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GPR155: Gene organization, multiple mRNA splice variants and expression in mouse central nervous system

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ABSTRACT

Emerging evidence suggests that GPR155, an integral membrane protein related to G-protein coupled receptors, has specific roles in Huntington disease and autism spectrum disorders. This study reports the structural organization of mouse GPR155 gene and the generation of five variants (*Variants* 1–5) of GPR155 mRNA, including so far unknown four variants. Further, it presents the level of expression of GPR155 mRNA in different mouse tissues. The mRNAs for GPR155 are widely expressed in adult mouse tissues and during development. In situ hybridization was used to determine the distribution of GPR155 in mouse brain. The GPR155 mRNAs are widely distributed in forebrain regions and have more restricted distribution in the midbrain and hindbrain regions. The highest level of expression was in the lateral part of striatum and hippocampus. The expression pattern of GPR155 mRNAs in mouse striatum was very similar to that of cannabinoid receptor type 1. The predicted protein secondary structure indicated that GPR155 is a 17-TM protein, and *Variant* 1 and *Variant* 5 proteins have an intracellular, conserved DEP domain near the *C*-terminal.

1. Introduction

An integral membrane protein termed GPR155 has been identified by systematic search for homologies to known G-protein coupled receptors (GPCRs). This protein appears to have specific roles in the brain based on the reports, characterizing its significant dysregulation in the caudate nucleus of Huntington disease patients and animal models [1,2] and in lymphoblastoid cells of humans with autism spectrum disorders [3]. At present, however, the gene organization, tissue distribution and localization of GPR155 in central nervous system have been elucidated at a less extent.

We report the structural organization of mouse GPR155 gene by sequencing all intron–exon boundaries and the generation of five variants of GPR155 mRNA including so far unknown four variants in the mouse (GenBank No. HM161763; HM161764; HM161765; HM161766). The sequence prediction shows that, unlike typical GPCRs, GPR155 is a 17-TM protein. *Variant* 1 and *Variant* 5 proteins have an intracellular, conserved DEP domain near the *C*-terminal. Further, this study presents the expression levels of GPR155 mRNA in different mouse tissues. Localization of the mRNA for GPR155 was performed by in situ hybridization, which reveals clues regarding the role of this receptor in motor, cognitive and limbic systems.

2. Materials and methods

2.1. In silico cloning and analysis of genomic structure of GPR155

The mouse DNA sequence from clone RP23-463K8 on chromosome 2 (GenBank No. AL954713), comprising several genes including the gene for GPR155, was analyzed with GENSCAN (http://genes.mit.edu/GENSCAN.html). The predicted peptide and coding sequences were set to query, by means of *tblastn* and *tblastx*, against *nucleotide collection (nr/nt)* database by BLAST search site (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Except for the reference mRNA and protein sequences (GenBank No. NM_001080707; NP_001074176), high homology was found with a protein sequence CAM25138. This sequence was used to obtain a protein-coding sequence, which was then subjected to *nucleotide blast* by BLAST search site. Multiple alignment, homology search and ORF prediction were performed by GENETYX 8.0 and SIM4 (http://pbil.univ-lyon1.fr/members/duret/cours/inserm210604/exercise4/sim4.html) for aligning cDNA and genomic DNA to identify exon and intron regions.

Multiple alignment of amino acid sequences predicted from GPR155 splice variants and analysis of interspecies homologies were performed with CLUSTALW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html). Analysis for signal peptide was performed by Signal IP 3.0 (http://www.cbs.dtu.dk/services/SignalP/). PROSITE (http://au.expasy.org/prosite/) and InterProScan (http://

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www.ebi.ac.uk/Tools/InterProScan/) served for the characterization of protein domains. Transmembrane stretches were predicted on the basis of Kyte and Doolittle hydrophobicity profile.

2.2. Expression of variants in different tissue

For PCR, mouse MTC Panel I (Clontech Laboratories Inc., Mountain View, CA) was used as template. The following primer pairs were used. (1) *Fwd3*(5′-gcttgcaaattccttctctggatcagc -3′)/*Rev2*(5′-gccagagatgatgattcccaccg-3′) to amplify part of the coding region common for all types of GPR155 mRNA variants; (2) *Fwd7*(5′-cagatagctcctaagcaccaggaagg -3′)/*Rev5*(5′-gtcactcaggctgctgaggcttactc-3′) to amplify the 3′-end of *Variant* 1, *Variant* 2, and *Variant* 5; (3) *Fwd5*(5′-ggccagtctcagaaccacaaaccagtaga-3′)/*Rev6*(5′-gcaccacacctg-tatgtcaaaggaatg-3′) to amplify a 712 bp fragment from the 3′-end of *Variant* 2; and (4) *Fwd4*(5′-ccttgtgggcgtgcttctgataactgg-3′)/*NMRev3*(5′-taggtttgagttgagggttggtcccgt-3′) to amplify the 3′-end of *Variant* 3 and *Variant* 4 (Fig. 1). The PCR mix (25 μl) contained 2.5 μl template cDNA, 1 μM forward and reverse primers, 200 μM dNTPs (Takara, Otsu, Japan), and 0.5 U Ex Taq HS (Takara). The parameters used for PCR were as follows: preheat 95 °C for 2 min; 25 cycles (40 cycles were used to amplify the 3′-end of *Variant* 2), first 95 °C for 0.5 min; second, 68 °C for 0.5 min; and third 72 °C for 0.5 min. To further amplify the 3′-end of *Variant* 3 and *Variant* 4, nested PCR was carried out with primers *Fwd5*/*NMRev3* (*Variant* 3) and *Fwd6*(5′-gagggctctgttttcttgggtgagttg-3′)/*Rev4*(5′-agtgcttgggattaacctagagccttc-3′) (*Variant* 4). The product from the first PCR was diluted 1:100 and used as a template for reamplification at 20 cycles with the same PCR parameters as described above. The products from the nested PCR were sequenced with ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems, Tokyo, Japan).

