Gene Expression Profiling of Hypothalamic Hamartomas: A Search for Genes Associated with Central Precocious Puberty

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Abstract

Background: Hypothalamic hamartomas (HHs) are congenital lesions composed of neurons and astroglia. Frequently, HHs cause central precocious puberty (CPP) and/or gelastic seizures. Because HHs might express genes similar to those required for the initiation of normal puberty, we used cDNA arrays to compare the gene expression profile of an HH associated with CPP with three HHs not accompanied by sexual precocity. ***Methods:*** Global changes in gene expression were detected using Affymetrix arrays. The results were confirmed by semiquantitative PCR, which also served to examine the expression of selected genes in the hypothalamus of female monkeys undergoing puberty. ***Results:*** All HHs were associated with seizures. Ten genes whose expression was increased in the HH with CPP were identified. They encode proteins involved in three key cellular processes: transcriptional regulation, cell-cell signaling, and cell adhesiveness. They include *IA-1* and *MEF2A*, two transcription factors required for neuronal development; *mGluR1* and *VILIP-1*, which encode proteins involved in neuronal communication, and *TSG-6* that encodes a protein involved in cell adhesiveness. Of these, expression of *mGluR1* also increases in the female monkey hypothalamus at puberty. ***Conclusions:*** Increased expression of these genes in HHs may be relevant to the ability of some HHs to induce sexual precocity.

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Introduction

Puberty results from the activation of a complex neuroendocrine machinery set in motion by a primary mechanism yet to be identified. Once under way, this activational process results in the diurnally timed resurgence of pulsatile gonadotropin hormone-releasing hormone (GnRH) secretion, which in turn stimulates the secretion of luteinizing hormone and follicle-stimulating hormone from the pituitary gland.

It appears now well-established that GnRH secretion during sexual development is mainly controlled by neuronal networks utilizing excitatory and inhibitory amino acids for neurotransmission. It is also believed that the pubertal activation of GnRH release depends on a coordinated increase in glutamatergic stimulation of GnRH neurons and a decrease in γ-aminobutyric acid inhibition [reviewed in 1]. In addition to these 'classical' neurotransmitters, the peptide metastatin/kisspeptin produced by discrete hypothalamic neuronal subsets [2] has

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Table 1. HHs used in this study and associated clinical characteristics

Patient	Age at surgery	Precocious puberty	Gender	Age at onset of puberty	GnRH treatment	Seizures
1	7 years, 8 months	Yes	M	3–4 years	Yes	Yes
2	9 years	No	M	–	–	Yes
3	5 years, 2 months	3 months after surgery	F	5 years, 3 months		Yes
4	37 years	No	M	–	–	Yes
5	14 years	Yes	F	5 years	Yes[1]	Yes

[1] Discontinued before surgery.

been recently identified as playing a pivotal role in the initiation of puberty [3, 4]. GnRH neuronal activity is not only controlled by these transsynaptic inputs, but also by glial cells that facilitate GnRH secretion via the activation of signaling pathways initiated by growth factors acting on receptors endowed with tyrosine- and serine-threonine kinase activity [reviewed in 1].

The search for those precipitating events responsible for the initiation of puberty may benefit not only from studying the normal process of puberty in animal models and humans, but also from the analysis of pathological conditions affecting pubertal development in humans. Hypothalamic hamartomas (HHs) represent one of these conditions. They are congenital nonneoplastic lesions containing mature brain tissue in a heterotopic location, commonly associated with the base of the hypothalamus [5]. In most cases, HHs contain neurons and astroglial cells of normal aspect in addition to astrocytes and ependymoglial-like cells [6]. If HH are symptomatic, they are frequently associated with central precocious puberty (CPP) and/or gelastic seizures, a form of ictal laughter [7].

In both sexes, sexual precocity caused by HH occurs much earlier (1–2 years of age) than idiopathic precocious puberty, which predominantly affects females. The mechanism by which they advance puberty so dramatically is still unknown. Because HHs appear to contain all the necessary components to initiate the pubertal process at a very early age, they might hold critical information concerning the transcriptional and/or signaling components underlying sexual precocity and the initiation of normal puberty in humans. In this study, we used cDNA microarrays to compare the gene expression profiles of an HH associated with CPP to that of HHs not accompanied by precocious puberty with the aim of identifying genes whose expression may be selectively altered in the HH with CPP. The results showed the genes most highly over-expressed in the HH associated with CPP were genes encoding transcriptional regulators required for neuronal differentiation and survival, and genes encoding proteins required for excitatory cell-cell communication.

