Title

Lysosomal Cathepsin Protease Gene Expression Profiles in the Human Brain During Normal Development

Journal

Journal of Molecular Neuroscience, 65(4)

ISSN

0895-8696

Authors

Hsu, Amy
Podvin, Sonia
Hook, Vivian

Publication Date

2018-08-01

DOI

10.1007/s12031-018-1110-6

Peer reviewed

HHS Public Access

Author manuscript

J Mol Neurosci. Author manuscript; available in PMC 2020 April 27.

Published in final edited form as:

J Mol Neurosci. 2018 August ; 65(4): 420–431. doi:10.1007/s12031-018-1110-6.

Lysosomal Cathepsin Protease Gene Expression Profiles in Human Brain during Normal Development

Amy Hsu[1], **Sonia Podvin**[1], **Vivian Hook**[1,2,*]

[1]Skaggs School of Pharmacy and Pharmaceutical Sciences

[2]Dept. of Neurosciences, School of Medicine, University of California San Diego, La Jolla, CA 92093, USA

Abstract

Cathepsin protease genes are necessary for protein homeostasis in normal brain development and function. The diversity of the fifteen cathepsin protease activities raises the question of what are the human brain expression profiles of the cathepsin genes during development from prenatal and infancy to childhood, adolescence, and young adult stages. This study, therefore, evaluated the cathepsin gene expression profiles in sixteen human brain regions during development by quantitative RNA-sequencing data obtained from the Allen Brain Atlas resource. Total expression of all cathepsin genes was lowest at the early prenatal stage which became increased at the infancy stage. During infancy to young adults phases, total gene expression was similar. Interestingly, the rank ordering of gene expression among the cathepsins was similar throughout the brain at the age periods examined, showing (a) high expression of cathepsins B, D, and F, (b) moderate expression of cathepsins A, L, and Z, (c) low expression of cathepsins C, H, K, O, S, and V, and (d) very low expression of cathepsins E, G, and W. Results show that the human brain utilizes well-defined, balanced patterns of cathepsin gene expression throughout the different stages of human brain development. Knowledge gained by this study of the gene expression profiles of lysosomal cathepsin proteases among human brain regions during normal development is important for advancing future investigations of how these cathepsins are dysregulated in lysosomal-related brain disorders that affect infants, children, adolescents, and young adults.

Keywords

lysosomes; cathepsin proteases; brain; human; gene expression; development

Introduction

The human brain undergoes dynamic changes in cellular functions throughout development from prenatal to infancy, childhood, adolescence, and young adult stages. The complex development and maturation of brain components requires lysosomal cathepsin proteases to maintain the balance of cellular protein homeostasis through protein degradation. Lysosomal cathepsin proteases are required for health and participate in numerous brain disorders that

*Address correspondence to: Dr. Vivian Hook, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Dr. MC0719, La Jolla, CA 92093-0719, phone 858-822-6682, vhook@ucsd.edu.

occur at different development age periods. Dysregulation of certain cathepsins occur in brain disorders including brain injuries (Hook et al. 2015; Stoka et al. 2016), lysosomal storage diseases (Jeyakumar et al. 2005), neurodegeneration (Nixon 2013; Hook et al. 2015; Yamashima 2016; Stoka et al. 2016), and others. It is, therefore, important to understand the normal expression patterns of cathepsin genes in human brain.

Lysosomal cathepsin proteases consist of cysteine, aspartyl, and serine protease subtypes (Barrett et al., 2004; Stoka et al., 2016). The cysteine cathepsins are the largest group composed of cathepsins B, C, F, H, K, L, O, S, V, W, and Z (also known as cathepsin X). The aspartyl cathepsin proteases consist of cathepsin D and cathepsin E. The serine cathepsin proteases are represented by cathepsin A and cathepsin G. These cathepsins possess a spectrum of proteolytic activities composed of endopeptidases, aminopeptidases, and carboxypeptidase types, each with preferences for particular cleavage sites of protein substrates. The breadth of the cathepsin proteolytic activities provides lysosomes with the ability to degrade diverse protein and peptide substrates to maintain cellular protein homeostasis.

The diversity of lysosomal cathepsin proteolytic activities and the dynamic brain functions controlled by different regions of the brain raises the question of what are the human brain expression patterns of the fifteen cathepsin genes at different developmental stages from prenatal to infancy, childhood, adolescence, and young age periods? This question can be answered by determining the gene expression profiles of the cathepsin genes at different development stages in human brain regions. The answer to the question may be that the cathepsins show differences in their expression patterns in different brain regions among the different age periods, or a uniform pattern of cathepsin gene expression profiles may exist in brain regions during development and maturation of the brain. To evaluate these alternative answers, this study compared the expression patterns of the fifteen cathepsin genes in sixteen human brain regions at different age periods of prenatal, infancy, childhood, adolescence, and young adult stages. Cathepsin gene expression was analyzed by quantitative Ref-Seq data from the Allen Human Brain Atlas resource (Hawrylycz et al., 2012; Sunkin et al., 2013). The Allen Human Brain Atlas is a public repository of gene expression data for all human genes in human brain (http://human.brain-map.org/).)

Results of this study demonstrated that total expression of all cathepsin genes was lowest at the early prenatal stage which became increased at the infancy stage. During infancy to young adult phases, total gene expression was similar. Of notable interest was the finding of the remarkable consistency in the proportion of high to moderate and low gene expression levels for the cathepsin genes during the different development periods in all brain regions examined. These results demonstrate the similar proportions of cathepsin gene expression patterns during human brain development. These findings suggest that a well-balanced pattern of lysosomal cathepsin gene expression occurs during normal human brain development for prenatal to infancy, childhood, adolescence, and young adult stages.