2.3. PCR-based cloning and analysis of GPR155 mRNA variants, and in situ hybridization histochemistry

2.3.1. Animals

All experiments were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and the Kansai Medical University local guidelines for animal experimentation. Every effort was made to minimize the number of animals used and their suffering.

2.3.2. RT-PCR

RNA was obtained from an adult male C57BL/6J mouse as described previously [4]. One microliter aliquot of reverse-transcribed product was amplified by PCR using forward and reverse primers. (1) *Fwd1*(5′-ctctgtgagcatcggcttccttctcc-3′) and *Rev1*(5′-gaggctttgccaattaagacgctgtag-3′) were used to amplify a 577 bp and 701 bp fragments from the 5′-noncoding region of GPR155 mRNAs; *Fwd2*(5′-gtctgattgttgaccagcaacttcaagg-3′) and *Rev1* were used to amplify 611 bp fragment containing the alternatively spliced second exon in the 5′-noncoding region after nested PCR with 1:100 diluted template from *Fwd1*/*Rev1* reaction; (2) *Fwd3* and *Rev2* were used to amplify 724 bp fragment in the common protein-coding region of the GPR155 mRNAs; (3) *Fwd6* and *Rev4* were used to amplify 629 bp fragment from the 3′-noncoding end of the *Variant* 4; (4) *Fwd7* and *Rev5* primers were used to amplify a 625 bp fragment of 3′-end of *Variant* 1, *Variant* 2 and *Variant* 5; and (5) *mCB1-Fwd*(5′-ggcagtaccatctcttctcacagctac-3′) and *mCB1-Rev*(5′-cagctaaacctgacagtatccgacagc-3′) primers were used to amplify 738 bp fragment from the 3′-end of mouse cannabinoid receptor 1 (GenBank No. NM_007726).

