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Research Articles

Proteomic Analysis of the Human Anterior Pituitary Gland

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Abstract

The pituitary function is regulated by a complex system involving the hypothalamus and biological networks within the pituitary. Although the hormones secreted from the pituitary have been well studied, comprehensive analyses of the pituitary proteome are limited. Pituitary proteomics is a field of postgenomic research that is crucial to understand human health and pituitary diseases. In this context, we report here a systematic proteomic

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profiling of human anterior pituitary gland (adenohypophysis) using high-resolution Fourier transform mass spectrometry. A total of 2164 proteins were identified in this study, of which 105 proteins were identified for the first time compared with high-throughput proteomic-based studies from human pituitary glands. In addition, we identified 480 proteins with secretory potential and 187 N-terminally acetylated proteins. These are the first region-specific data that could serve as a vital resource for further investigations on the physiological role of the human anterior pituitary glands and the proteins secreted by them. We anticipate that the identification of previously unknown proteins in the present study will accelerate biomedical research to decipher their role in functioning of the human anterior pituitary gland and associated human diseases.

Keywords: proteomics, anterior pituitary, diagnostics, biomarkers, ophthalmology, endocrinology

Introduction

The pituitary gland, often referred as the master endocrine gland, regulates various biological processes, including growth, water balance, reproductive functions, secretion and release of milk, response to stress, pigmentation, basal metabolism, and various other physiological activities in the body to maintain homeostasis. These functions are mediated through hormones secreted from adenohypophysis and neurohypophysis, which are two separate anatomical regions of the pituitary gland with distinct developmental origin, morphology, histology, ultrastructure, and physiological roles (Fig. 1A).

Anterior pituitary gland is the glandular portion of the pituitary secreting key hormones, such as growth hormone (GH), prolactin (PRL), luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and adrenocorticotropic hormone (ACTH). Posterior pituitary gland comprised specialized glial cells called pituicytes and axons of neurons arising from the hypothalamus releasing oxytocin and vasopressin.

The functions of the pituitary gland are classically regulated by the hypothalamus through stimulatory (e.g., gonadotropin-releasing hormone, thyrotropin-releasing hormone, and corticotropin-releasing hormone) and inhibitory factors (somatostatin and dopamine). In addition, neurotransmitters (serotonin, gamma-amino butyric acid, and vasoactive intestinal peptide) as well as cytokines (interleukin [IL]-1, IL-2, and IL-6) and neuropeptides have been found to regulate the synthesis and release of hormones from pituitary glands (Jorgensen et al., 2003; Karanth and McCann, 1991; Yasin et al., 1994).

Similarly, molecules expressed in the nonendocrine folliculostellate cells are also involved in the regulation of hormonal secretion from the anterior pituitary gland. For instance, S100, basic fibroblast growth factor, and IL-6 proteins secreted from folliculostellate cells stimulate the secretion of anterior pituitary gland hormones in a paracrine manner (Allaerts and Vankelecom, 2005).

Mass spectrometry (MS)-based studies using different fractionation techniques have been carried out to identify proteins from human pituitary glands. In one such study, 7596 proteins were shown to be expressed from human pituitary gland through MS-based analysis (Liu et al., 2017). In another study, 1660 proteins were reported using multidimensional separation by liquid chromatography (Liu et al., 2011). Furthermore, 1449 proteins were reported using multiple gel-based technologies (Zhao et al., 2005). Similarly, using a protein array containing 1005 antibodies, 316 proteins were identified from pituitary glands (Ribeiro-Oliveira et al., 2008).

Although the hormones secreted from the pituitary have been well studied, comprehensive analyses of the pituitary proteome are limited. Pituitary proteomics is a field of postgenomic research that is crucial to understand human health and pituitary diseases. In this context, we report here a systematic proteomic profiling of human anterior pituitary gland (adenohypophysis) using high-resolution Fourier transform MS.

Materials and Methods

Sample collection

The study was approved by the Scientific Ethics Committee of National Institute of Mental Health and Neuro Sciences (NIMHANS, Bangalore, India). Subsequently, pituitary glands were obtained from the Human Brain Tissue Repository (National Research Facility), NIMHANS, Bangalore, India. An informed written consent had been obtained from close relatives after having ascertained that no objection has been expressed by the deceased to any of his/her organs being used after his/her death for research/educational purposes. The Human Brain Tissue Repository has been authorized to collect the tissues during autopsy and to store them.

Anterior pituitary gland tissues were collected from three male victims of road traffic accidents. After death, the bodies were immediately transported and maintained at 4°C in the mortuary. The tissues were harvested between 9 and 17 h after death (Supplementary Table S1). Anterior pituitary gland ($n = 3$) tissues were dissected from human pituitary glands at the time of autopsy. Histological examination of a segment of pituitary glands was carried out to distinguish anterior pituitary gland to confirm the absence of adenomas and inflammation (Supplementary Fig. S1).

Protein extraction and normalization

Anterior pituitary gland tissues were homogenized individually in liquid nitrogen using mortar and pestle. Protein from these tissues was extracted in urea lysis buffer (9 M urea, 20 mM HEPES, 1 mM sodium orthovanadate, 1 mM β-glycerophosphate, and 2.5 mM sodium pyrophosphate). The lysates were subjected to centrifugation at 13,000 rpm for 15 min at 4°C. Supernatant from each tissue lysate was collected and protein estimation was carried out by bicinchoninic acid assays (Pierce, USA). Protein samples were normalized based on protein amounts, as verified on 10% sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE).

Five hundred micrograms of protein from each of the three tissue samples was pooled to get a 1.5 mg protein pool from