Materials and Methods

Allen Human Brain Atlas resource for human brain development expression of cathepsin genes.

The Allen Human Brain Atlas integrates genomic, anatomic, and age-related data (http://www.brain-map.org/)) (Hawrylycz et al., 2012; Sunkin et al., 2013) which was utilized for analyses of cathepsin gene expression levels in sixteen human brain regions during prenatal, infancy, childhood, adolescence, and young adult age periods. Data for human brain gene expression among the different age periods were conducted by the human brain development project of the Allen Brain Atlas, which provided RNA sequencing data (downloadable at http://www.brain-map.org/)) for quantitative gene expression analyses of the fifteen cathepsin protease genes in this study.

Human brain tissue samples from prenatal to infancy, childhood, adolescence, and young adult stages.

The Allen Human Brain Atlas describes the collection of the post-mortem human brain tissue samples from 42 brains that were obtained from donors (male and female) at ages representing early prenatal, late prenatal, infancy, childhood, adolescence, and young adult. Ages for each stage are shown in Table 1. Brain tissues were obtained from the Yale School of Medicine, the National Institute of Mental Health, the Albert Einstein College of Medicine, the University of Maryland, the University of Washington, Advanced Bioscience Resources, and the University of Newcastle. The ethnicities of subjects consisted of European, Asian, African American, Hispanic, and a mix of two or more cultural backgrounds. Negative selection criteria included aneuploidy or chromosomal abnormalities, maternal drug or alcohol abuse during pregnancy, potassium chloride, salt water, or urea injection into the amniotic sac during pregnancy, brain malformations or lesions, neuronal loss or abnormalities in the brain, samples that tested positive for Hepatitis B, Hepatitis C, or HIV, and postnatal specimens with excessive drug or alcohol abuse or neurological and psychiatric disorders or brain injuries.

The sixteen brain regions analyzed consisted of the amygdaloid complex, anterior (rostral) cingulate (medial prefrontal) cortex, cerebellar cortex, dorsolateral prefrontal cortex, hippocampus (hippocampal formation), inferolateral temporal cortex, mediodorsal nucleus of thalamus, orbital frontal cortex, posterior (caudal) superior temporal cortex, posteroventral (inferior) parietal cortex, primary auditory cortex (core), primary motor cortex, primary somatosensory cortex, primary visual cortex, striatum, and ventrolateral prefrontal cortex. Brain regions were histologically verified using the Nissl stain on tissue adjacent to the region of interest.

RNA-Seq analyses of cathepsin gene expression in human brain regions at different developmental stages.

RNA-Seq analyses were conducted for sixteen brain tissue regions; the protocol is illustrated in Figure 1. Brain tissues were dissected, RNA was extracted from pulverized tissue, and polyA+ RNA was purified from total RNA using magnetic beads linked to polyT-RNA. The purified mRNA was quantitated and spike-in RNA mixes were added for normalization of

gene expression across samples. RNA spike-in sequences were different for each brain region in order to ensure quality control by enabling detection of contaminated samples throughout the protocol. The RNA was subjected to first and second strand cDNA synthesis using reverse transcriptase and DNA polymerase I, respectively. Then, the ends of the DNA strands were repaired and adenylated at the 3'-ends. The resultant DNAs were sequenced and quantified using the Illumina Genome Analyzer IIx.

The genomic location for each read was mapped to a reference sequence and the gene expression of each gene of interest was measured by determining the number of reads that correspond to the specific location of that gene. The reads were normalized for read depth (the coverage of sequenced RNA fragments over the nown transcsript sequence), and gene length allowing for gene expression to be expressed in units of RPKM (reads per kilobase of exon model per million mapped reads) for RNA-Seq data. The RNA-Seq data was downloaded from the Allen Brain Atlas (http://www.brain-map.org/)) for analyses of the expression levels of the cathepsin protease genes.

The average gene expression level for each cathepsin protease in units of RPKM was calculated for the six age periods of early prenatal, late prenatal, infancy, childhood, adolescence, and young adult, in the sixteen brain regions for each age period. Also, the total cathepsin gene expression, the sum of expression levels of the fifteen cathepsin protease genes, was calculated for each age period as the mean ± standard error of the mean (s.e.m.). For each cathepsin, the percent of total expression (all fifteen cathepsins) was calculated as the mean ± standard error of the mean (s.e.m.). Statistical analyses utilized the Kruskal-Wallis non-parametric test, and the Dunn's multiple comparison post hoc test using GraphPad Prism 7. Statistical significance was assessed as $p < 0.05$.

Results

Cathepsin protease subtypes.

The cathepsin protease family members are composed of cysteine, aspartyl, and serine proteases. The fifteen human cathepsin protease genes are comprised of (a) the cysteine cathepsins B, C, F, H, K, L, O, S, V, W, and Z, (b) the aspartyl cathepsins D and E, and (c) the serine cathepsins A and G (supplemental Table S1). The expression of these cathepsin genes was analyzed in sixteen brain regions (illustrated in supplemental Figure S1) during prenatal, late prenatal, infancy, childhood, adolescence, and young adult stages. The ages for each of these developmental stages are shown in Table 1. Gene expression of the cathepsin proteases in human brain were measured by quantitative Ref-Seq analyses (illustrated in Figure 1, and explained in the methods).