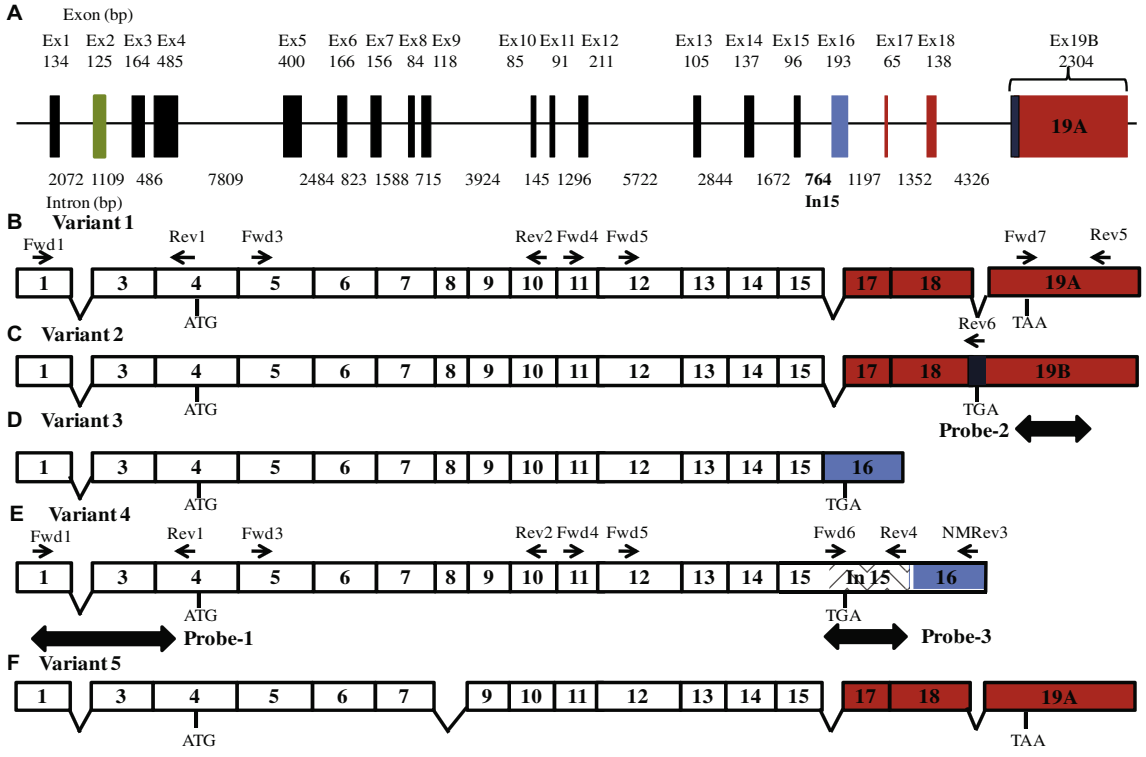

Fig. 1. Gene organization and mRNA splicing of mouse GPR155. (A) Scheme of exon–intron organization of GPR155. (B) *Variant* 1 encodes an 868-amino acid protein. (C) *Variant* 2 encodes a 783-amino acid protein. (D) *Variant* 3 encodes a 706-amino acid protein. (E) *Variant* 4 encodes a 715-amino acid protein. (F) *Variant* 5 encodes an 840-amino acid protein and. The arrows indicate the sites of primers for PCR. Positions of antisense DIG-labeled Probe-1, Probe-2 and Probe-3 are indicated by black left–right arrows.

2.3.3. Riboprobes synthesis

Four riboprobes were obtained in antisense and sense strand, namely: (1) Probe-1 – against 5′-noncoding region; (2) Probe-2 – against 3′-noncoding region of *Variant* 1, *Variant* 2, and *Variant* 5; (3) Probe-3 – against the nonspliced sequence between Exon 15 and Exon 16 of the *Variant* 4 of GPR155 mRNA; and (4) Probe-CB1 – against 3′-end of cannabinoid receptor 1.

2.3.4. In situ hybridization

Six male adult C57BL/6J mice were used as previously described [4]. To assist the identification of neurons and nuclei some horizontal sections were mounted and stained with Cresyl violet. Free-floating tissue sections were processed for in situ hybridization as described previously [4].

3. Results

3.1. Organization of mouse GPR155 gene

The computer-assisted analysis of exon–intron framework revealed that the GPR155 gene consists of nineteen exons (Fig. 1A). The genomic organization of GPR155 is shown schematically (Fig. 1). Five mRNA variants were noted in the present study. All of them showed deletion of Exon 2. It could be only detected by nested PCR amplification and its role in the expression of GPR155 remains to be elucidated.

Variant 1 consisted of seventeen exons encoding a protein with 868-amino acids. It shows deletion of Exon 2, Exon 16 and partial deletion of Exon 19. The TAA stop codon was located 1880 bp upstream of the ATTAAA polyadenylation signal in Exon 19 (Fig. 1B). *Variant* 2 has the same structure as the first variant but with additional 53 bp of the Exon 19. In that region is the TGA stop codon. This type of GPR155 mRNA has an open reading frame encoding a protein with 783-amino acids (Fig. 1C). *Variant* 3 corresponded to the GenBank sequence NM_001080707. It has fifteen exons encoding 706-amino acid protein. Polyadenylation signal (ATTAAA) was located 146 bp downstream of TGA stop codon in Exon 16 (Fig. 1D). *Variant* 4 was characterized by the presence of the 765 bp intronic sequence between Exon 15 and Exon 16 which comprised the TGA stop codon. The ATTAAA polyadenylation signal was located 883 bp downstream of the stop codon (Fig. 1E). *Variant* 5 has the same structure as *Variant* 1, but with an in frame deletion of Exon 8. All five types of mRNA have common ATG start codon located in Exon 4.

The alignment of the amino acid sequences of *Variant* 1, *Variant* 2, *Variant* 3, *Variant* 4, and *Variant* 5 reveal 98–99% sequence identity. The main difference in the amino acid sequences was found