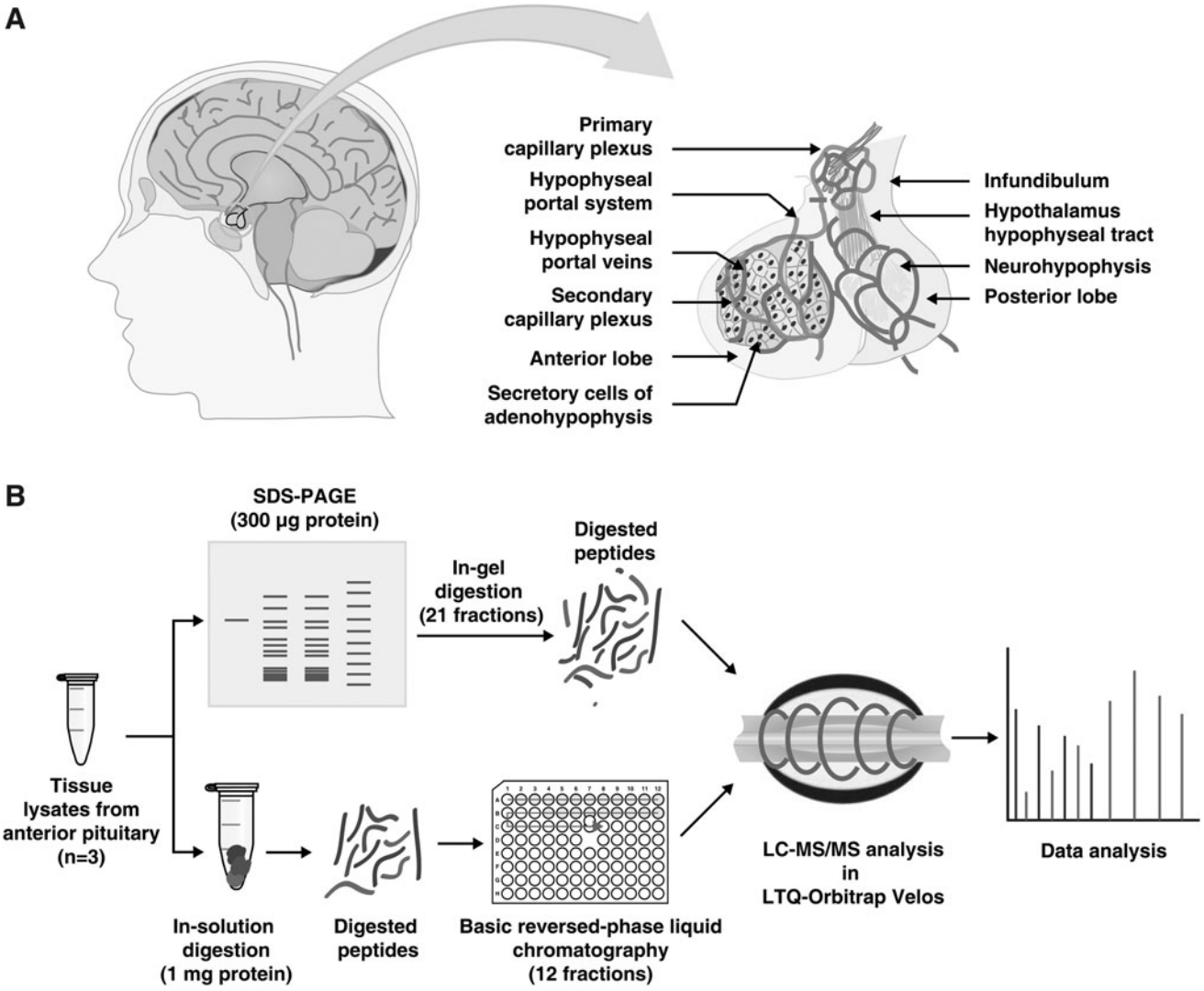

FIG. 1. Schematic representation of anatomical structure and proteomic analysis of human pituitary gland. **(A)** The detailed structure of human pituitary glands. **(B)** The methodology used using different fractionation techniques and LC-MS/MS analysis for the identification of proteins from human adenohypophysis. LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry.

adenohypophysis. Three hundred micrograms of protein from the pooled sample underwent in-gel digestion, while a 1 mg aliquot was subjected to in-solution digestion.

In-gel digestion

Protein-level fractionation was performed as described previously (Yelamanchi et al., 2016a). Briefly, 300 μg of protein from adenohypophysis was resolved on the 10% SDS-PAGE gel. The gel was stained with colloidal Coomassie blue stain and each lane was cut into 21 bands. Gel bands were completely destained with 40 mM ammonium bicarbonate in 40% acetonitrile. Reduction was carried out using 5 mM dithiothreitol and alkylation by 20 mM iodoacetamide. In-gel digestion was performed using trypsin (Promega, Madison, WI, USA), with the enzyme and substrate ratio of 1:20 (w/w), for 12 h at 37°C. In-gel digested peptides were subjected to peptide extraction followed by cleaning up using C_{18} stage tips (3M Empore high-performance extraction disks).

In-solution digestion and basic pH reversed-phase liquid chromatography (bRPLC)

In-solution digestion was carried out with 1 mg of protein from the pooled lysate essentially as described previously (Balakrishnan et al., 2014; Harish et al., 2015). Reduction and alkylation of cysteine residues were carried out using 5 mM dithiothreitol (60°C for 45 min) and 20 mM iodoacetamide, respectively. Reduced and alkylated samples were subjected to trypsin digestion with the enzyme and substrate ratio of 1:20 (w/w) at 37°C for 16 h. This was followed by cleaning up of the digested samples on Sep-Pak C_{18} columns (WAT051910; Waters Corporation, Milford, MA, USA). Samples were lyophilized (Operon, Gyeonggi-do, Korea) and stored at −80°C.

The in-solution digested peptides were subjected to basic pH reversed-phase liquid chromatography fractionation on XBridge (C_{18}, 5 μm 250×4.6 mm column) (Waters Corporation) coupled to an Agilent 1200 series HPLC system containing a binary pump, autosampler, UV detector, and a

fraction collector. Solvents such as 7 mM triethylammonium bicarbonate (TEABC) in water at pH 8.4 and 7 mM TEABC in 90% acetonitrile, pH 8.4, were used as mobile phase A and B, respectively. Fractionation was carried out on 1 mg equivalent peptides from adenohypophysis at a flow rate of 1 mL/min using the following gradient: 1% B for 0–5 min, 10% B for 5–10 min, 10–35% B for 10–40 min, and 100% B for 40–45 min. A total of 96 fractions collected were further pooled to obtain a total of 12 fractions. The fractions were vacuum dried and stored at −80°C.

Liquid chromatography–mass spectrometry/mass spectrometry analysis

Anterior pituitary gland protein fractions were subjected to liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) analysis on LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Bremen, Germany) connected to an Easy nano-LC II system (Thermo Scientific) for peptide separation through reversed-phase chromatography method. The peptides were loaded onto a trap column (2 cm × 75 μm, 5 μm) and fractionated on an analytical column (10 cm × 75 μm, 3 μm). The columns were packed in-house using C_{18} material (Magic C_{18} AQ, 100 Å). The sample was loaded on a trap column at a flow rate of 3 μL/min using 0.1% formic acid (solvent A). The peptides were separated on an analytical column at LC gradient of 5% to 30% solvent B (100% acetonitrile, 0.1% formic acid) over a period of 65 min at a flow rate of 350 nL/min.