Total cathepsin expression in sixteen brain regions from the prenatal stage to infancy, childhood, adolescence, and young adult stages.

Total cathepsin geene expression data (Figure 2) showed significant elevation of total cathepsin expression from early prenatal to infancy stages in the brain regions of amygdaloid complex, anterior cingulate cortex, dorsolateral prefrontal cortex, interolateral temporal cortex, orbital frontal cortex, posteroventral parietal cortex, primary somatosensory cortex,

primary visual cortex, and striatum (Figure 2 a, b, d, f, j, m. n, o). Significant increases in total cathepsin expression occurred from early prenatal to childhood in amygdaloid complex, anterior cingulate cortex and dorsolateral prefrontal cortex, interolateral temporal cortex, posterior superior temporal cortex, posteroventral parietal cortex, primary auditory cortex, primary motor cortex, primary visual cortex, and ventrolateral prefrontal cortex (Figure 2 a, b, d, f, i, j, k, l, n, p). Increases in total cathepsin expression occurred in the adolescence compared to early prenatal periods in dorsolateral prefrontal cortex, hippocampus, interolateral temporal cortex, orbital frontal cortex, posteroventral parietal cortex, and ventrolateral prefrontal cortex (Figure 2 d, e, f, h, j, p). The young adult stage showed increases in total cathepsin expression compared to early prenatal in all brain regions except cerebellar cortex and mediodorsal nucleus of thalamus (Figure 2 c, g). Further, the four developmental periods of infancy, childhood, adolescence, and young adult displayed similar levels of total cathepsin expression in the brain regions examined (no significant differences).

Profiles of cathepsin gene expression levels among human brain regions from prenatal to infancy, childhood, adolescence, and young adult stages.

The fifteen cathepsin genes were compared to illustrate their proportions of expression relative to total cathepsin expression, calculated as the sum of the fifteen cathepsin genes expressed (Figure 2). Results showed similar rank orders of relative abundances of cathepsin gene expression levels among the brain regions and developmental periods examined, described in detail below.

High expression was observed for cathepsins B (CTSB), cathepsin F (CTSF), and cathepsin D (CTSD) in all brain regions and all developmental stages examined (Figure 2, and Table 2). CTSB, CTSD, and CTSF comprised 12–32%, 25–49%, and 25–49% of total cathepsin expression among the sixteen brain regions during the six different age periods (Table 2).

Moderate levels of expression were observed for cathepsin L (CTSL), cathepsin Z (CTSZ), and cathepsin A (CTSA), among all brain regions and developmental age periods (Figure 2). CTSL, CTSZ, and CTSA represented 4–9%, and 3–12%, and 5–9% of total cathepsin expression among the brain regions and development stages (Figure 2, and Table 2).

The relative abundances of these six cathepsins of high and moderate levels of expression, but not those of low abundances, were visible in graphs displaying total cathepsin expression of the fifteen cathepsin genes (Figure 2). To visualize the relative proportions of the lower expressing cathepsins, such genes were graphed separately (Figure 3). Results showed similar relative levels of expression for each cathepsin among the brain regions and developmental stages examined. Low expression was displayed by cathepsin H (CTSH), cathepsin K (CTSK), and cathepsin O (CTSO) with expression ranges of 0.3–6.4%, 0.5–6.2%, and 1.5–3.9% of total cathepsin expression, respectively (Table 2). Lower expression was observed for cathepsin C (CTSC), cathepsin S (CTSS), and cathepsin V (CTSV) which showed ranges of expression of 0.15–1.7%, 0.22–1.3%, and 0.05–2.2% of total cathepsin expression, respectively (Table 2). Very low expression was observed for cathepsin E (CTSE), cathepsin G (CTSG), and cathepsin W (CTSW) which had ranges of expression of

0.001–0.02%, 0.003 −0.24%, and 0.01–0.17%, respectively, of total cathepsin expression (Table 2).

The high, moderate, low, and very low levels of expression among the cathepsin genes spanned four orders of magnitude (Table 2 and supplemental Table 2).

High expressing cathepsins B, F, and D during prenatal to young adult stages.

The high expressing CTSB, CTSF, and CTSD genes were examined individually in the brain regions at different development age periods (Figures 4–6).

Cathepsin B, a cysteine protease, showed increased expression during the periods of infancy, childhood, adolescence, and young adult compared to the early prenatal stage. These development periods showed 2–3 fold greater expression than in the early prenatal stage in most of the sixteen brain regions, except for cerebellar cortex and mediodorsal nucleus of thalamus (Figure 4 c, g). Expression of CTSB was similar among the stages of infancy, childhood, adolescence, and young adult in many brain regions (variations were not significant). Cathepsin F, a cysteine cathepsin, is abundantly expressed in human brain regions from prenatal through young adult stages. CTSF expression increased from early prenatal to the stages of infancy, childhood, adolescence, and young adult among the brain regions examined (Figure 5). Similar levels of CTSF expression were displayed during infancy through young adult stages (it is noted that variations were not significant).

Cathepsin D, an aspartyl cathepsin, increased during the transition from early prenatal to the stages of infancy through young adult in most brain regions (Figure 6), with the exception of cerebellar cortex (but not all age periods compared to early prenatal were significant). Expression of CTSD during infancy, childhood, adolescence, and young adult stages were generally similar. Higher expression during late prenatal compared to early prenatal was apparent, but was not statistically significant.