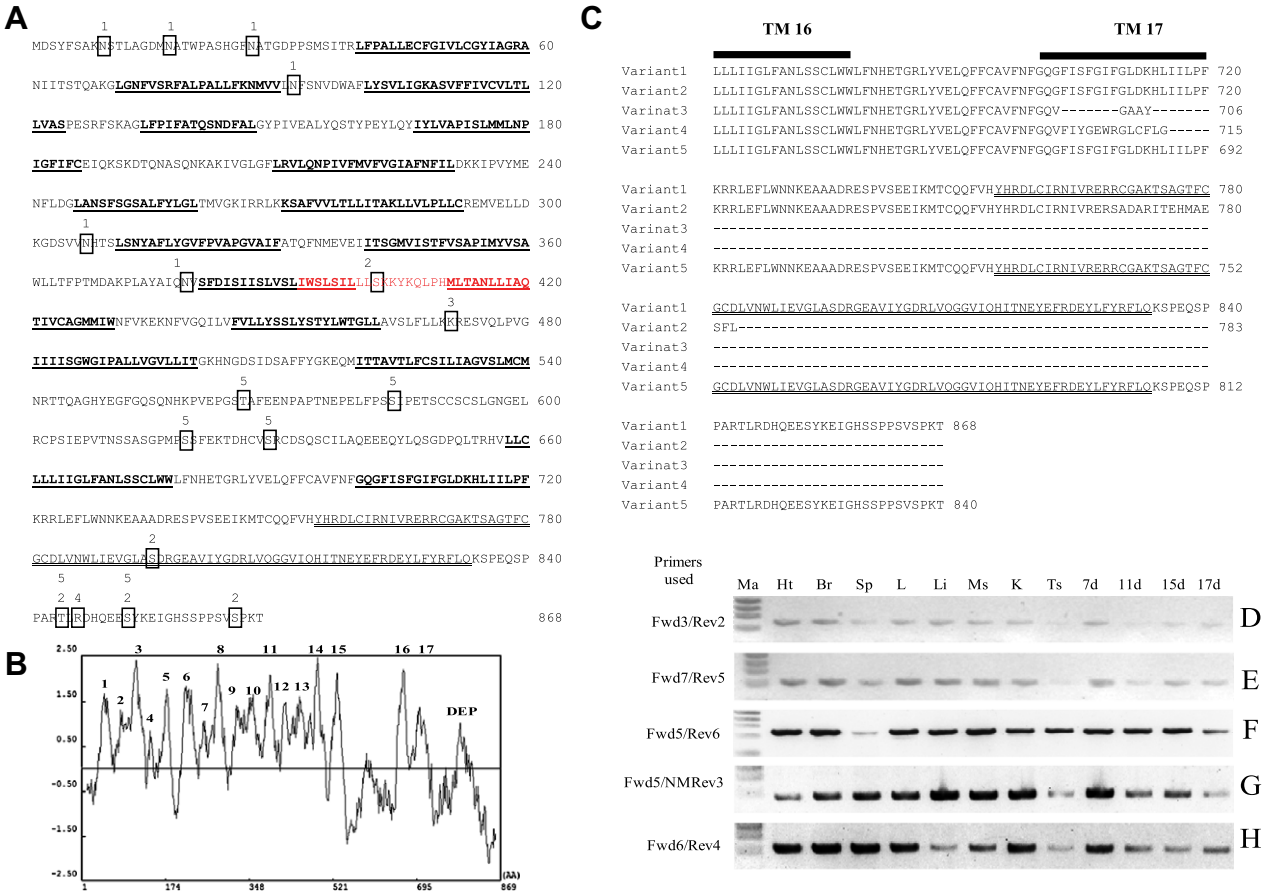

Fig. 2. (A) The predicted amino acid sequence of mouse GPR155 *Variant* 1 protein. Protein motifs are marked with squares and numbered accordingly: (1) *N*-glycosylation site; (2) protein kinase C phosphorylation site; (3) cAMP- and cGMP-dependent protein kinase phosphorylation site; (4) tyrosine kinase phosphorylation site; and (5) casein kinase II phosphorylation site. There are 17 transmembrane domains shown in bold and underlined. The conserved DEP domain is double-underlined. The deleted 28 amino acids of *Variant* 5 are shown in red. (B) Kyte and Doolittle hydrophobicity profile of GPR155 *Variant* 1 protein. A series of numbered hydrophobic stretches provide this protein with 17-TM regions. (C) Multiple sequence alignment of the *C*-terminal of GPR155 protein variants. The position of the DEP domain is double-underlined. (D–H) Expression of GPR155 mRNAs in mouse tissues. The expression level of common protein-coding region for all splice variants (D), 3′-noncoding regions of *Variant* 1, *Variant* 2 and *Variant* 5 (E), 3′-region of *Variant* 2 (F) and 3′-noncoding regions of *Variant* 3 (G) and *Variant* 4 (H) of GPR155 mRNA are compared by PCR amplification using mouse MTC Panel I as cDNA template. Lanes (Ht) heart; (Br) brain; (Sp) spleen; (L) lung; (Li) liver; (Ms) muscle; (K) kidney; (Ts) testis; (7d) 7-day embryo; (11d) 11-day embryo; (15d) 15-day embryo; (17d) 17-day embryo; and Ma, marker ϕX 174/HaeIII.

near the *C*-terminal (Fig. 2C) and *Variant* 5 lacks 28 amino acids from position 392 to 420 (Fig. 2A). The consensus *N*-glycosylation sites and phosphorylation sites are shown on Fig. 2A. It appears that all GPR155 protein variants lack signal peptide. All sequences have a conserved domain of amino acids from position 39 to 360, typical for the membrane transport proteins and auxin efflux carrier proteins. In contrast to other protein variants, *Variant* 1 and *Variant* 5 have a DEP domain at their *C*-terminal end from amino acid 755 to 833 (Fig. 2C). The amino acid sequence of the DEP domain is conserved between species and between other DEP domain-containing proteins in mouse. Analysis of the hydrophobicity profile (Kyte and Doolittle) revealed that *Variant* 1 protein has 17 transmembrane stretches (Fig. 2B), which is also supported from the localization of predicted *N*-glycosylation and phosphorylation sites. The *N*-terminal of the protein bears three consensus *N*-glycosylation sites and most probably has extracellular location. The *C*-terminal of the protein has many phosphorylation sites, the DEP domain and most probably is located intracellularly.