Each peptide fraction was run over a total time period of 70 min. The spray voltage was set to 2.0 kV, and the data were acquired in a data-dependent manner. Twenty most intense precursor ions were chosen for fragmentation in higher energy collisional dissociation (HCD) mode. The scans were acquired using Orbitrap mass analyzer at a resolution of 60,000 and 15,000 for MS and MS/MS, respectively, at 400 m/z. Thirty-nine percent of normalized collision energy was used to fragment the peptides. The automatic gain control for full MS was set to 1×10^6 ions and for MS/MS was set to 5×10^4 ions with a maximum time of accumulation of 100 and 300 ms, respectively. The lock mass option was enabled for accurate mass measurements. Polydimethylcyclosiloxane (m/z, 445.1200025) ions were used for internal calibration.

Data analysis

LC-MS/MS data analysis was carried out by using Proteome Discoverer platform, version 2.1 (Thermo Scientific). The data were searched against NCBI Human RefSeq 81 database, which contained 110,386 unique protein sequences with known contaminants using SequestHT and Mascot (Version 2.4) search algorithms. The search parameters used were set as indicated—precursor mass tolerance was set to 20 ppm and fragment mass tolerance to 0.05 Da. Oxidation of methionine and acetylation at protein N-terminus was set as variable modification, while carbamidomethylation of cysteine was set as fixed modification.

Other search parameters included two missed cleavages by trypsin and 1% false discovery rate (FDR). Gene Ontology (GO) terms on biological processes, cellular components, and signal peptides were acquired from Human Protein Reference Database (HPRD) (Goel et al., 2012; Keshava Prasad et al., 2009). Signal peptide domains were also fetched from SignalP version 4.1 (http://cbs.dtu.dk/services/SignalP), for the proteins that lack domain information from HPRD. The data were compared with two published studies on human proteome, comprising one such study from our group (Kim et al., 2014; Wilhelm et al., 2014). Furthermore, human anterior pituitary gland data were also compared with Human Protein Atlas (HPA; http://proteinatlas.org) (Uhlen et al., 2015) containing antibody-based proteomic evidence.

Data availability

The MS data from anterior pituitary gland were submitted to the ProteomeXchange Consortium (http://proteomexchange.org) via the PRIDE partner repository (Vizcaino et al., 2013) with the data set identifier PXD005819.

Results and Discussion

LC-MS/MS-based proteomic analysis of anterior pituitary gland was performed using two different fractionation techniques, as illustrated in Figure 1B. A total of 33 separate LC-MS/MS runs were analyzed using high-resolution Fourier transform mass spectrometer. The analysis resulted in the identification of 2164 proteins that were supported by 12,076 peptides and 120,971 peptide spectrum matches. A complete list of peptides and proteins identified from anterior pituitary gland is given in Supplementary Table S2.

A total of 1395 proteins were identified with multiple peptide evidence. We identified 200 N-terminally acetylated peptides, which enabled us to confirm translational start sites of 187 proteins. A representative MS/MS spectrum for peptides of some of these proteins is depicted in Figure 2. Functional categorization of human anterior pituitary gland proteome was performed based on biological processes and cellular components using the data acquired from HPRD (Goel et al., 2012; Keshava Prasad et al., 2009). GO classification of human anterior pituitary gland proteins is shown in Figure 3A and B.

Anterior pituitary gland proteome was compared with our recent study describing a draft map of human proteome, which comprised proteomes from 30 different human tissues or primary cells (Kim et al., 2014). We also compared human anterior pituitary gland data with another MS-based study that comprises human proteins from 60 tissues, 13 body fluids, 147 cell lines, and public repositories (Wilhelm et al., 2014). Besides these, the data were also mapped to HPA that includes proteins identified from 45 healthy tissues (Uhlen et al., 2015).

Protein expression data from human pituitary glands have not been appended in all these three databases till date. We identified two proteins that are not found in these databases, including putative V-set and immunoglobulin domain-containing-like protein IGHV4OR15-8-like (LOC102723407) and ES1 protein homolog, mitochondrial (LOC102724023). The data from the current study will serve as a baseline for future studies that can help in understanding the organ physiology under normal and disease conditions.

Proteins that are previously not reported

Proteomics of human pituitary gland has been carried out previously by some of the research groups using the high-resolution MS-based approach (Table 1). In this study, we identified an additional 105 proteins from the human anterior pituitary gland that were not reported in any of the earlier studies (Supplementary Table S3).