Moderately expressing cathepsins L, Z, and A during prenatal to young adult stages.

The moderately expressing cathepsins CTSL, CTSZ, and CTSA showed elevated expression at the postnatal age periods compared to the prenatal stage.

Cathepsin L, a cysteine protease, increased during transition from prenatal to the later infancy to young adult age periods (supplemental Figure S2); the exceptions were the cerebellar cortex and the mediodorsal nucleus of thalamus. In several brain regions, CTSL expression was higher in late prenatal compared to early prenatal (Fig. S2 a, e, g, j, k, l, m, n, o, and p), but not all were significant. Similar levels of CTSL expression were displayed during infancy, childhood, adolescence, and young adult in most brain regions.

Cathepsin Z, a cysteine protease, increased from the prenatal stage compared to the ages of infancy, childhood, adolescence, and young adult in many brain regions, with the exceptions of the anterior cingulate cortex and cerebellar cortex (supplemental Figure S3). Expression levels of CTSZ were generally similar in most brain regions during the age periods from infancy to young adult (no significant differences).

Cathepsin A, a serine protease, displayed increased expression during transition from prenatal to infancy through young adult stages (supplemental Figure S4), except for the cerebellar cortex. The general levels of cathepsin A expression at infancy to young adult stages were similar.

Low expressing cathepsins H and O during prenatal to young adult stages.

The gene expression levels of CTSH, a cysteine protease, increased from the prenatal stage to those at infancy through young adult (supplemental Figure S5), except in the cerebellar cortex. Several brain regions showed decreased CTSH expression during transition from childhood or adolescence stages to young adult, although not statistically significant (regions shown in Figure S5 b, d, e, f, g, h, l, m, n, p).

Expression of CTSO, a cysteine protease, increased from the prenatal stage to the infancy and childhood stages, with the exception of cerebellar cortex and mediodorsal nucleus of thalamus (supplemental Figure S6). CTSO expression from childhood to young adult decreased in several brain regions, but was not statistically significant.

The remainder of the low and very low expressing cathepsins displayed expression values in the range of 0.001–6.4% of total cathepsin expression (supplemental Table 2).

Consistent rank order groups of cathepsin gene expression levels in brain regions at prenatal to young adult stages.

The relative abundances of cathepsin expression were observed as four groups consisting of high (green), moderate (yellow), low (blue), and very low (gray), illustrated in a color-coded manner in supplemental Table 3. The data show remarkable consistency in the rank order of the four groups of cathepsin gene expression abundances among the brain regions and developmental stages examined. Notably, 99.6% of the 1,440 cathepsin gene expression values (16 brain regions, 15 cathepsin genes, and 6 age periods) are represented by the four rank ordered groups of high, moderate, low, and very low expression abundances. The high expressing group consists of CTSB, CTSF, and CTSD. The moderate expressing group consists of CTSA, CTSL, and CTSZ. The low expressing group consists of CTSC, CTSH, CTSK, CTSO, CTSS, and CTSV. The very low expression group contains CTSE, CTSG, and CTSW. The consistent cathepsin gene expression patterns suggest that the human brain expresses a defined profile of cathepsin genes in different brain regions during development from prenatal to young adult stages.

Discussion

Lysosomes utilize cathepsin proteases to maintain cellular protein homeostasis by protein degradation. The fifteen cathepsin proteases are comprised of eleven cysteine cathepsins, two aspartyl cathepsins, and two serine cathepsins. The complexity of lysosomal functions requiring multiple cathepsins with diverse proteolytic activities leads to the question of what are the gene expression profiles of cathepsin genes at different developmental stages among human brain regions? For this reason, this study investigated the cathepsin gene expression profiles in human brain regions at the prenatal to infancy, childhood, adolescence, and young adult age periods.

Differences in total expression of all cathepsin genes was observed in early development. Total expression of all cathepsin genes was lowest at the early prenatal stage which became increased at the infancy stage. During infancy to young adult phases, total gene expression was similar.

Of interest was the remarkable consistency in the profiles of high to moderate, low, and very low gene expression levels for the fifteen cathepsin genes among the brain regions and ages studied. The four groups of gene expression levels observed in the brain regions and developmental stages examined were (a) high expression of cathepsins B, F, and D, (b) moderate expression of cathepsins L, Z, and A, (3) low expression of cathepsins H, K and O, with still lower expression of cathepsins C, S, and V, and (4) very low expression of cathepsins E, G, and W. The unique finding of this study was the consistency in the relative levels of high to very low cathepsin expression across all brain regions during different developmental periods. these results show that lysosomal functions in human brain regions utilize a well-defined pattern of cathepsin gene expression during human brain development.

The gene expression levels of the fifteen cathepsins span four orders of magnitude among the brain regions and developmental stages evaluated (Table 2). Groups of cathepsins with high, moderate, low, lower, and extremely low expression comprised 12–49%, 3–12%, 0.3 −6.4%, 0.05–2.2%, and 0.001–0.24% of total cathepsin expression, respectively, among the brain regions and ages examined. These groups of relative expression abundances for the fifteen cathepsins were consistent from prenatal to infancy, childhood, adolescence, and young adult ages for all the brain regions studied.