Patients and Methods

Patients

HHs from 4 patients were used for DNA array analysis and semiquantitative PCR. In addition, the expression of selected genes from a fifth HH was analyzed by semiquantitative PCR only. In all cases, the HHs were surgically resected due to intractable seizure activity. With the exception of patient 3 and 5, all other patients were males. Only patients 1 and 5 exhibited CPP before surgery (table 1). However, the evolution of these two cases was different, because the HH of patient 5 was removed at 14 years of age (9 years after CPP was diagnosed), during the course of normal puberty and 1 year after discontinuation of treatment with a GnRH agonist. The HH of patient 1 was resected much sooner (at approximately 8 years of age), 4 years after the manifestation of CPP, and while the patient was still being treated with a GnRH agonist. The 3 other patients had seizures, but not CPP at the time of HH resection. Patient 3 (female) exhibited CPP 3 months *after* surgery (table 1). A fragment of each HH was routinely processed for histopathological examination, and tissue samples not needed for diagnostic purposes were stored at –80°C for future reference or research.

These preexisting samples were sent to Beaverton, Oreg., USA as unlinked, anonymous specimens for RNA processing. The experimental protocol for this project was approved by the Institutional Review Board for Human Subjects of the Oregon Health & Science University.

Nonhuman Primates

The hypothalamic tissue used in this study derived from early juvenile (8.9–11.7 months of age, n = 5), late juvenile (1.2–1.8 years of age, n = 4), early pubertal (2–3 years of age, n = 4) and mid-pubertal (3–4 years of age, n = 6) female monkeys *(Macaca mulatta)* that had been euthanized for a variety of reasons and obtained through the Oregon National Primate Research Center (ONPRC) Necropsy Program. The developmental stages of the animals were defined according to the criteria proposed by Watanabe and

Table 2. Genes showing a 2-fold increase or more in an HH associated with precocious puberty (HH-1) in comparison to HHs not accompanied by sexual precocity (HH-2 to 4)

Accession No.	Gene identity	1- vs. 2-fold increase	1- vs. 3-fold increase	1- vs. 4-fold increase	Mean fold increase
U30872.1	Centromere protein F (mitosin)	4.29	3.25	6.5	4.68
NM_002196.1	Insulinoma-associated 1 (IA1)	4.29	2.3	10.56	5.72
NM_005587.1	MEF2A transcription enhancer factor 2	3.48	2	2.83	2.77
NM_000838.2	Glutamate receptor metabotropic 1 α (mGluR1A)	3.48	2.14	3.73	3.13
NM_003385.1	Visinin-like 1 (VILIP-1)	3.73	2.3	4.59	3.54
AI040163	Calcium channel, voltage-dependent, β_2-subunit	3.73	2	3.03	2.92
AF047033.1	Solute carrier family 4, sodium bicarbonate cotransporter, member 7 (SLC4A7)	2.64	2.14	2.64	2.47
AJ300461.1	Transmembrane protein 16C (C11ORF25)	5.28	4	6.5	5.26
R41498	T cell activation leucine repeat-rich protein	3.25	2.83	3.25	3.11
NM_007115	Tumor necrosis factor alpha-stimulated gene 6 *(TSG-6)*	3.73	2.3	3.73	3.25

Terasawa [8]. All procedures were approved by the ONPRC Animal Care and Use Committee in accordance with the NIH guidelines for the use of animals in research.

RNA Extraction
Total RNA from the HH samples and the nonhuman primate brain was extracted as outlined in online supplementary note 1 (www.karger.com/doi/10.1159/000111815). RNA from the human placenta and human fetal brain was purchased from BD Biosciences (San Jose, Calif., USA); total RNA from human hypothalami was obtained from Ambion (Austin, Tex., USA).

Sample Preparation and Microarray Hybridization
Microarray assays were performed by the Affymetrix Microarray Core of the OHSU Gene Microarray Shared Resource, as described in online supplementary note 2 (www.karger.com/doi/10.1159/000111815).

Microarray Data Analysis
Image processing and expression analysis were carried out using Affymetrix Microarray Suite (MAS) 5.0 software, as described in online supplementary note 3 (www.karger.com/doi/10.1159/000111815). The array data have been deposited in NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/; accession number GSE7142).

Semiquantitative PCR Validation of Array Results
Five genes showing an increased expression of 2-fold or greater in the HH associated with CPP (patient 1, male) versus the HHs from the 2 male patients without CPP (patients 2 and 4), and the HH from a female patient showing puberty after surgery (HH-3) were selected for PCR verification of the array results. The same genes were also examined in an additional HH (patient 5) not subjected to array analysis. The five genes studied were *IA-1, VILIP-1, TSG-6, mGluR1A,* and *MEF2A* (table 2). Reverse transcription of total RNA (50 ng) from each sample and PCR amplification was performed as outlined in online supplementary note 4 (www.karger.com/doi/10.1159/000111815). Other genes examined were *GnRH, TGFα, KiSS1,* and *GPR54* using primers and PCR conditions described in online supplementary note 4. Densitometric analysis of the gels was performed using Quantity One software (Bio-Rad, Hercules, Calif., USA). The results are expressed as arbitrary units resulting from dividing the densitometric value obtained for each mRNA of interest by the corresponding cyclophilin value found in each sample.

