNA synthesis and adapter (5’-CTAATACGA CTCACTATAGGGCTCGAGCGGCCGCCCCG GGCAGGT-3’, 3’-H2N-CCCGTCCA-PO4–5’) ligation were carried out according to the manufacturer’s protocol (Clontech). The PCR was carried out with an adapter primer 1 (AP1, 5’-CCATCCTAATACGACTCACTATAGGGC-3’) and gene specific primers for GS9544 (5’-CTCAGACCACCCCTCCTCCCACGCA-3’), GS9589 (5’-TGTCTCCTTCCAGTAGCAGCAC CGGTAAA-3’) and GS9573 (5’-AGACAGACC CTCCAAAGATTCA-3’). The PCR products were sequenced with AP1 or gene specific primers.

Northern hybridization

Poly(A) RNA from the human pituitary gland and liver (Clontech) were fractionated on a formaldehyde-agarose gel and transferred to a Hybond N+ membrane according to the manufacturer’s recommendation. This membrane or Northern LIGHT Human Multiple mRNA Blot I (Life Technologies, Gaithersburg, MD, USA) containing human heart, brain, liver, pancreas, skeletal muscle and lung mRNA, was hybridized with the probes based on the putative open reading frame (ORF)

region of the screened cDNA clones labeled with the enhanced chemiluminescence (ECL) direct system (Amersham Pharmacia). The membranes were hybridized and washed according to the protocol provided with the ECL direct system. The membranes were exposed to RX-U diagnostic film with an intensifier screen at room temperature for 1 h or more. The probes were obtained by PCR using the primers listed in Table 1.

Results and discussion

Gene expression profile of the human pituitary gland

To understand the molecular basis of the synthesis and secretion of pituitary hormones, we constructed a gene expression profile of the human pituitary gland. This profile shows the relative activities of the genes in maintaining the tissue specificity. From the 3’-directed cDNA library of the human pituitary gland, 1015 GSs were obtained (Table 2). These sequences were compared and found to make up 527 independent GS species. Among the 527 GS species, 263 GS species represented by 658 GSs were derived from genes registered in the DDBJ/GenBank/EMBL database and 264 GS species represented by 357 GSs were derived from novel genes.

In Table 3, the frequencies of the recurrently appearing genes in the human pituitary gland are

Figure 1 Expression and structure of PGSF1s. (A) Schematic structure of PGSF1s. (Top) Structure of the PGSF1 gene. (Below) cDNA structure of PGSF1s. The tall and short rectangles in the cDNA structure represent the putative open reading frame (ORF) and non-coding sequence respectively. Shaded rectangles indicate the conserved region with soybean nodulin families (see D). AW583046 is alternatively spliced at exon 2 and has a 53-bp insertion causing a frame shift in the putative ORF. (B) Amino acid sequence alignment of PGSF1s. (C) Multi-tissue Northern hybridization for PGSF1s and beta-actin. (D) Amino acid sequence alignment of PGSF1s and soybean nodulin families. The amino acids identical with PGSF1s are highlighted. NO2B_SOYBN: soybean nodulin 26B; NO23_SOYBN: soybean nodulin 23; NO44_SOYBN: soybean nodulin 44.

compared with those in 63 other tissues in the BodyMap database, and their tissue specificities for the pituitary gland are indicated by pituitary TS ratios. To characterize the active genes in the human pituitary gland, we categorized these GS species mainly according to their cellular localization with subclassification, together with their frequencies in the pituitary gland, as summarized in Table 2. The PRL gene was most actively expressed, followed by the growth hormone (GH) gene. All of the genes for pituitary hormones listed (PRL, GH, luteinizing hormone (LH) beta, glycoprotein alpha, follicle-stimulating hormone (FSH) beta) were expressed specifically in the pituitary gland and were most frequently expressed in the human pituitary gland (23.4%) (Tables 2 and 3).

The third most abundant transcript was that for the chromogranin B (CHGB) gene. CHGB is a secretory granule protein related to the regulated secretory pathway. Two other secretory granule protein genes, CPE and 7B2, were predominantly expressed in the pituitary gland and neural tissues concordant with their known characters. These secretory granule protein genes were included in the fourth most frequently expressed group (2.3–3.4%) (Tables 2 and 3).

With respect to genes specific to both the pituitary gland and neural tissues, all the previously well characterized genes are either pituitary hormones or secretory granule proteins, with the exception of the cAMP-dependent catalytic alpha subunit (PRKACA); therefore, other little-characterized genes expressed predominantly in

Figure 2 Expression and structure of PGSF2. (A) Schematic structure of PGSF2 and IGDC1. The tall and short rectangles represent the putative ORF and non-coding sequence respectively. Each hatched pattern indicates a different nucleotide sequence. Signal peptide, Ig-like domains, transmembrane domains (TMs), and probes used for the Northern hybridization are indicated. (B) Amino acid sequence alignment of PGSF2, IGDC1 and InhBP-s. The identical amino acids are highlighted. (C) Multi-tissue Northern hybridization with the 3' probe unique to PGSF2 and the 5' probe.

the pituitary gland and neural tissues, such as KIAA0343 and KIAA0512, may well be related to the regulated secretory pathway.