Expression of the lysosomal cathepsin protease genes is the first step for the production of active proteolytic enzymes utilized for protein homeostasis, achieved through lysosomal degradation of unwanted cellular proteins. Functional cathepsins must be generated through the steps of gene expression, mRNA translation, and post-translational processing required to generate active proteases from their inactive zymogens. Levels of gene expression may or may not correspond to the level of protein translated into active protein by the cell. Nonetheless, the gene expression data of this study demonstrate a remarkable consistency in relative levels of gene expression for the 15 cathepsin proteases of lysosomes in 16 different brain regions during human brain development. This group of cathepsins mediate proteolysis of proteins by different cleavages within peptide sequences as endopeptidases, removal of N-terminal residues as aminopeptidases, and removal of C-terminal residues as carboxypeptidases, with specificity for particular amino acid residues. In future studies, it will be valuable to compare the proteolytic activities of these cathepsins with their gene expression profiles during human brain development.

Significantly, the consistency in the rank order profiles of gene expression for the cathepsin proteases in human brain during the different prenatal to young adult periods suggest that alterations in these normal levels of cathepsin expression may lead to dysfunctions in the brain. Indeed, there is much evidence for brain disorders associated with dysregulation of cathepsins. Examples of several cathepsins involved in brain disorders occurring during infant to young adult stages are described below.

Cathepsin B is elevated in traumatic brain injury (TBI) that results in behavioral dysfunctions and brain neuropathology (Hook et al. 2014a, 2015). TBI occurs at all ages from infants to children, adolescents, and adult periods of life. TBI results from falls, car accidents, sports injuries, military-related injuries, and related impact injuries to the brain. The important function of cathepsin B in TBI has been demonstrated by knockout of the cathepsin B gene in TBI mice, which results in improvements in TBI-induced motor dysfunction and amelioration of brain lesions (Hook et al. 2014a, 2015). Cathepsin B is also involved in emotionality of anxiety-related and depression-like behaviors, as demonstrated by effects on these behaviors in cathepsin B knockout mice (Czibere et al. 2011). These behavioral features of mental disorders occur throughout life, including adolescence, young adults, and adults of all ages.

Lysosomal storage diseases afflict infants and children, a result of inherited mutations in genes involved in lysosomal structure and function (Jeyakumar et al. 2005). The neuronal ceroid lipofuscinosis (NCL) disorders of aberrant lysosomal storage afflicts children, juvenile, and adult ages (Stoka et al. 2016). The clinical features of childhood NCLs display mental disturbances, motor deterioration, seizures, loss of vision and early death. Among the NCL mutations, mutations of the cathepsin D (CTSD) (Ketscher et al. 2016; Doccini et al. 2016), and cathepsin F (CTSF) genes are involved (Smith et al. 2013).

Cathepsin K has been linked to schizophrenia, a mental disorder occurring in adolescence and young adults, supported by findings that knockout of the cathepsin K gene in mice results in reduced anxiety as well as spatial learning and memory (Bernstein et al. 2008; Dauth et al. 2011).

Cathepsin Z hypomethylation has been suggested as a risk factor for multiple sclerosis (MS) (Allan et al. 2017). In the EAE (experimental autoimmune encephalomyelitis) model of MS, mice deficient in cathepsin Z reduced the ability of mice to generate Th17 responses, involving reduced neuroinflammation, that are critical in the pathogenesis of EAE and MS. MS usually affects those between 20 and 50 years of age, and the average age of onset is approximately 34 years.

Lysosomal dysfunction in aging and the elderly participates in numerous brain neurodegenerative and neurological disorders including Alzheimer's disease (Kindy et al., 2012; Nixon 2013; Hook et al. 2014b; Yamashima 2016; Stoka et al. 2016; de Magalhaes et al. 2009; Pislar and Kos 2014), progranulin-mediated dementia (Zhou et al. 2017), Parkinson's disease (Moors et al. 2016; McGlinchey et al. 2015), Huntington's disease (Liang et al. 2011; Kim et al. 2006), amyotrophic lateral sclerosis (ALS) (Kikuchi et al. 2003), epilepsy (Ni et al. 2012), TBI and TBI-related stroke and ischemia (reviewed in Hook et al. 2015), and others. Dysregulated lysosomal cathepsins can result in apoptosis, autophagy, protein homeostasis, inflammation, and many detrimental cellular conditions. It will be important in future studies to examine the pattern of cathepsin expression profiles during elderly ages in health and brain disorders.

Cathepsin proteases also function in organelles other than lysosomes, including secretory vesicles where cathepsin L and cathepsin V participate in generating active peptide

neurotransmitters from inactive precursors (Funkelstein et al. 2012; Hook et al. 2018). Cathepsins are found to function in the cytoplasm, nucleus, at the plasma membrane, and extracellularly (Kilinc et al. 2010; Talukdar et al. 2016). Thus, expression of cathepsin protease genes in brain represents not only lysosomal functions, but is involved in biological functions of cathepsins in non-lysosomal cellular compartments (Reier et al. 2010).

In conclusion, results of this study show that during human brain development, the majority of human brain regions utilize a well-defined pattern of lysosomal cathepsin gene expression profiles. The remarkable consistency in expression profiles of the fifteen cathepsins among brain regions during prenatal to young adult age periods demonstrated that a well-defined, balanced profile of cathepsin gene expression is utilized for human brain development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

This work was supported by a grant from the National Institutes of Health 9NIH) (R01 NS094597) awarded to V. Hook. A. Hsu was supported by a NIH/NIA grant t32 AG26757 (Dilip V. Jeste, PI), and the Stein Institute for research on Aging and the Center for Healthy Aging at the University of California, San Diego. We thank the Allen Human brain Atlas for providing the extensive efforts and gene expression data as a public resource (http://www.brain-map.org/).)