Semiquantitative PCR Analysis of IA-1, TSG-6 and mGluR1A mRNA Expression in Monkey Hypothalamus
Five hundred nanograms of total RNA from each sample were reverse transcribed as indicated above. The PCR primers (online suppl. note 5, www.karger.com/doi/10.1159/000111815), chosen using Primer Express software (PR Applied Biosystems) were selected from monkey cDNA sequences (http://www.hgsc.bcm.tmc.edu/projects/rmacaque).

Statistics
Differences between two groups were analyzed using the Student t test. When comparing several groups, the differences were analyzed by one-way ANOVA followed by the Student-Newman-Keuls multiple comparison test for unequal replications.

Results

Identification of Genes Predominantly Expressed in an HH Associated with CPP
To identify genes sharing a similar expression profile in HH-1 (the HH from patient 1 who had CPP and seizures) in comparison to HH-2 to 4 (the HHs from patients 2–4 who had seizures, but not CPP at the time of surgery), we used a K-means clustering algorithm provided by J-Express 2.0 software (http://www.ii.uib.no/~bjarted/jexpress). A group of 10 genes whose expres-

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Table 3. Cellular and molecular functions of genes showing a 2-fold increase or more in an HH associated with precocious puberty (HH-1) in comparison to HHs not accompanied by sexual precocity (HH-2 to 4)

Gene identity	Cellular process	Molecular function	Tissue specificity	TRG
Centromere protein F (mitosin)	Mitosis regulation	Chromosome segregation: microtubule binding protein	Embryonic tissue, malignant tissue	Yes
Insulinoma-associated 1 (IA1)	Transcriptional regulation	Transcription repressor of NeuroD/beta2	Fetal brain, fetal pancreas, neuroendocrine tumors	Yes
MEF2A transcription enhancer factor 2	Transcriptional regulation	Activates transcription	Muscle, brain	Yes
Glutamate receptor metabotropic 1 α (mGluR1A)	Cell-cell communication	Metabotropic glutamate receptor	Brain	No
Visinin-like 1 (VILIP-1)	Cell-cell communication	Calcium sensor protein	Brain, pancreas	Yes
Calcium channel, voltage-dependent, β_2-subunit	Cell-cell communication	Voltage-gated calcium channel	Brain, heart, pancreas	No
Solute carrier family 4, sodium bicarbonate cotransporter, member 7 (SLC4A7)	Cell-cell communication	Coupled transport of sodium and bicarbonate: maintains intracellular pH	Brain, testis, spleen	No
Transmembrane protein 16C (C11ORF25)	Cell-cell communication	Transporter for unidentified substrate	Ear, brain	Yes
T cell activation leucine repeat-rich protein	Cell-cell communication	Membrane protein	Brain (adult and fetal), kidney, ovary	No
Tumor necrosis alpha-stimulated gene 6 (TSG-6)	Cell adhesiveness, cell-cell communication	Hyaluronic acid binding protein	Brain, ovary	Yes

TRG = Tumor-related gene.

sion was consistently greater (2-fold or more) in HH-1 than in HH-2 to 4 was selected (table 2). The functions of the encoded proteins were determined using three different search tools: the Swiss-Prot database (www.expasy.ch), GOMiner (http://discover.nci.nih.gov/gominer/) and IHOP (www.ihop-net.org/UniPub/iHOP/). This analysis indicated that the genes overexpressed in HH-1 encode proteins involved in three fundamental cellular processes: transcriptional regulation, cell-cell communication and cell adhesiveness (table 3). With the possible exception of *CENP-F* (mitosin), a cell cycle-regulated nuclear protein [9] found at very low levels in the normal brain (http://source.stanford.edu), but that is overexpressed in malignant tissues, including astrocytomas and meningiomas [10 and references therein], all other genes are highly expressed in the normal developing brain in either neurons, astrocytes or both (table 3).

Validation of the Array Results by Semiquantitative PCR

Based on their perceived importance in the control of HH cell function, five of the genes overexpressed in HH-1 were selected for PCR validation: *IA-1* and *MEF2A*, two transcriptional regulators [11, 12]; *mGluR1* and *VILIP-1*, two genes encoding proteins involved in cell-cell signalling [13, 14], and *TSG-6*, a gene encoding a multifunctional secreted protein that participates in cell-cell adhesion via binding to hyaluronan, a component of the extracellular matrix [15].