The present study revealed that the amino acid sequences of GPR155 are conserved among species (88%–95% identity) including human, mouse, rat, dog and cow. The identity of amino acid sequences was highest (95%) between mouse and rat. The identity between mouse GPR155 *Variant* 1 and human sequences was 89% (data not shown).

3.2. Expression levels of GPR155 mRNAs in tissue

Different primer sets were used (Fig. 1) to amplify fragments of the 3′-noncoding ends and of the coding region, common for all types of mRNA (Fig. 2D–H).

In tissues used here, *Variant* 3 and *Variant* 4 could only be detected by means of nested PCR amplification (Fig. 2G–H). The expression of *Variant* 1, *Variant* 2, *Variant* 5 and the part of the coding region was demonstrated by means of conventional PCR amplification. The expression of *Variant* 1, *Variant* 2, and *Variant* 5 was detectable at high level in brain, heart, lung, liver, muscle, kidney and 7-day embryo; a low level of expression was detectable in spleen, 11-day, 15-day, and 17-day embryo and the lowest level in testis (Fig. 2E and F). The level of expression of the common protein-coding region was similar to that of the 3′-noncoding end of *Variant* 1, *Variant* 2, and *Variant* 5 (Fig. 2D).

Using primers towards the 3′-noncoding region of *Variant* 3 along with the expected fragment (532 bp), a longer fragment (1296 bp) was visible. This fragment was sequenced and identified as containing a nonspliced sequence between Exon 15 and Exon 16 of *Variant* 3. The expression pattern of these two types of GPR155 mRNA was almost equal in all tissues with slight prevalence of *Variant* 3 (Fig. 2G and H).

3.3. Localization of GPR155 mRNAs in mouse brain and spinal cord

In mouse brain, the identical pattern of hybridization was obtained for DIG-labeled riboprobes Probe-1 (see Fig. 1 for probe map) and Probe-2. It should be noted that the intensity of hybridization signal was higher for Probe-1 than Probe-2. Positive hybridization signal given by Probe-3 was notable only in the hippocampal formation (Fig. 4C′), cerebellum (Fig. 4L′) and piriform cortex, and at a very low level in the lateral striatum. Hereafter, the description of GPR155 distribution is based on the in situ hybridization with Probe-1 (Figs. 3 and 4).

3.3.1. Olfactory system

In the olfactory bulb, intense hybridization signal was found in the mitral cell layer, granular cell layer and glomerular layer. In the