References

Allan ERO, Campden RI, Ewanchuk BW, Tailor P, Balce DR, McKenna NT, & Yates RM (2017) A role for cathepsin Z in neuroinflammation provides mechanistic support for an epigenetic risk factor in multiple sclerosis. J. Neuroinflammation, 14, 103. [PubMed: 28486971]

Barrett AJ, Rawlings ND, and Woessner JF (2004). Handbook of Proteolytic Enzymes, 2nd edition, Elsevier Academic Press, Amsterdam.

Bernstein HG, Bogerts B, Lendeckel U (2008) Cathepsin K and metabolic abnormalities in schizophrenia. Arterioscler Thromb Vasc Biol, 28, e163. [PubMed: 19020311]

Czibere L, Baur LA, Wittmann A, Gemmeke K, Steiner A, Weber P, & Deussing JM (2011) Profiling trait anxiety: transcriptome analysis reveals cathepsin B (Ctsb) as a novel candidate gene for emotionality in mice. PLoS One, 6, e23604. [PubMed: 21897848]

Dauth S, Sîrbulescu RF, Jordans S, Rehders M, Avena L, Oswald J, & Brix K (2011). Cathepsin K deficiency in mice induces structural and metabolic changes in the central nervous system that are associated with learning and memory deficits. BMC Neurosci, 12, 74. [PubMed: 21794126]

de Magalhães JP, Curado J, Church GM (2009) Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 25, 875–881. [PubMed: 19189975]

Doccini S, Sartori S, Maeser S, Pezzini F, Rossato S, Moro F, & Simonati A (2016). Early infantile neuronal ceroid lipofuscinosis (CLN10 disease) associated with a novel mutation in CTSD. J. Neurol, 263, 1029–1032. [PubMed: 27072142]

Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, & Jones AR (2012). An anatomically comprehensive atlas of the adult human brain transcriptome. Nature, 489, 391–399. [PubMed: 22996553]

Funkelstein L, Lu WD, Koch B, Mosier C, Toneff T, Taupenot L, & Hook V (2012) Human cathepsin V protease participates in production of enkephalin and NPY neuropeptide neurotransmitters. J. Biol. Chem, 287, 15232–15241. [PubMed: 22393040]

Hook V, Bandeira N (2015) Neuropeptidomics mass spectrometry reveals signaling networks generated by distinct protease pathways in human systems. J Am Soc Mass Spectrom 26, 1970–80. [PubMed: 26483184]

Hook G, Jacobsen JS, Grabstein K, Kindy M, Hook V (2015). Cathepsin B is a new drug target for traumatic brain injury therapeutics: evidence for E64d as a promising lead drug candidate. Front. Neurol, 6, 178. [PubMed: 26388830]

Hook GR, Yu J, Sipes N, Pierschbacher MD, Hook V, Kindy MS (2014a). The cysteine protease cathepsin B is a key drug target and cysteine protease inhibitors are potential therapeutics for traumatic brain injury. J. Neurotrauma, 31, 515–529. [PubMed: 24083575]

Hook G, Yu J, Toneff T, Kindy M, Hook V (2014b) Brain pyroglutamate amyloid-β is produced by cathepsin B and is reduced by the cysteine protease inhibitor E64d, representing a potential Alzheimer's disease therapeutic. J. Alzheimers Dis, 41, 129–149. [PubMed: 24595198]

Jeyakumar M, Dwek RA, Butters TD, Platt FM (2005) Storage solutions: treating lysosomal disorders of the brain. Nat. Rev. Neurosci, 6, 713–725. [PubMed: 16049428]

Stoka V, Turk V, Turk B (2016). Lysosomal cathepsins and their regulation in aging and neurodegeneration. Ageing Res. Rev, 32, 22–37. [PubMed: 27125852]

Ketscher A, Ketterer S, Dollwet-Mack S, Reif U, Reinheckel T (2016). Neuroectoderm-specific deletion of cathepsin D in mice models human inherited neuronal ceroid lipofuscinosis type 10. Biochimie, 122, 219–226. [PubMed: 26232697]

Kikuchi H, Yamada T, Furuya H, Doh-ura K, Ohyagi Y, Iwaki T, Kira J (2003) Involvement of cathepsin B in the motor neuron degeneration of amyotrophic lateral sclerosis. Acta Neuropathol, 105, 462–468. [PubMed: 12677446]

Kilinc M, Gürsoy-Ozdemir Y, Gürer G, Erdener SE, Erdemli E, Can A, Dalkara T (2010) Lysosomal rupture, necroapoptotic interactions and potential crosstalk between cysteine proteases in neurons shortly after focal ischemia. Neurobiol. Dis, 4, 293–302.