The increased abundance of *IA-1* mRNA detected by PCR in HH-1 as compared to HH-2 and 4 (all from male patients) was essentially identical to that detected by DNA arrays (fig. 1a). HH-3, derived from a female patient who developed CPP shortly after HH resection, exhibited an abundance of *IA-1* mRNA intermediate between HH-
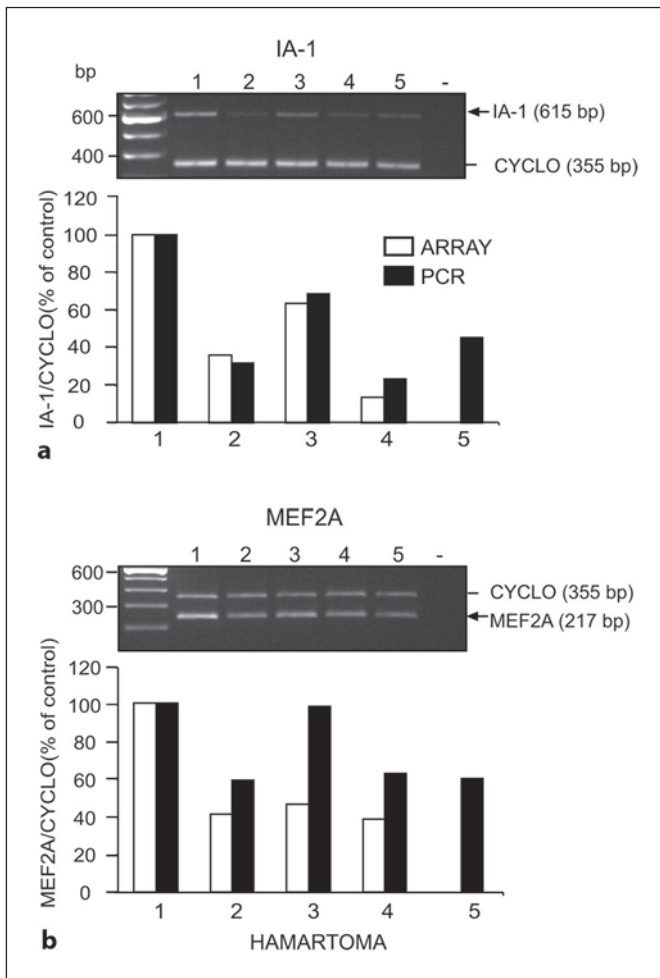
Fig. 1. Selective overexpression of the mRNAs encoding the transcriptional regulators IA-1 (**a**) and MEF2A (**b**) in an HH associated with CPP (HH-1) as compared with three HHs not accompanied by CPP (HH-2 to 4), and one HH (HH-5) removed 9 years after the occurrence of CPP. White bars represent the relative levels of *IA-1* and *MEF2A* mRNAs detected by cDNA arrays, using the mRNA levels found in HH-1 as 100%. Black bars represent relative expression levels detected by semiquantitative PCR, normalized to the cyclophilin mRNA content of each sample, and calculated using the mRNA levels in HH-1 as 100%. The gels depicting the PCR products used to calculate these densitometric values are shown on top of each bar graph. – = PCR, no RT control.

1, HH-2 and HH-4, suggesting that *IA-1* expression might have been already increasing at the time of surgery. *IA-1* mRNA levels were not elevated in HH-5, which was removed from a female patient 9 years after the diagnosis of CPP was made.

Consistent with the array results, *MEF2A* mRNA content measured by PCR was higher in HH-1 than in HH-2

and 4 (fig. 1b). However, *MEF2A* mRNA levels in HH-1 were similar to those in HH-3, a finding at variance with the array results that detected a 2-fold increase in *MEF2A* expression in HH-1 as compared to HH-3 (table 2). Like *IA-1* mRNA, *MEF2A* expression was as low in HH-5 as in HH-2 and 4.

The differences in *mGluR1A* and *VILIP-1* mRNA abundance between HH-1 versus HH-2, 3 and 4, assessed by semiquantitative PCR, were similar to those estimated by the arrays (fig. 2a, b). HH-5 had *VILIP-1* mRNA levels as high as in HH-1, but *mGluR1A* mRNA values as low as in HH-2, 3 and 4, suggesting that not all genes with increased expression in HHs associated with CPP remain permanently activated. As in the case of *IA-1* and *mGluR1A*, there was excellent agreement between the *TSG-6* mRNA levels detected by PCR and the arrays (fig. 2c). *TSG-6* mRNA prevalence in HH-5 was as low as in HH-2, 3 and 4.

Expression of GnRH, TGFα, KiSS1 and GPR54 in HHs

Because earlier reports showed the presence of GnRH [16 and references therein] and *TGFα* [17] in HHs, expression of these genes was also examined. In addition, we sought to determine if *KiSS1* and *GPR54* are expressed in HHs, because of recent findings indicating that activation of GPR54 receptors by kisspeptin, the processed protein product of the *KiSS1* gene, is a critical transsynaptic input to GnRH neurons required for the initiation of puberty [3, 4].

As shown in figure 3a and b, both *GnRH* and *TGFα* mRNAs were detected in all five HHs examined, regardless of their association with CPP. *GnRH* mRNA levels were higher in HH-2, despite the lack of association of this HH with precocious puberty, and TGFα mRNA abundance was highest in HH-3, which resulted in CPP after resection of the tumor. *KiSS1* mRNA was undetectable in all five HHs (fig. 3c), but *GPR54* mRNA was expressed in some HHs (HH-1 to 3), and absent in others (HH-4 and 5) (fig. 3d). Thus activation of GPR54 receptors within HHs does not appear to be a primary mechanism by which HHs induce sexual precocity.