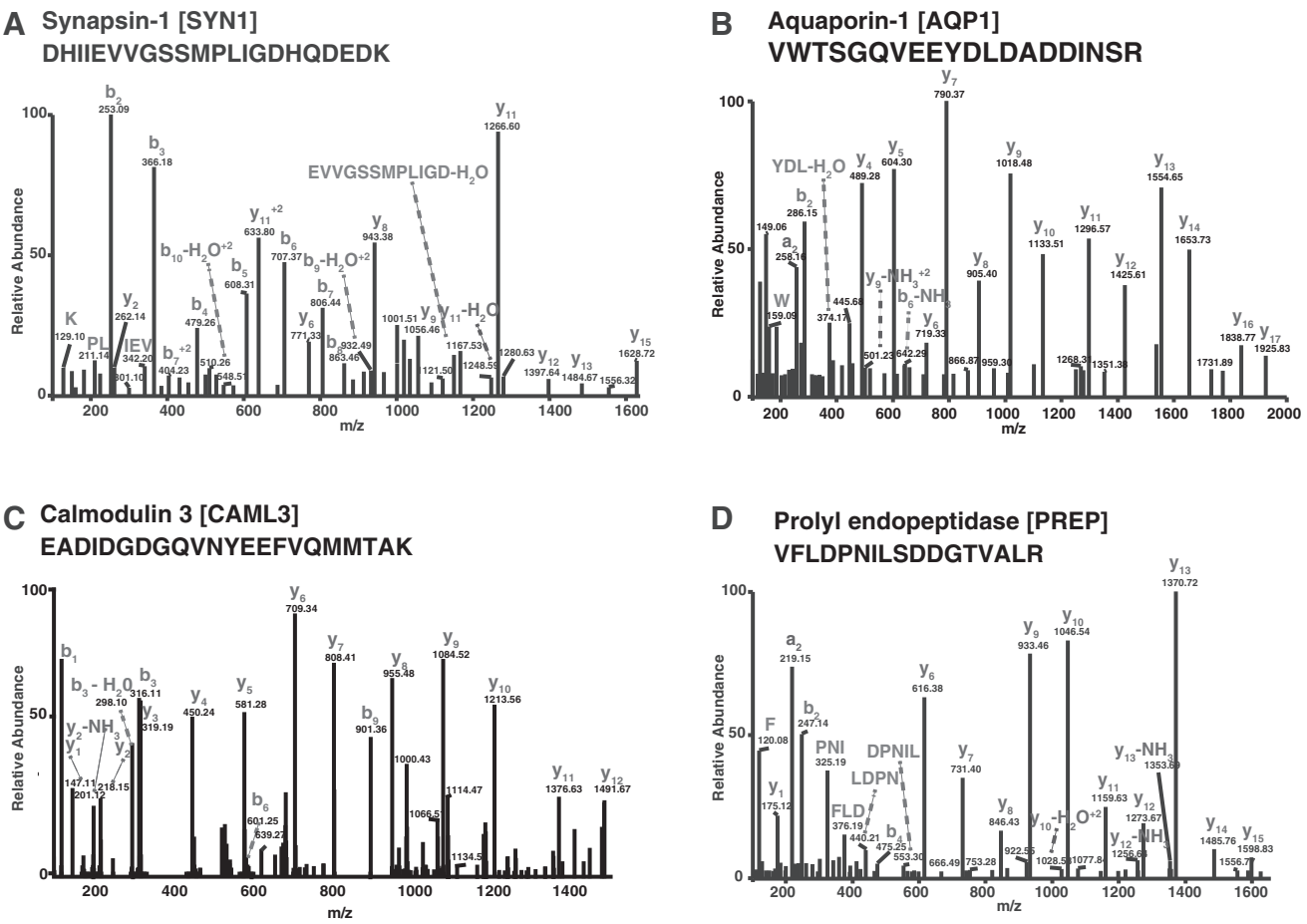

FIG. 2. Representative MS/MS spectra. MS/MS spectra for peptides corresponding to proteins identified from human pituitary gland—**(A)** synapsin-1 [SYN1]; **(B)** aquaporin-1 [AQP1]; **(C)** calmodulin [CALM3]; and **(D)** prolyl endopeptidase [PREP]. The fragmented ions are indicated as b, y, and a ions. Internal fragmented ions are also clearly represented.

Anterior pituitary gland proteins associated with aryl-hydrocarbon receptor signaling

We identified a large number of aryl-hydrocarbon receptor (AHR) signaling network proteins from this study. AHR upon stimulation with ligand activates a series of signaling cascades that are known to be associated with distinct physiological processes in the body, including detoxification process, immunoregulation, gene regulation, and homeostasis (Yelamanchi et al., 2016). Activated AHR has been reported to regulate the mRNA expression of PRL, lutropin subunit beta (LHB), and chromogranin A (CHGA) in pituitary gland (Cao et al., 2011; Moran et al., 2012). Aryl-hydrocarbon interacting protein (AIP) interacts with ligand-bound AHR in the cytosol and translocates into the nucleus leading to transcriptional repression of AHR (Ramadoss et al., 2004).

Previous reports have shown that decreased AHR expression and mutation in AIP gene are associated with GH-secreting pituitary adenomas (Iacovazzo et al., 2016; Jaffrain-Rea et al., 2009). We identified 24 proteins to be enriched in the AHR signaling pathway. The pathway map highlighting the proteins identified in this study is shown in Figure 4.

Significance of anterior pituitary gland proteins with secretory potential

The anterior pituitary gland is well known for secreting distinct hormones. Proteins destined to be secreted from cell usually harbor an N-terminal 15–30 amino acid sequence called a signal peptide, which regulates the translocation/secretion of secretory proteins through the secretory pathway. As the pituitary gland is a secretory endocrine gland, it is reasonable to investigate other lesser known proteins with secretory potential. Therefore, we analyzed signal peptide containing proteins expressed in the pituitary gland. Signal peptide information was available for 336 pituitary gland proteins in HPRD and we combined these data with infor mation from SignalP. In total, 480 proteins were found to contain signal peptides in our study (Supplementary Table S4).

All the known peptide hormones of anterior pituitary gland were identified in this study. A literature search was carried out to analyze the functional significance of anterior pituitary gland proteins with secretory potential. Secretogranin-3 (SCG3), a secretory protein, has been found to be localized in secretory granules of endocrine cells in adenohypophysis (Sakai et al., 2003).

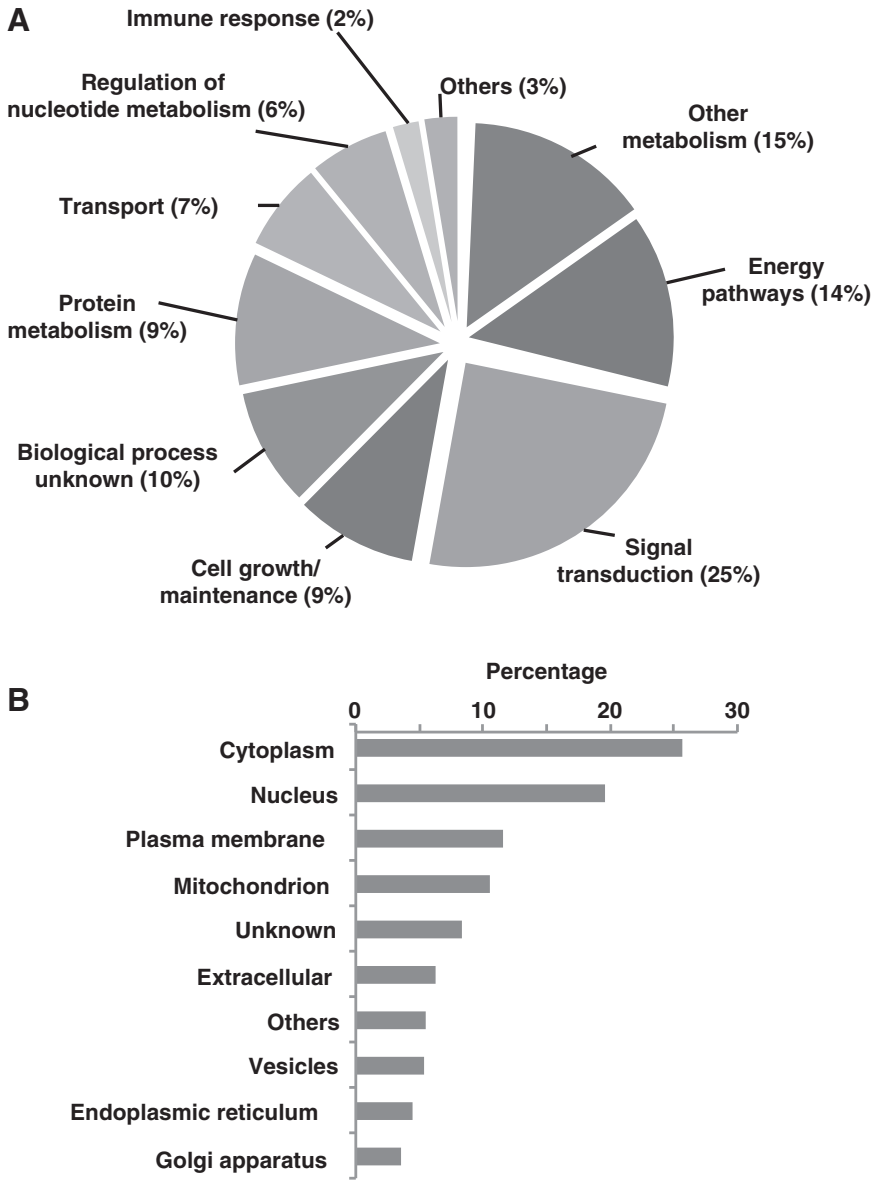

FIG. 3. GO analysis. **(A)** Biological processes such as signal transduction, protein metabolism, and transport were the GO terms derived from human adenohypophysis proteins; **(B)** cellular components, including cytoplasm, nucleus, extracellular and plasma membrane, are illustrated. GO, Gene Ontology.