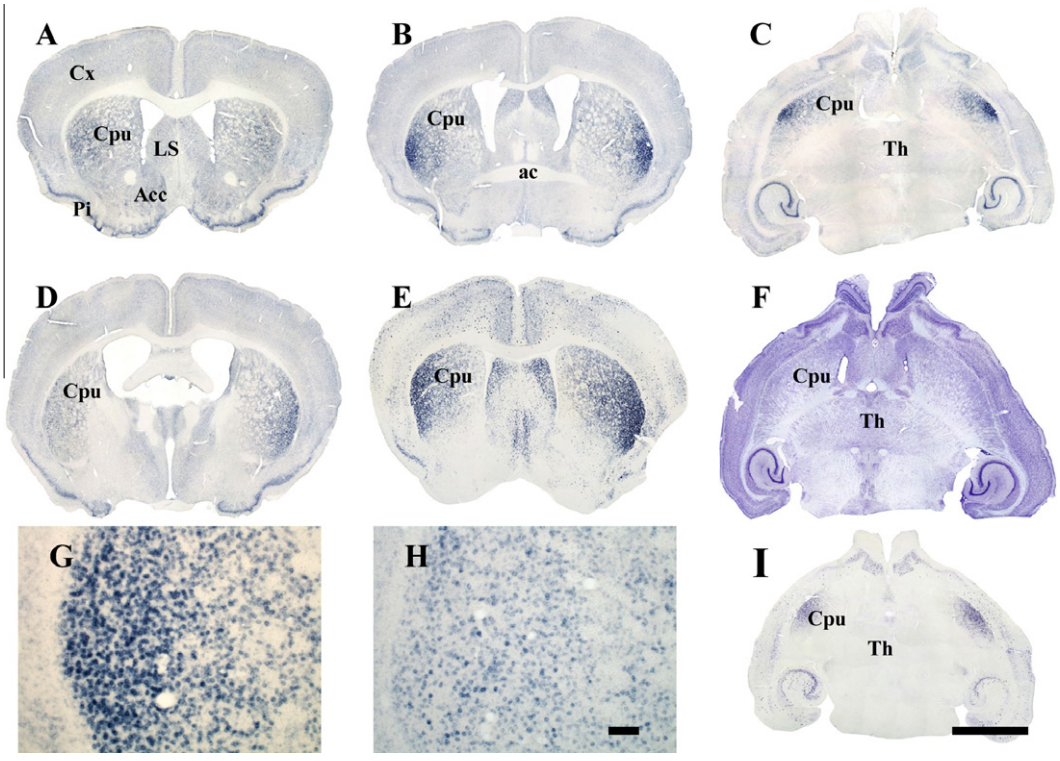

Fig. 3. Localization of GPR155 mRNA in adult mouse forebrain structures. Low-magnification merged photomicrographs (A–F) of coronal and horizontal brain section hybridized with Probe-1. (G and H) are high magnification images of section (B) and (D). (E and I) are low-magnification merged photomicrographs of sections hybridized with Probe-CB1 and section (F) is stained with Cresyl violet. Scale bar = 1 mm (A–D, I) and 100 μm (G and H). Acc, accumbens nucleus; ac, anterior commissure; Cpu, caudate-putamen (striatum); LS, lateral septal nucleus; Pi, piriform cortex; and Th, thalamus.

anterior olfactory nucleus, the labeling was low to moderate (Fig. 4A). High intensity hybridization signal was found in the olfactory tubercle and piriform cortex (Fig. 3A, B, D, and E).

3.3.2. Cerebral cortex and amygdaloid region

Moderate expression of GPR155 mRNA could be observed in layers II and III in the neocortex. In deeper layers V and VI, the level of expression was somewhat lower (Fig. 4B). The expression level in all amygdaloid nuclei was moderate to high. The highest hybridization signal was observed in the basolateral and lateral amygdaloid nuclei (Fig. 4E and F).

3.3.3. Basal ganglia

In the striatum, the hybridization signal was higher than in any other region. Signal intensities showed distinct regional variations. Pronounced lateral-to-medial gradient of hybridization signal can be seen in frontal and horizontal sections hybridized with Probe-1 (Fig. 3). The highest expression level of GPR155 mRNA was observed in the clusters of medium-sized projection neurons located in the lateral striatum. The labeling density gradually decreased medially and ventrally. The cannabinoid receptor CB1 mRNA was also primarily localized to the medium-sized neurons dominating in the lateral striatum. The cells expressing CB1 mRNA occupied a broader area including the region in which the cells expressing GPR155 mRNA were present (Fig. 3E and I).

In the accumbens nucleus and globus pallidus, cells with low to moderate expression levels were scattered throughout.

3.3.4. Septal – hippocampal regions

In the lateral septal nucleus, moderately hybridized neurons were seen, while in medial septal nucleus and nucleus of diagonal band, the expression of GPR155 mRNA was low (Fig. 3A and B).

In the pyramidal cell layer of the hippocampus, most of the cells displayed very intense hybridization signal (Fig. 4C). A number of cells with moderate to low expression level were also scattered across the stratum oriens, stratum radiatum, and stratum lacunosum-moleculare. High expression of GPR155 mRNA was observed in the granule cell layer of dentate gyrus and some moderately labeled neurons could be seen in the molecular layer and especially in the hilar region of dentate gyrus (Fig. 4D).

3.3.5. Thalamic and hypothalamic regions

The hybridization signals in thalamic and hypothalamic nuclei were relatively low compared to other brain regions. However,