Ontogeny of IA-1, mGluR1A and TSG-6 Expression in the Female Monkey Hypothalamus

Because *IA-1, mGluR1A* and *TSG-6* expression is consistently increased in HH-1, we sought to determine if similar changes occur in the primate hypothalamus during the normal onset of puberty. Female rhesus monkeys were used, because the intrinsic neuroendocrine mecha-
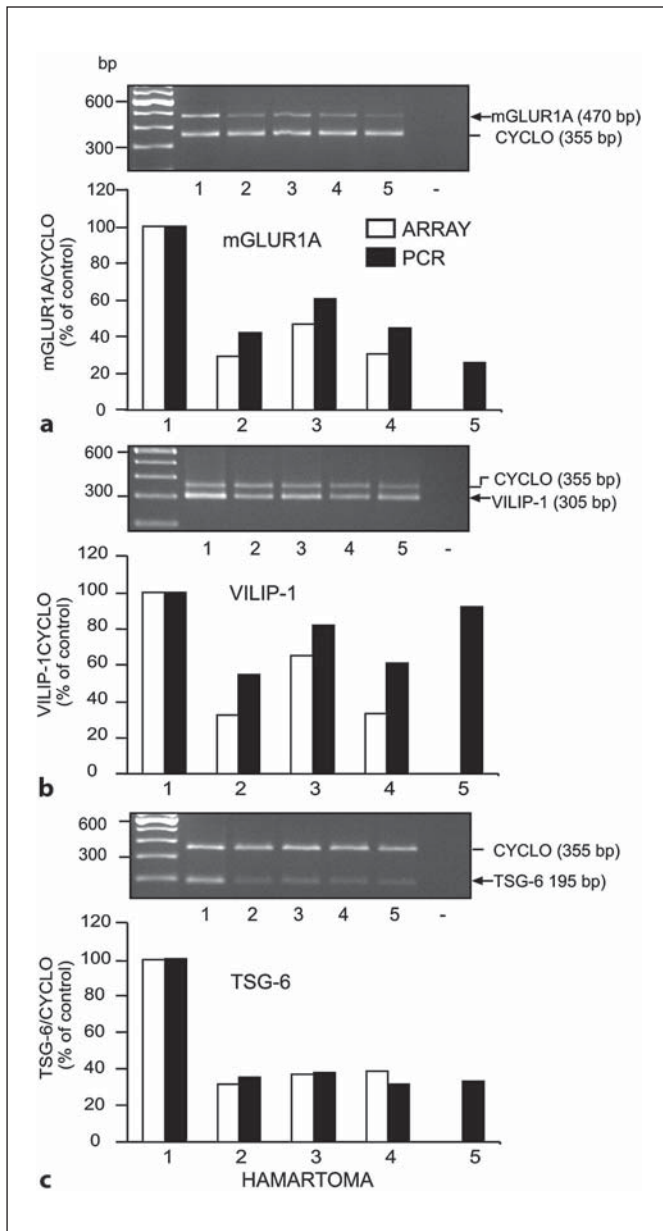
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Fig. 2. Selective overexpression of the mRNAs encoding the cell-cell communication molecules mGluR1A (**a**), VILIP-1 (**b**) and TSG-6 (**c**) in an HH associated with CPP (HH-1) as compared with three HHs not accompanied by CPP (HH-2 to 4), and one HH (HH-5) removed 9 years after the occurrence of CPP. White bars represent the relative levels of *mGLuR1A, VILIP-1* and *TSG-6* mRNAs detected by cDNA arrays, using the mRNA levels found in HH-1 as 100%. Black bars represent relative expression levels detected by semiquantitative PCR, normalized to the cyclophilin mRNA content of each sample, and calculated using the mRNA levels in HH-1 as 100%. The gels depicting the PCR products used to calculate these densitometric values are shown on top of each bar graph. – = PCR, no RT control.
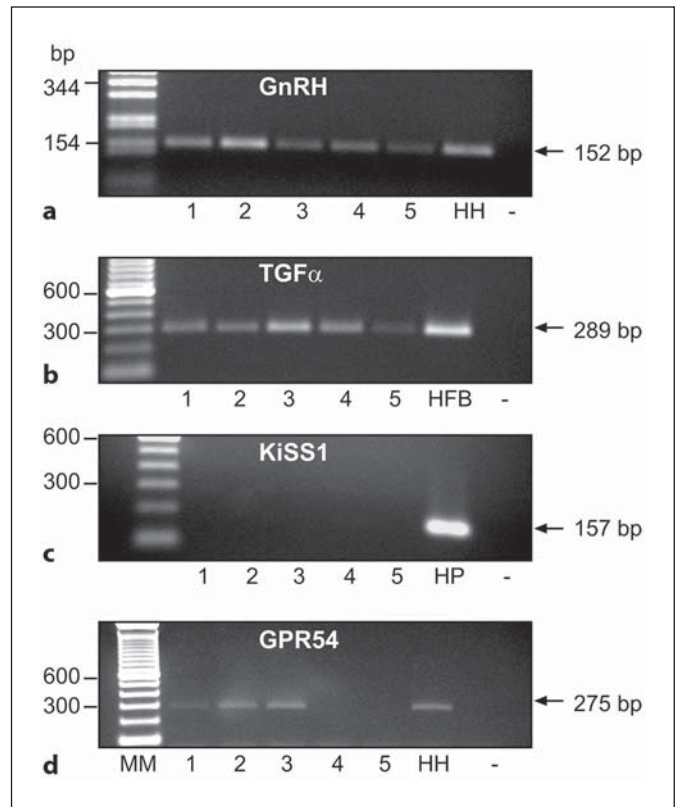