Previously, it has been reported that CHGA, another secretory protein identified in this study, colocalized with SCG3 to endocrine cells of pituitary gland and pancreas. Similar to most hormones, CHGA and chromogranin B are synthesized as preprohormone and proteolytically processed into multiple smaller peptide hormones with distinct biological functions. CHGA can give rise to peptide hormones that regulate hormone secretion from the parathyroid chief cells (Russell et al., 1994), insulin secretion from the pancreatic islets (Tatemoto et al., 1986), and catecholamine release from the adrenal medulla (Mahata et al., 1997).

VGF nerve growth factor inducible (*VGF*) is a secreted neuroendocrine hormone with an important central metabolic and reproductive role (Jethwa and Ebling, 2008). Mice lacking VGF are infertile and have dramatically reduced fat mass and body weight despite consuming more calories per gram body weight (Hahm et al., 1999). As a preprohormone, VGF is proteolytically processed by neuroendocrine convertases (PCSK1 and PCSK2) into multiple smaller isoforms, each possessing distinct biological functions in the central nervous system with regard to modulating food intake (Jethwa et al., 2007) and reproduction (Succu et al., 2004). A partial list of hitherto signal peptide containing proteins from human anterior pituitary gland is provided in Table 2.

Folliculostellate proteins identified from human anterior pituitary gland

Anterior pituitary gland comprises different groups of endocrine cells that regulate various functional activities in the body through the secretion of hormones. It also harbors

TABLE 1. SUMMARY OF PREVIOUS STUDIES ON HUMAN PITUITARY PROTEOMICS

	Sample preparation	*# Proteins*	*#Peptides*	*MS/MS spectra*	*Instrument and resolution used*	*Search algorithm*	*Reference*
1	Immunoaffinity depletion and high-pH HPLC separation	7596	121,315	436,104	TripleTOF 5600 (MS1 and MS2 40000 and 20000)	Mascot	Liu et al. (2017)
2	In-gel digestion and 2D-HPLC (SCX and RPLC)	1660	42,055	89,314	LTQ-Orbitrap XL	SEQUEST	Liu et al. (2011)
3	In-gel digestion (isoelectric focusing and SDS-PAGE)	1449	6295	—	LCQ Deca XP Plus ion trap	SEQUEST	Zhao et al. (2005)
4	In-solution digestion	1007	14,301	—	Q-TOF	ProteinLynx Global Server	Krishnamurthy et al. (2011)
5	2D gel electrophoresis	316	—	—	BD powerblot western array	—	Ribeiro-Oliveira et al. (2008)
6	In-gel digestion isoelectric focusing	127	—	—	LCQ Deca	SEQUEST	Giorgianni et al. (2003)
7	2D gel electrophoresis	28	—	—	Voyager DE-RP and LCQ Deca	—	Zhan and Desiderio (2003)

HPLC, high-performance liquid chromatography; 2D, two dimensional; SDS-PAGE, sodium dodecyl sulfate/polyacrylamide gel electrophoresis; MS, mass spectrometry; SCX, strong cation exchange; RPLC, reverse phase liquid chromatography.

another group of nonendocrine star-shaped cells with long cytoplasmic processes known as the folliculostellate cells. These cells are restricted to anterior pituitary gland portion of the pituitary gland and are known to form gap junctions and network-like structures surrounding the endocrine cells (Inoue et al., 1999).

In this study, we identified a number of proteins that have been described to be expressed in folliculostellate cells. Annexin A1 (ANXA1), expressed in these nonendocrine cells, is known to inhibit the release of ACTH from adenohypophysis (Tierney et al., 2003). Macrophage migration inhibitory factor, a proinflammatory cytokine, functions as an autocrine or paracrine factor in folliculostellate cells and provides protection against infection or inflammation (Tierney et al., 2005). Glutamine synthetase (GLUL), which has been identified in folliculostellate cells, utilizes ammonia for the synthesis of glutamine. Hence, folliculostellate cells can also function as scavengers of ammonia (Shirasawa and Yamanouchi, 1999).

Matrix metalloproteinase-9 (MMP9) is reported to regulate the proliferation of folliculostellate cells (Ilmiawati et al., 2012). Metalloproteinase inhibitor 2 (TIMP2) is secreted from the folliculostellate cells and promotes the survival of endocrine cells of the pituitary gland (Matsumoto et al., 1993). Ciliary neurotrophic factor (CNTF) receptor subunit alpha identified in this study induces the proliferation of folliculostellate cells on stimulation with CNTF (Perez Castro et al., 2000). Previous reports have shown that CNTF forms gap junctions between these cells (Sakuma et al., 2002). Protein S100-B (S100B) has been reported to regulate the secretion of PRL from lactotrophs (Ishikawa et al., 1983).

Anterior pituitary gland proteins associated with diseases

Anterior pituitary gland proteins have been reported to be involved in various human diseases. Beta-2-syntrophin (SNTB2), follicle stimulating hormone subunit beta (FSHB), and neuronal pentraxin-2 (NPTX2) have been reported to be differentially expressed in prolactinomas (Evans et al., 2008). A calcium binding protein secretagogin (SCGN), which is highly expressed in pituitary glands, has been reported to be associated with nonfunctional pituitary adenomas (Gartner et al., 2001). The cochaperone, AIP, has been shown to interact with many biologically significant proteins, including guanine nucleotide-binding protein subunit alpha-13 (GNA13), which destabilizes AIP-AhR complex, thus regulating AhR translocation to the nucleus.

In addition, AIP interaction with cGMP-dependent 3′, 5′-cyclic phosphodiesterase (PDE2A) has been shown to decrease mitogenic effects by reducing cAMP levels. This indicates that AIP has tumor suppressor activity in pituitary tumor cells (Trivellin and Korbonits, 2011).