Kim YJ, Sapp E, Cuiffo BG, Sobin L, Yoder J, Kegel KB, & DiFiglia M (2006) Lysosomal proteases are involved in generation of N-terminal huntingtin fragments. Neurobiol. Dis, 22, 346–356. [PubMed: 16423528]

Kindy MS, Yu J, Zhu H, El-Amouri SS, Hook V, Hook GR (2012) Deletion of the cathepsin B gene improves memory deficits in a transgenic Alzheimer's disease mouse model expressing AβPP containing the wild-type β-secretase site sequence. J. Alzheimers Dis, 29, 827–840. [PubMed: 22337825]

Liang Q, Ouyang X, Schneider L, Zhang J (2011) Reduction of mutant huntingtin accumulation and toxicity by lysosomal cathepsins D and B in neurons. Mol. Neurodegener, 6, 37. [PubMed: 21631942]

McGlinchey RP, Lee JC (2015) Cysteine cathepsins are essential in lysosomal degradation of α-synuclein. Proc. Natl. Acad. Sci. USA, 112, 9322–9327. [PubMed: 26170293]

Moors T, Paciotti S, Chiasserini D, Calabresi P, Parnetti L, Beccari T, van de Berg WD (2016) Lysosomal Dysfunction and α-Synuclein Aggregation in Parkinson's Disease: Diagnostic Links. Mov. Disord, 31, 791–801. [PubMed: 26923732]

Ni H, Yan JZ, Zhang LL, Feng X, Wu XR (2012) Long-term effects of recurrent neonatal seizures on neurobehavioral function and related gene expression and its intervention by inhibitor of cathepsin B. Neurochem. Res, 37, 31–39. [PubMed: 21842268]

Nixon RA (2013) The role of autophagy in neurodegenerative disease. Nat. Med 19, 983–997. [PubMed: 23921753]

Pišlar A, Kos J (2014) Cysteine cathepsins in neurological disorders. Mol. Neurobiol, 49, 1017–1030. [PubMed: 24234234]

Reiser J, Adair B, Reinheckel T (2010) Specialized roles for cysteine cathepsins in health and disease. J. Clin. Invest, 120, 3421–3431. [PubMed: 20921628]

Smith KR, Dahl HH, Canafoglia L, Andermann E, Damiano J, Morbin M, & Bahlo M (2013). Cathepsin F mutations cause Type B Kufs disease, an adult-onset neuronal ceroid lipofuscinosis. Hum. Mol. Genet, 22, 1417–1423. [PubMed: 23297359]

Sunkin SM, Ng L, Lau C, Dolbeare T, Gilbert TL, Thompson CL, & Dang C (2013). Allen Brain Atlas: an integrated spatio-temporal portal for exploring the central nervous system. Nucleic Acids Res, 41, D996-D1008. [PubMed: 23193282]

Talukdar R, Sareen A, Zhu H, Yuan Z, Dixit A, Cheema H, & Saluja AK (2016) Release of Cathepsin B in Cytosol Causes Cell Death in Acute Pancreatitis. Gastroenterology, 151, 747–758.e5. [PubMed: 27519471]

Yamashima T (2016) Can ‘calpain-cathepsin hypothesis’ explain Alzheimer neuronal death? Ageing Res. Rev, 32, 169–179. [PubMed: 27306474]

Zhou X, Paushter DH, Feng T, Sun L, Reinheckel T, Hu F (2017) Lysosomal processing of progranulin. Mol. Neurodegener, 12, 62. [PubMed: 28835281]