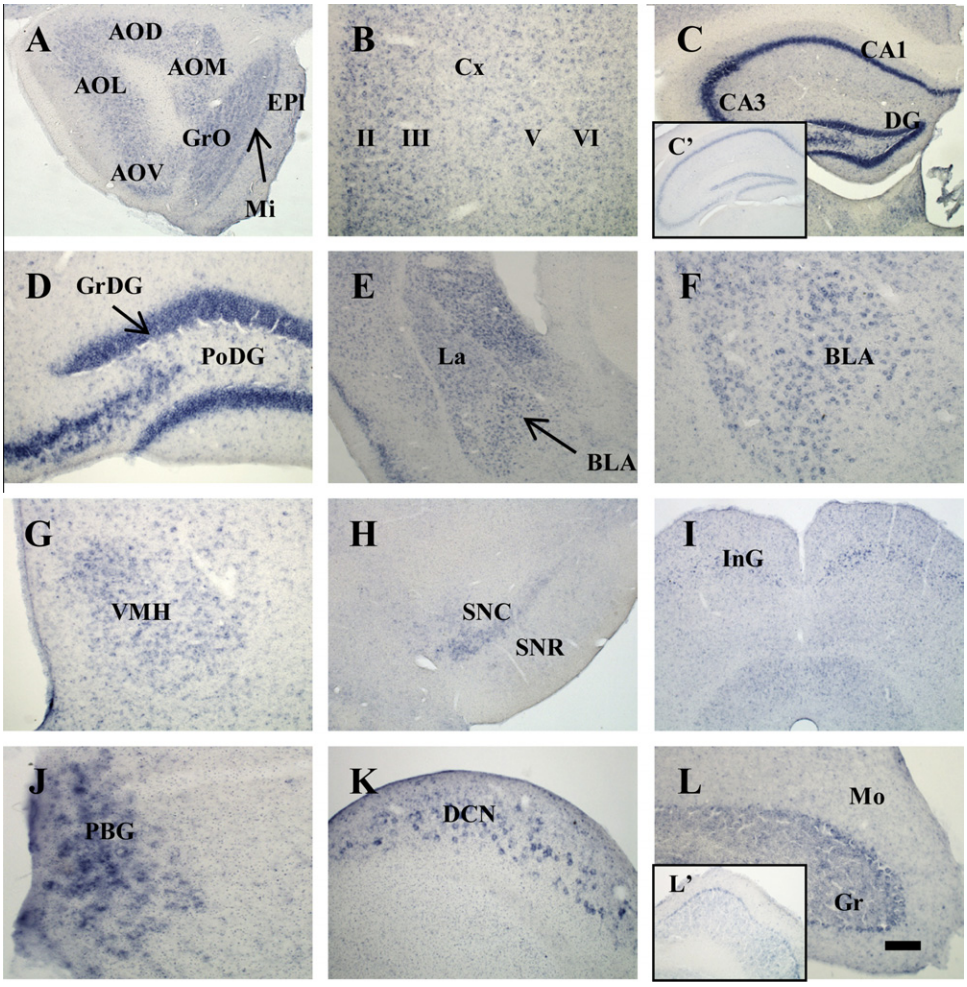

Fig. 4. Expression of GPR155 mRNA detected by in situ hybridization with Probe-1 in mouse olfactory bulb (A), cerebral cortex (B), hippocampus (C and D), amygdaloid nuclei (E and F), VMH (G), SCN (H), superior colliculus (I), PBG (J), DCN (K) and cerebellum (L). Low level of expression of GPR155 mRNA of *Variant* 4 was detected by in situ hybridization with Probe-3 in hippocampus (C′) and cerebellum (L′). AOD, anterior olfactory nucleus, dorsal; AOL, anterior olfactory nucleus, lateral; AOM, olfactory nucleus, medial; AOV, olfactory nucleus, ventral; BLA, anterior basolateral amygdaloid nucleus; Cx, cerebral cortex; DCN, dorsal cochlear nucleus; DG, dentate gyrus; EPI, external plexiform layer of olfactory bulb; Gr, granular cell layer of cerebellum; GrDG, granular cell layer of dentate gyrus; GrO, outer granular layer; InG, intermediate gray layer of superior colliculus; La, lateral nucleus of the amygdale; Mi, mitral cell layer of olfactory bulb; Mo, molecular cell layer of cerebellum; PBG, parabigeminal nucleus; PoDG, polymorphic layer of dentate gyrus; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulate; and VMH, ventromedial hypothalamic nucleus. Scale bar = 100 μm (D, F, G, J, K, L) and 200 μm (A, B, C, E, H, I).

low to moderate labeling was detected in the paraventricular, anterodorsal and anteroventral thalamic nuclei. Low expression level was found in the medial habenular nucleus. The ventromedial hypothalamic nucleus revealed moderate expression level of GPR155 mRNA (Fig. 4G).

3.3.6. Cerebellum

The cerebellar cortex displayed a moderate level of expression of GPR155 mRNA (Fig. 4L). The labeling was predominantly seen in Purkinje cells and in the granular cell layer. Small number of cells revealing low level of hybridization signal could be seen in the molecular layer.

3.3.7. Midbrain, brainstem and spinal cord

Hybridization signals in the midbrain were relatively low, although cells within the parabigeminal nucleus (Fig. 4J) and intermediate gray layer of the superior colliculus (Fig. 4I) displayed a moderate to high level of GPR155 mRNA expression. In the substantia nigra pars compacta, cells displaying moderate to high levels of expression were seen (Fig. 4H).

In the pons and medulla oblongata, the hybridization signal was found at a very low level. Only in scattered cells in the dorsal cochlear nucleus, high expression level of GPR155 mRNA could be observed (Fig. 4K).

In the cervical part of the spinal cord, moderately labeled small neurons could be observed in laminae I and II of the dorsal horn. In laminae V, VII, and X, some large neurons were labeled at a moderate level.