Fig. 3. The genes encoding GnRH (**a**), TGFα (**b**), KiSS1 (**c**) and GPR54 (**d**) are not selectively expressed in an HH associated with CPP (HH-1) in comparison to three HHs not accompanied by CPP (HH-2 to 4), and one HH (HH-5) removed 9 years after the occurrence of CPP. Notice that while *GnRH* and *TGFα* are expressed in all HHs, *KiSS1* is not expressed in any. In contrast *GPR54* mRNA is found in three of the five HHs studied. Also notice that *TGFα* mRNA abundance is higher in HH-3, which was resected from a patient who underwent CPP not before, but shortly after surgery. MM = Molecular marker; – = PCR, no RT control; HH = human hypothalamus; HFB = human fetal brain; HP = human placenta.

nisms underlying the onset of puberty in this species are similar to those operating in humans [18]. The content of *mGluR1A* mRNA increased ($p < 0.05$) in the hypothalamus (fig. 4a), but not in the cerebral cortex (inset), at the initiation of puberty, remaining moderately elevated thereafter. Neither *IA-1* nor *TSG-6 mRNA* abundance increased in the hypothalamus during postnatal development (fig. 4b, c).

Identification of Genes with Decreased Expression in HH with CPP

Fifteen genes showed a 2-fold or greater decrease in expression in HH-1 as compared with HH-2 to 4 (online
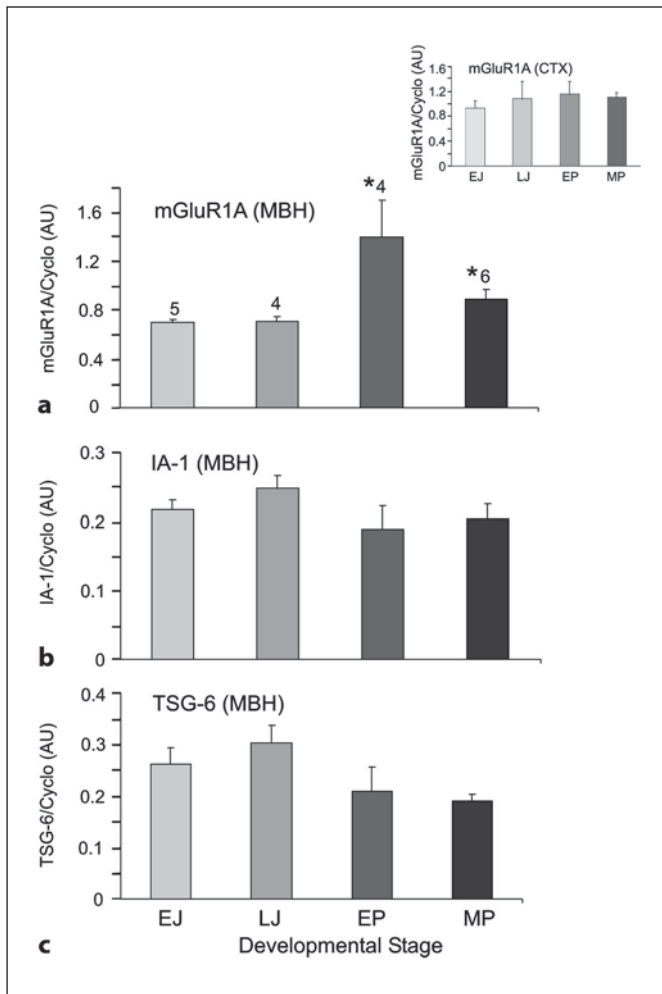
Fig. 4. Expression of *mGluR1A* (**a**), but not that of *IA-1* (**b**) or *TSG-6* (**c**), increases in the medial basal hypothalamus (MBH) of female rhesus monkeys at the time of puberty, as assessed by semi-quantitative PCR. Values are expressed as the ratio between each gene of interest and the levels of cyclophilin mRNA detected in each sample. The increase in hypothalamic mGluR1A mRNA content was not observed in the cerebral cortex (CTX, **inset** in **a**). * $p < 0.05$ vs. early juvenile group. EJ = Early juvenile (8.9–11.7 months of age); LJ = late juvenile (1.2–1.8 years of age); EP = early puberty (2–3 years of age); MP = mid-puberty (3–4 years of age). Bars are mean and vertical bars represent SEM. Numbers on top of bars in **a** indicate the number of animals per group.