The fourth most abundant transcript was that for the GNAS1 gene. The gene encodes three proteins, Gs- α , an extra large G protein (XL- α -s) and NESP55, transcribed from three different promoters (Kozasa *et al.* 1988, Kehlenbach *et al.* 1994, Ischia *et al.* 1997). Activities for Gs- α have been detected in various tissues, although expressions of XL- α -s and NESP55 are limited to neuroendocrine tissues, and inactive or active mutations cause endocrinological abnormalities, e.g. Albright hereditary osteodystrophy (Patten *et al.* 1990, Weinstein *et al.* 1992) and McCune-Albright syndrome (Schwindinger *et al.* 1992). In agreement with the activities for Gs- α , their expressions as a whole were not limited to the pituitary gland

and neural tissues in the BodyMap database (Table 3).

Most of the other recurrently appearing genes were not expressed specifically in the pituitary gland or tissue groups, and thus were considered housekeeping genes. Ribosomal protein genes (9.9%) and nuclear protein genes (4.8%) were frequently expressed. Some of these genes may serve as better internal references for comparing expression levels among tissues, since beta-actin and glyceraldehyde-3-phosphate dehydrogenase, two commonly used references, were expressed infrequently and unequally among tissues in the BodyMap database.

Hu *et al.* (2000) also recently reported an expression profile of the human pituitary gland. Their profile was based on seven thousand 5' ESTs from cDNAs, one third of which contained the first

Figure 3 Expression of KIAA0512. Multi-tissue Northern hybridization for KIAA0512.

ATG. They compared it with expression profiles of the hypothalamus and adrenal gland. In agreement with our observation, GH and PRL were expressed in over 1% of the total ESTs analyzed. Other recurrently appearing, known genes in Table 3 were also observed in their profile, but not the genes SEPP1, NACA, EIF4G2, TNFRSF12, PBP, ANXA6, PRKACA or several of the uncharacterized genes, such as Pumilio homolog 1, MLN51, KIAA0343 and KIAA0512. Among the pituitary hormones, thyrotropin (TSH) beta and pro-opiomelanocortin (POMC) did not appear in our profile, PRL was 10 times more abundant in our profile, and FSH beta did not appear in their profile. To assess the qualities of these profiles, comparisons of well-characterized pituitary hormones would be of use.

Because TSH beta cDNA lacks an *MboI* site (Tatsumi *et al.* 1988), it does not appear as a GS. The extremely high expression of PRL in our profile was not due to specific endocrinological conditions

for the following reason. The high level of PRL in our samples is unlikely to be caused by overexpression of this mRNA in one or two of the subjects, even if suffering from a prolactinoma or another metabolic disturbance. The pituitary mRNA used in our study was derived from 21 individuals, each contributing approximately the same amount of RNA. Hence high expression of PRL in a small number of individuals would be diluted by the RNA from the other individuals in the total sample. The absence of POMC may reflect a low content of ACTH in the anterior pituitary gland (Matsuyama *et al.* 1971, Moldow & Yalow 1978, Ishikawa *et al.* 1987). In agreement with our profile, previously measured expression levels for FSH beta and LH beta showed similar quantitative mRNA measurements (Dalkin *et al.* 1989).

Our method has two major advantages over other gene expression profiling methods. First, compared with the gene expression profiling methods obtained from a single tissue or from several tissues, the BodyMap database of 63 other human tissues is currently available, and comparison of the pituitary-gland expression profile composed using the same method as used for the BodyMap database should readily reveal candidates for tissue-specific transcripts. Secondly, the length of most GSs was under 1 kb and over 30 bp, allowing us to avoid artifacts in library construction while providing probes for further analyses. Indeed, this method has been successful in isolating tissue-specific genes in various other tissues (Okubo *et al.* 1995, Kita *et al.* 1996, Nishida *et al.* 1996, Yokoyama *et al.* 1996, Maeda *et al.* 1997, Shimizu-Matsumoto *et al.* 1997, Itoh *et al.* 1998). We therefore concluded that our profile is reliable, although verification through future analyses of other gene expression profiles obtained from other methods or other primates will be needed.

Human pituitary gland-specific gene expression profile

To identify genes that may play important roles in the pituitary gland, we selected GS species expressed recurrently and specifically in the pituitary gland (Table 4). Eight of the 11 GS species were from known genes. Five of these (PRL, GH, LH beta, glycoprotein alpha and FSH beta) were anterior pituitary hormones, two (CHGB and PRKACA) were specific to the endocrine tissues,

and the remaining one (KIAA0512) has not been characterized.

By screening a human pituitary gland cDNA library with the GS sequences, we obtained cDNA clones for GS9544, GS9589, and GS9573 (Table 4). GS9651 represented a unique sequence in the human genome draft sequence (International Human Genome Sequencing Consortium 2001, Venter *et al.* 2001), but screenings for cDNA clones were unsuccessful. Northern hybridization showed that GS9544 and GS9589 were pituitary-specific and GS9573 (KIAA0512) was pituitary-predominant.