4. Discussion

Our results indicated that five different mRNA types for mouse GPR155 are transcribed from 19 exons located on chromosome 2 in the mouse. The predicted secondary structure of GPR155 *Variant* 1 protein, based on the hydrophobicity profile of Kyte and Doolittle, has 17 transmembrane domains, similar to the transmembrane architecture of human GPR155 (UniProtKB/Swiss-Prot Q7Z3F1). The *N*-terminal is most probably located to the outer surface of the cellular membrane and the *C*-terminal has intracellular localization. This orientation is suggested from the uneven number of transmembrane helices and from the position of the three consensus *N*-glycosylation sites near the *N*-terminal and five phosphorylation sites near the *C*-terminal.

We found that *Variant* 1 and *Variant* 5 proteins have a DEP domain and these variants are quite different from *Variants* 2, 3 and 4 in this respect. The DEP domain is present in a number of signaling molecules, including Regulator of G-protein Signaling (RGS) proteins. RGS regulates signal transduction by increasing the GTPase activity of G-protein alpha subunits, thereby driving them into their inactive GDP-bound form [5]. We are aware of intriguing findings that demonstrate the major role of the DEP domains in mediating the interaction of an RGS protein to the *C*-terminal tail of GPCRs, thus placing RGS in close proximity with its substrate G-protein alpha subunit [6–8].

All variants have a conserved amino acid sequence typical for membrane transport proteins including auxin efflux carrier proteins in plants that influence processes like the establishment of embryonic polarity and plant growth. These proteins are 600–700 amino acids long and exhibit 8–12 transmembrane segments [9].

GPR155 mRNA subtypes were expressed in many tissues including brain and during embryonic development. In adult mouse brain, GPR155 mRNAs showed a regionally distinct and unique distribution revealed by in situ hybridization. The GPR155 was widely distributed in the forebrain (cerebral cortex, basal ganglia and hippocampal formation) and has more restricted distribution in the midbrain and hindbrain. It shared very similar distribution in the lateral striatum with the CB1 receptors [10–14]. According to their location, most of the cells expressing GPR155 mRNA in the lateral striatum are likely to express CB1 receptors and GABA. From the in situ hybridization results obtained with three different riboprobes, it could be assumed that *Variant* 1 is the dominant GPR155 mRNA in mouse brain.

The most striking aspect of the GPR155 in mouse brain was the dense localization in discrete parts of the basal ganglia. The striatum displayed very high levels of expression, however, the hybridization signal gradually decreased from lateral-to-medial parts. The cellular pattern of distribution of GPR155 mRNA in striatal projection neurons resembled that described by Hohmann and Herkenham [15] for CB1 receptor. The area of receptor enrichment closely coincides with the part of the striatum that receives afferent inputs from primary motor and sensory cortex in the rat [16]. These data provide anatomical support that GPR155 might be involved in the modulation of sensorimotor and limbic inputs like CB1 receptors. The function of CB1 receptors is linked to depolarization-induced suppression of GABA-mediated inhibition [13,17]. Recently, the preferential expression of GPR155 mRNA in the striatum was revealed by serial analysis of gene expression and it was demonstrated that the regional enrichment is conserved in human [2]. The important role of GPR155 in the motor brain function was evident from its down-regulation in the striatum of Huntington disease animal models [2] and Huntington disease patients [1]. Hodges [1] revealed by means of laser-captured microdissection that this down-regulation is due to the dysfunction of the affected neurons for the time before they eventually die. Similar decrease was observed in the expression of CB1 receptor mRNA in the lateral striatum of transgenic Huntington disease mice [18], thus implicating some common features in the expression and function of GPR155 and CB1 receptor. Moderate expression of mRNA for GPR155 in the substantia nigra and cerebellum also implicates its significant role in motor control.

The GPR155 protein might also play a role in sensory information processing and memory as it was highly expressed in the cerebral cortex, hippocampus, septal nuclei, amygdaloid complex and significantly dysregulated in a condition like autism spectrum disorders, characterized by impaired social interaction, impaired communication, restricted and repetitive interests and activities [3].

In addition, other sites that mediate sensory information like the olfactory bulb, superior colliculus, parabigeminal nucleus and dorsal cochlear nucleus also show moderate expression of GPR155 mRNA. The GPR155 expression in the superior colliculus and parabigeminal nucleus might suggest its functional roles in the regulation of visual motor activity.

5. Conclusions

In summary, mouse GPR155 mRNA comprised five variants including newly identified four variants. They are translated into five different proteins. The most abundantly expressed *Variant* 1 is probably a 17-TM membrane protein. The mRNAs for GPR155 are widely expressed in adult mouse tissues and during development. The GPR155 mRNAs are highly expressed in forebrain regions and have more restricted distribution in the midbrain and hindbrain regions. The highest level of expression was in the lateral part of striatum and the hippocampus. The expression pattern of GPR155 mRNAs in mouse striatum was very similar to that of CB1 receptors in rat. Many neurons that are identifiable as GABAergic might express GPR155, implicating its important role in GABAergic neurotransmission.

Acknowledgments

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