suppl. table 1, www.karger.com/doi/10.1159/000111815). The greatest decrease observed was in glutathione S-transferase M5 *(GSTM5)* mRNA levels. GSTM5 is an enzyme that contributes to the detoxification of carcinogens, products of oxidative stress, and environmental toxins, by conjugation with glutathione [19]. Like the

other 14 genes with decreased expression in HH-1, the contribution of *GSTM5* to HH-induced puberty is obscure.

Discussion

The present study demonstrates that expression of a discrete cohort of genes involved in transcriptional control, cell-cell communication and cell adhesiveness is increased in an HH associated with sexual precocity in comparison with HHs that do not elicit advanced sexual development. The common feature linking all cases (including HH-5, which was not analyzed by DNA arrays) is that all patients had seizures, and all had been unsuccessfully treated with antiepileptic drugs prior to surgery. However, because HHs are only resected when they give rise to intractable seizures, the number of HHs available for analysis was necessarily small and variable with regard to age at surgery, gender of the patients, and pubertal history.

Despite these differences, the selective increase in gene expression observed in HH-1 was remarkably consistent with the neuroendocrine clinical features of each patient. Thus HH-2 and 4, derived from patients in whom sexual precocity never occurred, exhibited essentially identical differences in gene expression when compared to HH-1. The differences between HH-1 and HH-3 were uniformly less accentuated, and they were more variable in the case of the few HH-5 genes examined. It is, therefore, possible that the initiation of puberty observed in patient 3 after surgery may have been related to activation of the same genes whose expression is increased in HH-1. The increased prevalence of TGFα mRNA found in this HH is consistent with this possibility. Judging from the limited information gathered from HH-5, it would also appear that gene activation is not a permanent feature of HH neuropathology. The fact that sexual precocity was arrested in patients 1 and 5 by long-term GnRH antagonist treatment is also an important factor that needs to be taken into account when interpreting the results, because the GnRH treatment itself may have caused some of the changes in gene expression we detected.

The present results do not identify gene defects that may be responsible for the development of HHs, but suggest that HHs associated with CPP have a gene expression profile distinct from that of HHs not eliciting premature sexual development. They also suggest that genes showing increased expression in the HH associated with CPP may be more useful for an understanding of this condi-

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tion than genes with a decreased expression. Examination of additional HHs associated with sexual precocity is necessary to verify the validity of these concepts.

We recently used DNA microarrays to query the hypothalamus of female rhesus monkeys during pubertal development (Roth et al., unpubl. data), and quantitative proteomics to identify hypothalamic proteins that might be down- or upregulated in a mouse model of delayed puberty [20]. The results of these studies showed that expression of certain genes previously described as being involved in 'tumor suppression' – but that otherwise play a role in maintaining normal cell differentiation processes – increases in the hypothalamus at the time of monkey puberty (Roth et al., unpubl. data) or decreases in mice with delayed puberty [20]. A prominent example of a tumor suppressor gene now found to play a critical role in the initiation of puberty is *KiSS1* [3, 4]. Before its function in the control of puberty was discovered, *KiSS1* was known as a suppressor of tumor metastases [21].

Of the 10 genes showing increased expression in HH-1, six had been earlier proposed to have roles in tumor suppression. One of them is *CENP-F* [10]. Two others, *IA-1* (insulinoma-associated-1, also known as *INSM1*) and *MEF2A*, are transcription factors. IA-1 is a zinc finger transcriptional repressor involved in neuronal differentiation with expression restricted to the embryonic nervous system and neuroendocrine tumors [11 and references therein]. MEF2A, is a transcription factor also required for neuronal differentiation and survival [12], in addition to dendritic arborization and dendritic spine formation [22]. Two additional genes related to tumor formation play a physiological role in cell-cell communication. One of them, *VILIP-1* (visinin-like protein-1) is a member of the family of neuronal calcium sensor proteins [14]; loss of *VILIP-1* expression is associated with accelerated tumor cell invasiveness [23]. *C11ORF25* encodes an eight-transmembrane protein with similarity to three other genes located on chromosomes 11 and 12; like other members of the family, *C11ORF25* is amplified in malignant tumors [24]. C11ORF25 is predicted to be involved in the intracellular transport of yet to be identified molecules. Finally, *TSG-6* (tumor necrosis factor-stimulated gene 6) was also highly expressed in HH-1. TSG-6 is a multifunctional protein secreted in response to TNFα stimulation and elevated cAMP levels that binds to hyaluronan, a component of the extracellular matrix [15]. As a downstream component of p53-mediated cell cycle arrest [25], *TSG-6* is a potential tumor suppressor gene. It thus appears that in both normal puberty and HHs there is an activation of genes that, having diverse cellular func-

tions, share the common feature of having been earlier identified as involved in tumor suppression.