Mutational studies have shown that AIP is associated with familial isolated pituitary adenoma (Vierimaa et al., 2006), pituitary adenoma with neuronal choristoma, and sporadic pituitary adenoma. Similarly, mutation in G-protein subunit alpha S (GNAS) gene is reported to be associated with the McCune-Albright syndrome (Weinstein et al., 1991). cAMP-dependent protein kinase type I-alpha regulatory subunit (PRKAR1A) is associated with Carney complex-related pituitary adenomas. Tissue alpha-L-fucosidase (FUCA1)

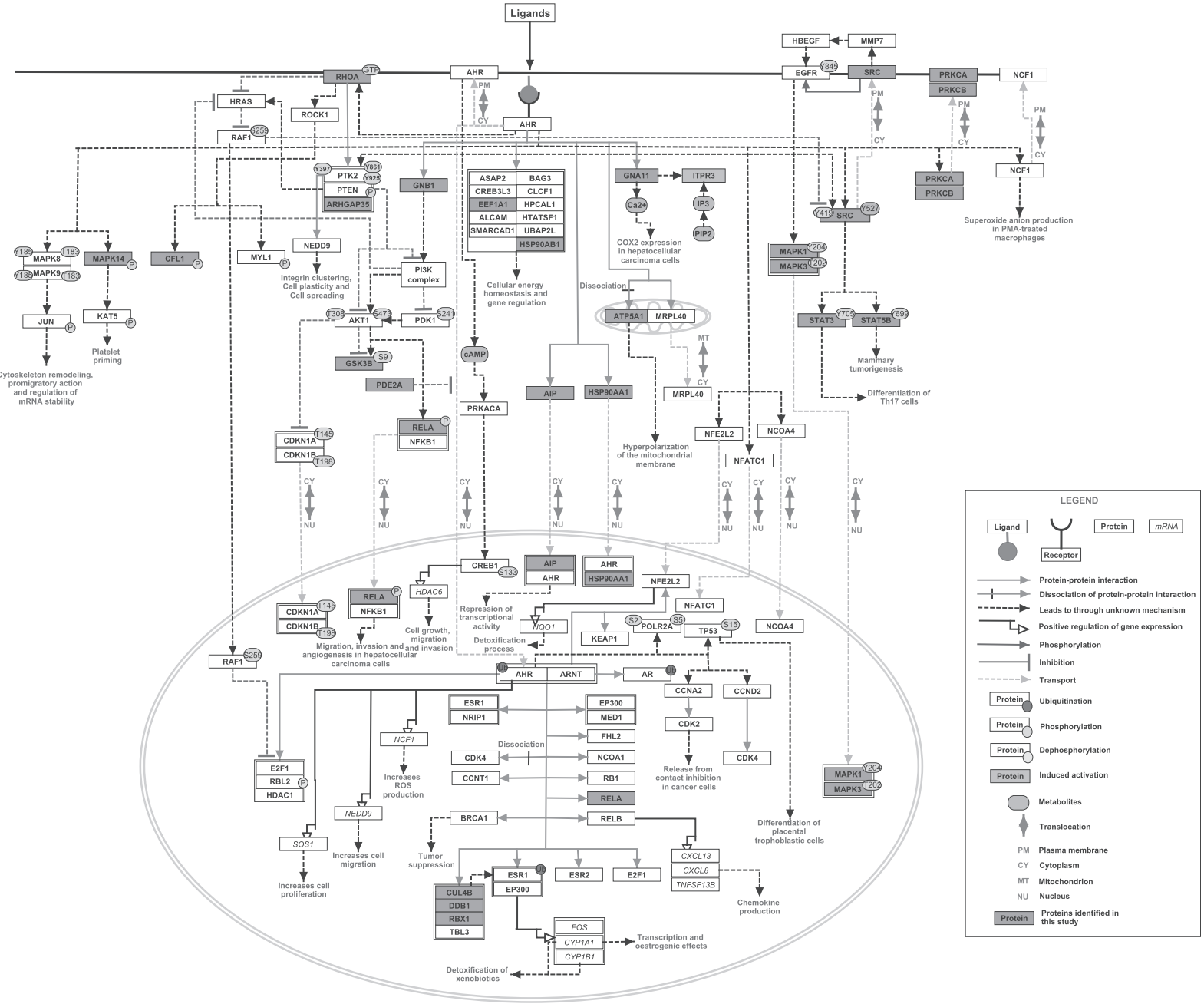

FIG. 4. Aryl-hydrocarbon signaling pathway. The signaling pathway map exhibiting distinct reactions under receptor activation. Twenty-four proteins were identified to be associated with this pathway, as highlighted.

Table 2. A Partial List of Signal Peptide Containing Proteins Identified from Human Anterior Pituitary Gland

	Gene symbol	*Protein*	*RefSeq accession*	*Molecular function*
1	*SYNPR*	Synaptoporin	NP_001123475.1	Auxiliary transport protein activity
2	*SORT1*	Sortilin 1	NP_002950.3	Receptor activity
3	*CDH2*	Cadherin-2	NP_001783.2	Cell adhesion molecule activity
4	*HPX*	Hemopexin precursor	NP_000604.1	Protein binding
5	*GM2A*	Ganglioside GM2 activator	NP_000396.2	Transporter activity
6	*VASN*	Vasorin	NP_612449.2	Growth factor binding
7	*FUCA1*	Tissue alpha-L-fucosidase	NP_000138.2	Hydrolase activity
8	*MRC2*	C-type mannose receptor 2	NP_006030.2	Receptor activity
9	*IPO9*	Importin-9	NP_060555.2	Transporter activity
10	*NIPSNAP3B*	Protein NipSnap homolog 3B	NP_060846.2	Transporter activity
11	*CMPK1*	UMP-CMP kinase isoform a	NP_057392.1	Catalytic activity
12	*EPHX1*	Epoxide hydrolase 1	NP_001129490.1	Hydrolase activity
13	*SERINC1*	Serine incorporator 1	NP_065806.1	Protein binding
14	*KNG1*	Kininogen-1	NP_000884.1	Protease inhibitor activity
15	*SLC22A13*	Solute carrier family 22 member 13	NP_004247.2	Auxiliary transport protein activity

has previously been reported to be associated with colorectal cancer (Otero-Estevez et al., 2013). SNTB2, PDE2A, and FUCA1 have not previously been reported from human pituitary gland.

We compared the proteomic data from anterior pituitary gland with the genes associated with sporadic pituitary adenoma from the genome-wide association study database that comprises information related to mutations in genes and the disease association. We identified protocadherin-related 15 (PCDH15) from this study. Previous reports have shown that mutation in *PCDH15* gene is associated with Usher syndrome and hearing loss. Furthermore, *PCDH15* gene expression was known to be linked with pituitary development in mice (Ye et al., 2015).