Characterization of novel pituitary-specific transcripts

For GS9544, two splice variants of 0.7 kb and 0.9 kb were isolated from the human pituitary cDNA library. They encoded two putative proteins, pituitary gland specific factor 1a (PGSF1a) of 128 amino acids (aa) and PGSF1b of 91 aa (Fig. 1A,B). A BLAST search against the human genome draft sequence localized PGSF1s to chromosome 19 (accession no. (ACC#) NT_011255) (International Human Genome Sequencing Consortium 2001). In the EST database, a short partial human cDNA from the islets of Langerhans cDNA library represented another splice variant (ACC# AW583046) (Fig. 1A). The cDNA probe for the two PGSF1s (PGSF1a and PGSF1b) hybridized strongly with pituitary transcripts of approximately 1 kb and weakly with pancreas transcripts of approximately 1.4 kb by multi-tissue Northern hybridization (Fig. 1C). These are presumably endocrine-specific transcripts expressed most specifically in the pituitary gland. They may be proteins related to secretory granules, one of the most abundant members in the gene expression profile of the human pituitary gland. PGSF1s have a homology of 33% within 51 aa with the soybean nodulin 26B-carboxy terminal domain (Jacobs *et al.* 1987) (Fig. 1D). Nodulins are proteins associated with the peribacteroid membrane of soybean root nodules, and the carboxyl terminal domain is in the cytoplasmic fraction of the nodule. Although the functions of this domain have not been identified (Panter *et al.* 2000), the fact that this domain is conserved between humans and soybeans may well indicate that it has a novel function.

Full-length cDNA for GS9589 was 2 kb in length and encoded a putative protein, PGSF2, of 242 aa (Fig. 2A). A BLAST search revealed that the 5' 0.8 kb of this cDNA was identical with exons 1–5 of the immunoglobulin (Ig)-like domain containing 1 (IGDC1) (Frattini *et al.* 1998) and was a human counterpart for the recently identified rat inhibin binding protein short isoform (InhBP-S) (Bernard & Woodruff 2001) (Fig. 2B). The IGDC1 protein consists of an N-terminal signal peptide, twelve Ig-like domains and a C-terminal transmembrane domain (Fig. 2A). PGSF2 is a splice variant of IGDC1, and is probably secreted. A 3' probe unique to PGSF2 hybridized with pituitary-specific transcripts of approximately 2 kb by multi-tissue Northern hybridization (Fig. 2C). While the 3' probe hybridized with pituitary-specific transcripts, a 5' probe hybridized with a 2 kb band and a longer band encoding PGSF2 and IGDC1 respectively (Fig. 2C). The InhBP long isoform (InhBP-L), an ortholog of IGDC1, was identified as an inhibin A-specific receptor (Bernard & Woodruff 2001). Because transcripts for InhBP-S and InhBP-L have been detected specifically in the rat pituitary gland and testis (Bernard & Woodruff 2001) and transcripts for IGDC1 have been detected in the human testis and prostate (Frattini *et al.* 1998), both PGSF2/InhBP-S and IGDC1/InhBP-L are expressed specifically in tissues with secretory granules in humans and rats. A BLAST search against the human genome draft sequence localized PGSF2 to the chromosome X q25–26.2 region. The gene for X-linked recessive panhypopituitary dwarfism lies in this region (Lagerstrom-Fermer *et al.* 1997). If PGSF2 is responsible for this disease, it would be involved in the development or functions of the pituitary gland.

Full-length cDNA for GS9573 matched the cDNA encoding KIAA0512, except that the former cDNA had an insertion of 44 bp at the 5' noncoding region. The KIAA0512 protein is a putative protein of 632 aa, possesses an armadillo/beta-catenin-like repeat motif, and has homology of 61% within 307 aa with a putative protein (ACC# AF211175). By multi-tissue Northern hybridization, KIAA0512 was expressed as 3.2 kb and 4.5 kb transcripts in all tissues examined, but at different levels, with the highest expression occurring in the pituitary gland and heart (Fig. 3). Because the putative protein (ACC# AF211175) bound to non-erythroid alpha-spectrin *in vivo* (a

note in the GenBank database) and the spectrin families are structural proteins that are located adjacent to membrane or Golgi body, the KIAA0512 protein may form a complex with spectrin and other spectrin binding proteins (for example, ankyrin, actin or band 4.1), and may act as a transport component for the vesicles.

Consequently, the expression profile of the human pituitary gland and its comparison with the expression profiles of other tissues provided an alternative way of isolating genes expressed specifically in the human pituitary gland. Expanding the numbers of GSs will further enable us to identify less abundant pituitary-specific genes. In the present study, we obtained three transcripts from two novel GSs expressed specifically in the human pituitary gland. With the help of recently developed oligonucleotide-based, PCR-mediated multi-tissue quantitative expression analyses (Heid *et al.* 1996, Kato 1997, Hall *et al.* 1998, Kawamoto *et al.* 1999), which are more rapid and specific than Northern hybridization, we will be able to select pituitary-specific genes more easily in the future. These genes will serve as promising candidates for pituitary diseases, such as congenital pituitary abnormalities and autoantigens for lymphocytic hypophysitis.

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