An additional gene encoding a protein involved in cell-cell communication and found to be upregulated in HH-1 is much less well-characterized. This gene, T cell activation leucine-rich repeat containing 8 *(LRRC8)* is a member of a highly conserved family of leucine-rich repeat proteins; LRRC8 may function as a receptor for an unknown ligand [26].

Two genes encoding proteins involved in transmembrane ion mobilization are also overexpressed in HH-1: a splice variant of the L-type high voltage-activated $Ca_v1.2$ channel β-subunit [27], and the sodium bicarbonate cotransporter *NBC3* (SLC4A7) [28]. The β-subunits of L-Ca^{2+} channels are critical modulators of the channel's gating activity, and are required for the correct targeting of the $Ca_v1.2$ complex to the cell membrane [29]. Sodium-coupled bicarbonate transporters are essential for the homeostatic maintenance of intracellular pH [28]. Mice lacking *NBC3* develop blindness and hearing loss due to degeneration of the corresponding sensory receptors, a condition similar to Usher syndrome [30].

Among the genes showing increased expression in HH-1, *IA-1* and *VILIP-1* deserve special mention because of their reported role in pancreatic β-cell/intestinal endocrine cell differentiation [31] and insulin secretion [32], respectively. Like in the pancreas, *IA-1* might function in HHs to promote the differentiation of neurosecretory cells. *IA-1* is highly expressed in the fetal brain, pancreas, and neuroendocrine tumors, but is absent in adult tissues [11, 33]. *VILIP-1*, a neuronal calcium sensor protein [14], is also expressed in pancreatic β-cells where it modulates insulin secretion instead of cell differentiation [32]. *VILIP-1* also appears to mediate metabotropic receptor-induced synaptic plasticity [34]. Conceivably, coupling of mGluR1 to VILIP-1 may set in motion cAMP- and cGMP-dependent pathways [14] that enhance the secretion of substances, such as PGE_2, able to stimulate GnRH release. These considerations suggest that the defining feature distinguishing HHs able to induce CPP from HHs not associated with sexual precocity is the presence of active neuroendocrine cells in HHs causing CPP. Of interest in this context is a recently described case of precocious puberty in a 2-year-old girl caused by a pancreatic neuroectodermal tumor [35].

Previous studies showed that HHs are composed of mature neuronal and glial elements [36, 37]. The functions of the 10 genes found to be overexpressed in HH-1 provide molecular support to this concept, because all of them, with the possible exception of *CENP-F*, are involved

in the regulation of differentiated neural cell functions [12, 13, 22, 27, 28, 38]. Noteworthy, the overexpression of *TSG-6*, a gene encoding a secreted protein that binds to the extracellular matrix component glycosaminoglycan hyaluronan [15], may reflect the presence of growth factor-producing glia in HH-1, because TSG-6 synthesis is strongly upregulated by glial growth factors, such as bFGF, TGFα, TNFα and TGFβ [39].

Also of interest is the finding that all five HHs contained *GnRH* and *TGFα* mRNA, but only HH-1 and HH-5 were associated with precocious puberty before surgery, suggesting that GnRH neurons and TGFα-producing glia in HHs require a set of additional genes to become engaged in the cascade of events that allow HHs to induce sexual precocity. Interestingly, HH-3 had not only the gene expression profile most similar to HH-1, but also exhibited elevated TGFα mRNA levels, suggesting that this combination may have contributed to the prompt, and surprising, initiation of puberty seen in this patient after removal of the HH. The additional genes required for HHs to induce precocious puberty do not appear to include the KiSS1/GPR54 signaling complex because none of the HHs expressed KiSS1 mRNA, and the presence of GPR54 receptors was unrelated to the ability of the HH to elicit sexual precocity.

The onset of normal female puberty in nonhuman primates was not accompanied by increased hypothalamic expression of *IA-1* or *TSG-6*, but it did show an increased abundance of *mGluR1A* mRNA. These findings suggest that either the increases in *IA-1* and *TSG-6* mRNA content observed in HH-1 do not occur during normal puberty, or that they reflect the presence in the HH of a subset of differentiated neurons and glial cells that represent only a small fraction of cells in the whole hypothalamus. Such a small population of cells would be difficult to detect by PCR analysis of the whole medial basal hypothalamus. It is also possible that the alterations in gene expression observed in HH-1 are gender-dependent, and that, because of this, are not readily detectable in female monkeys undergoing normal puberty. Further studies are necessary not only to resolve these issues, but also to determine if expression in an in vitro context of key genes overexpressed in HH-1 results in the production of GnRH secretagogues able to accelerate the onset of puberty.

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