We also identified aminophospholipid transporter, class I, type 8A (ATP8A2) and Wiskott–Aldrich syndrome protein family member 3 (WASF3) in this study when compared with expression Quantitative Trait Loci (eQTL) analysis. Previous reports have shown that mutation in ATP8A2 was found to be linked to several human neurological disorders such as cognitive disorders, optic dystrophy, hypotonia, and cerebellar ataxia mental retardation and dysequilibrium (CAMRQ) syndrome (McMillan et al., 2018). Furthermore, overexpression of *WASF3* gene is known to play an active role in the development of cancer (Teng et al., 2013).

Conclusions

Pituitary gland is known as the master gland and regulates most of the endocrine functions in the human body. It should be noted that the proteins analyzed in this study were from the male anterior pituitary gland and it is indeed possible that the female anterior pituitary gland could be different in terms of its proteome.

The present data set will serve as a vital resource for further investigations on the physiological role of human pituitary glands and the proteins secreted by them. Notably, experimental standards for high-throughput proteomics have been evolving for over a decade (Hogan et al. 2006), and advances in omics analyses for health and disease have now become crucial for a system-scale understanding of the pituitary gland pathophysiology (Zhan and Desiderio, 2016). Moreover, we anticipate that the identification of novel proteins in this study will accelerate biomedical research to decipher their role in the functioning of human pituitary gland and associated human diseases.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

References

Allaerts W, and Vankelecom H. (2005). History and perspectives of pituitary folliculo-stellate cell research. Eur J Endocrinol 153, 1–12.

Balakrishnan L, Nirujogi RS, Ahmad S, et al. (2014). Proteomic analysis of human osteoarthritis synovial fluid. Clin Proteomics 11, 6.

Cao J, Patisaul HB, and Petersen SL. (2011). Aryl hydrocarbon receptor activation in lactotropes and gonadotropes interferes with estradiol-dependent and -independent preprolactin, glycoprotein alpha and luteinizing hormone beta gene expression. Mol Cell Endocrinol 333, 151–159.

Evans CO, Moreno CS, Zhan X, et al. (2008). Molecular pathogenesis of human prolactinomas identified by gene expression profiling, RT-qPCR, and proteomic analyses. Pituitary 11, 231–245.

Gartner W, Lang W, Leutmetzer F, Domanovits H, Waldhausl W, and Wagner L. (2001). Cerebral expression and serum detectability of secretagogin, a recently cloned EF-hand Ca(2+)-binding protein. Cereb Cortex 11, 1161–1169.

Giorgianni F, Desiderio DM, and Beranova-Giorgianni S. (2003). Proteome analysis using isoelectric focusing in immobilized pH gradient gels followed by mass spectrometry. Electrophoresis 24, 253–259.

Goel R, Harsha HC, Pandey A, and Prasad TS. (2012). Human protein reference database and human proteinpedia as resources for phosphoproteome analysis. Mol Biosyst 8, 453–463.

Hahm S, Mizuno TM, Wu TJ, et al. (1999). Targeted deletion of the Vgf gene indicates that the encoded secretory peptide precursor plays a novel role in the regulation of energy balance. Neuron 23, 537–548.

Harish G, Mahadevan A, Pruthi N, et al. (2015). Characterization of traumatic brain injury in human brains reveals distinct cellular and molecular changes in contusion and pericontusion. J Neurochem 134, 156–172.

Hogan JM, Higdon R, and Kolker E. (2006). Experimental standards for high-throughput proteomics. OMICS 10, 152–157.

Iacovazzo D, Caswell R, Bunce B, et al. (2016). Germline or somatic GPR101 duplication leads to X-linked acrogigantism: A clinico-pathological and genetic study. Acta Neuropathol Commun 4, 56.

Ilmiawati C, Horiguchi K, Fujiwara K, and Yashiro T. (2012). Matrix metalloproteinase-9 expression in folliculostellate cells of rat anterior pituitary gland. J Endocrinol 212, 363–370.

Inoue K, Couch EF, Takano K, and Ogawa S. (1999). The structure and function of folliculo-stellate cells in the anterior pituitary gland. Arch Histol Cytol 62, 205–218.

Ishikawa H, Nogami H, and Shirasawa N. (1983). Novel clonal strains from adult rat anterior pituitary producing S-100 protein. Nature 303, 711–713.

Jaffrain-Rea ML, Angelini M, Gargano D, et al. (2009). Expression of aryl hydrocarbon receptor (AHR) and AHR-interacting protein in pituitary adenomas: Pathological and clinical implications. Endocr Relat Cancer 16, 1029–1043.

Jethwa PH, and Ebling FJ. (2008). Role of VGF-derived peptides in the control of food intake, body weight and reproduction. Neuroendocrinology 88, 80–87.

Jethwa PH, Warner A, Nilaweera KN, et al. (2007). VGF-derived peptide, TLQP-21, regulates food intake and body weight in Siberian hamsters. Endocrinology 148, 4044–4055.

Jorgensen H, Kjaer A, Knigge U, Moller M, and Warberg J. (2003). Serotonin stimulates hypothalamic mRNA expression and local release of neurohypophysial peptides. J Neuroendocrinol 15, 564–571.

Karanth S, and McCann SM. (1991). Anterior pituitary hormone control by interleukin 2. Proc Natl Acad Sci U S A 88, 2961–2965.

Keshava Prasad TS, Goel R, Kandasamy K, et al. (2009). Human protein reference database—2009 update. Nucleic Acids Res 37, D767–D772.

Kim MS, Pinto SM, Getnet D, et al. (2014). A draft map of the human proteome. Nature 509, 575–581.

Krishnamurthy D, Levin Y, Harris LW, Umrania Y, Bahn S, and Guest PC. (2011). Analysis of the human pituitary proteome by data independent label-free liquid chromatography tandem mass spectrometry. Proteomics 11, 495–500.

Liu X, Guo Z, Sun H, Li W, and Sun W. (2017). Comprehensive map and functional annotation of human pituitary and thyroid proteome. J Proteome Res 16, 2680–2691.

Liu Y, Zhuang D, Hou R, et al. (2011). Shotgun proteomic analysis of microdissected postmortem human pituitary using complementary two-dimensional liquid chromatography coupled with tandem mass spectrometer. Anal Chim Acta 688, 183–190.

Mahata SK, O'Connor DT, Mahata M, et al. (1997). Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. J Clin Invest 100, 1623–1633.

Matsumoto H, Ishibashi Y, Ohtaki T, Hasegawa Y, Koyama C, and Inoue K. (1993). Newly established murine pituitary folliculo-stellate-like cell line (TtT/GF) secretes potent pituitary glandular cell survival factors, one of which corresponds to metalloproteinase inhibitor. Biochem Biophys Res Commun 194, 909–915.

McMillan HJ, Telegrafi A, Singleton A, et al. (2018). Recessive mutations in ATP8A2 cause severe hypotonia, cognitive impairment, hyperkinetic movement disorders and progressive optic atrophy. Orphanet J Rare Dis 13, 86.

Moran TB, Brannick KE, and Raetzman LT. (2012). Aryl-hydrocarbon receptor activity modulates prolactin expression in the pituitary. Toxicol Appl Pharmacol 265, 139–145.

Otero-Estevez O, Martinez-Fernandez M, Vazquez-Iglesias L, Paez de la Cadena M, Rodriguez-Berrocal FJ, and Martinez-Zorzano VS. (2013). Decreased expression of alpha-L-fucosidase gene FUCA1 in human colorectal tumors. Int J Mol Sci 14, 16986–16998.

Perez Castro C, Nagashima AC, Pereda MP, et al. (2000). The gp130 cytokines interleukin-11 and ciliary neurotropic factor regulate through specific receptors the function and growth of lactosomatotropic and folliculostellate pituitary cell lines. Endocrinology 141, 1746–1753.

Ramadoss P, Petrulis JR, Hollingshead BD, Kusnadi A, and Perdew GH. (2004). Divergent roles of hepatitis B virus X-associated protein 2 (XAP2) in human versus mouse Ah receptor complexes. Biochemistry 43, 700–709.

Ribeiro-Oliveira A, Jr., Franchi G, Kola B, et al. (2008). Protein western array analysis in human pituitary tumours: Insights and limitations. Endocr Relat Cancer 15, 1099–1114.

Russell J, Gee P, Liu SM, and Angeletti RH. (1994). Inhibition of parathyroid hormone secretion by amino-terminal chromogranin peptides. Endocrinology 135, 337–342.

Sakai Y, Hosaka M, Hira Y, et al. (2003). Immunocytochemical localization of secretogranin III in the anterior lobe of male rat pituitary glands. J Histochem Cytochem 51, 227–238.

Sakuma E, Herbert DC, and Soji T. (2002). Leptin and ciliary neurotrophic factor enhance the formation of gap junctions

between folliculo-stellate cells in castrated male rats. Arch Histol Cytol 65, 269–278.
Shirasawa N, and Yamanouchi H. (1999). Glucocorticoids induce glutamine synthetase in folliculostellate cells of rat pituitary glands in vivo and in vitro. J Anat 194 (Pt 4), 567–577.
Succu S, Cocco C, Mascia MS, et al. (2004). Pro-VGF-derived peptides induce penile erection in male rats: Possible involvement of oxytocin. Eur J Neurosci 20, 3035–3040.
Tatemoto K, Efendic S, Mutt V, Makk G, Feistner GJ, and Barchas JD. (1986). Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. Nature 324, 476–478.
Teng Y, Ghoshal P, Ngoka L, Mei Y, and Cowell JK. (2013). Critical role of the WASF3 gene in JAK2/STAT3 regulation of cancer cell motility. Carcinogenesis 34, 1994–1999.
Tierney T, Christian HC, Morris JF, Solito E, and Buckingham JC. (2003). Evidence from studies on co-cultures of TtT/GF and AtT20 cells that annexin 1 acts as a paracrine or juxtacrine mediator of the early inhibitory effects of glucocorticoids on ACTH release. J Neuroendocrinol 15, 1134–1143.
Tierney T, Patel R, Stead CA, Leng L, Bucala R, and Buckingham JC. (2005). Macrophage migration inhibitory factor is released from pituitary folliculo-stellate-like cells by endotoxin and dexamethasone and attenuates the steroid-induced inhibition of interleukin 6 release. Endocrinology 146, 35–43.
Trivellin G, and Korbonits M. (2011). AIP and its interacting partners. J Endocrinol 210, 137–155.
Uhlen M, Fagerberg L, Hallstrom BM, et al. (2015). Proteomics. Tissue-based map of the human proteome. Science 347, 1260419.
Vierimaa O, Georgitsi M, Lehtonen R, et al. (2006). Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science 312, 1228–1230.
Vizcaino JA, Cote RG, Csordas A, et al. (2013). The PRoteomics IDEntifications (PRIDE) database and associated tools: Status in 2013. Nucleic Acids Res 41, D1063–D1069.
Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, and Spiegel AM. (1991). Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. N Engl J Med 325, 1688–1695.
Wilhelm M, Schlegl J, Hahne H, et al. (2014). Mass-spectrometry-based draft of the human proteome. Nature 509, 582–587.
Yasin SA, Costa A, Forsling ML, and Grossman A. (1994). Interleukin-1 beta and interleukin-6 stimulate neurohypophysial hormone release in vitro. J Neuroendocrinol 6, 179–184.
Ye Z, Li Z, Wang Y, et al. (2015). Common variants at 10p12.31, 10q21.1 and 13q12.13 are associated with sporadic pituitary adenoma. Nat Genet 47, 793–797.
Yelamanchi SD, Kumar M, Madugundu AK, et al. (2016). Characterization of human pineal gland proteome. Mol Biosyst 12, 3622–3632.
Yelamanchi SD, Solanki HS, Radhakrishnan A, et al. (2016). Signaling network map of the aryl hydrocarbon receptor. J Cell Commun Signal 10, 341–346.
Zhan X, and Desiderio DM. (2003). Heterogeneity analysis of the human pituitary proteome. Clin Chem 49, 1740–1751.
Zhan X, and Desiderio DM. (2016). Editorial: Systems biological aspects of pituitary tumors. Front Endocrinol (Lausanne) 7, 86.
Zhao Y, Giorgianni F, Desiderio DM, Fang B, and Beranova-Giorgianni S. (2005). Toward a global analysis of the human pituitary proteome by multiple gel-based technology. Anal Chem 77, 5324–5331.

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Abbreviations Used

2D = two dimensional
ACTH = adrenocorticotropic hormone
AHR = aryl-hydrocarbon receptor
AIP = aryl-hydrocarbon interacting protein
ANXA1 = annexin A1
CHGA = chromogranin A
CNTF = ciliary neurotrophic factor
CRH = corticotropin-releasing hormone
GH = growth hormone
GLUL = glutamine synthetase
GNA13 = guanine nucleotide-binding protein subunit alpha-13
GO = Gene Ontology
HPA = Human Protein Atlas
HPLC = high-performance liquid chromatography
HPRD = Human Protein Reference Database
IL = interleukin
LC-MS/MS = liquid chromatography–mass spectrometry/mass spectrometry
MMP9 = matrix metalloproteinase-9
NIMHANS = National Institute of Mental Health and Neuro Sciences
NPTX2 = neuronal pentraxin-2
PCDH15 = protocadherin-related 15
SCG3 = secretogranin-3
SCGN = secretagogin
SDS-PAGE = sodium dodecyl sulfate/polyacrylamide gel electrophoresis
SNTB2 = beta-2-syntrophin
TEABC = triethylammonium bicarbonate