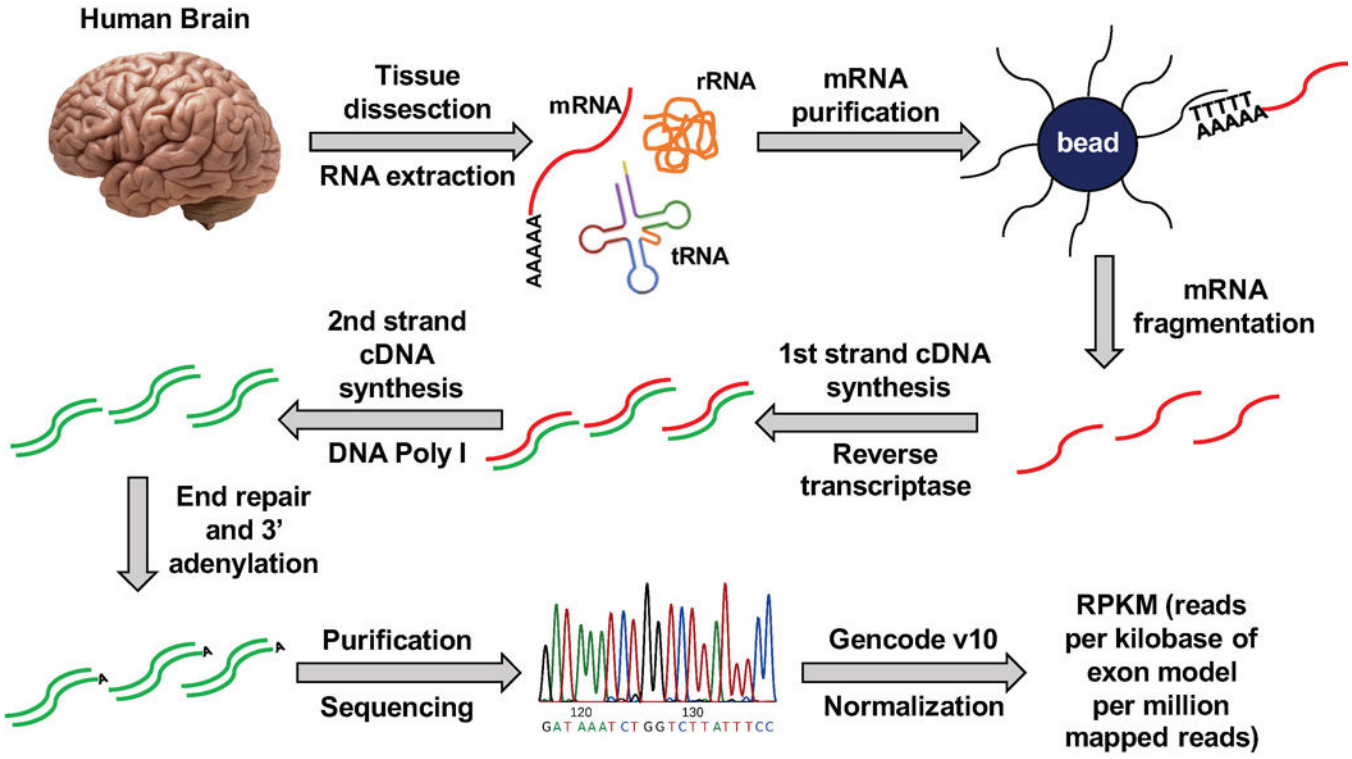

Figure 1. Human Brain Gene Expression Analyses by RNA-Seq.

Gene expression analyses for the fifteen cathepsin protease genes was conducted by RNA-Seq analyses, illustrated here (as described in the methods). Human brain tissues collected from tissue banks were dissected, subjected to RNA extraction and mRNA purification, synthesis of 1st and 2nd strand cDNAs with 3' adenylation, followed by DNA sequencing and mRNA levels quantitated by RPKM (reads per kilobase of exon model per million mapped reads).

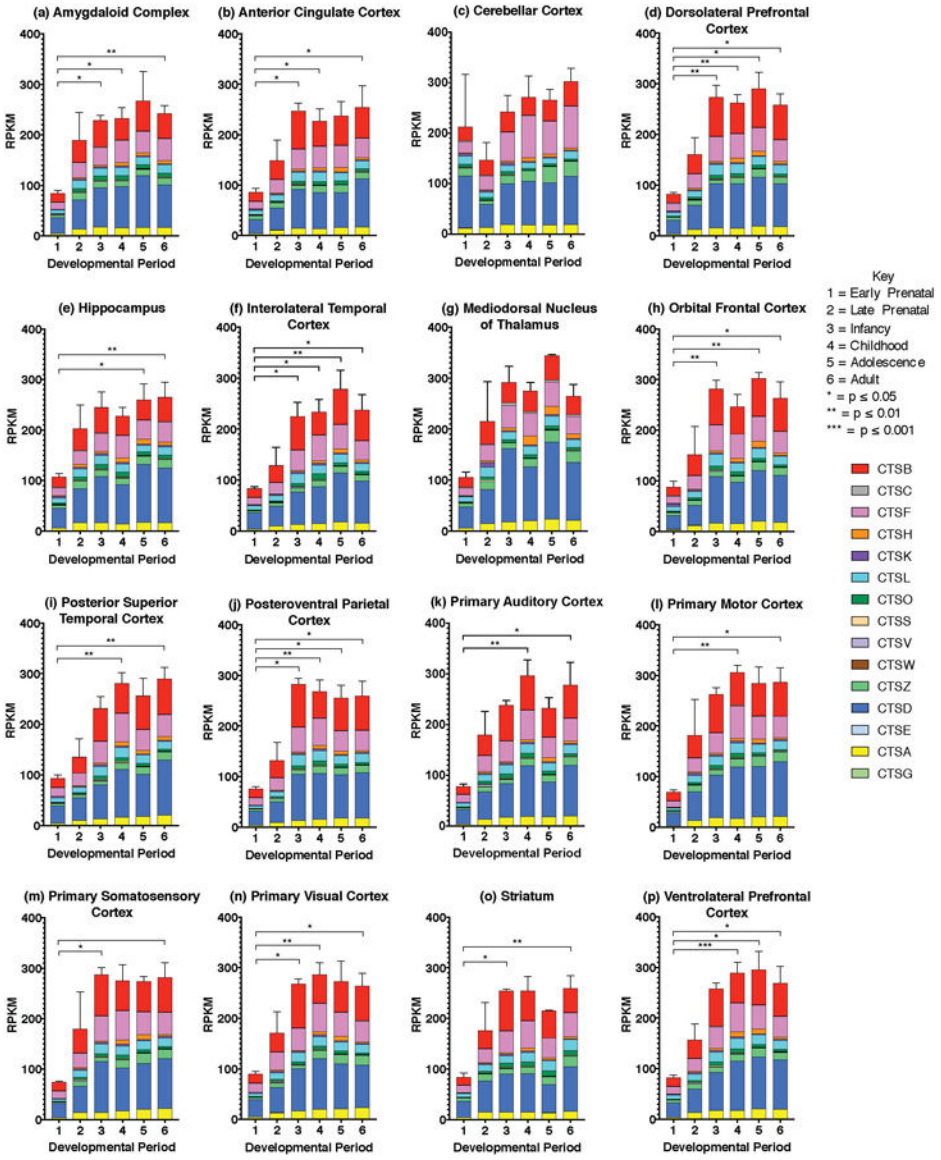

Figure 2. Total Expression of Cathepsin Genes in Human Brain during Development from Prenatal to Young Adult.

The expression of the fifteen cathepsin genes was graphed as a portion of the total cathepsin expression (sum of all fifteen cathepsin genes) expressed as RPKM (listed in supplemental Table 2) in sixteen brain regions, panels (a) to (p), during six development age periods of (1) early prenatal, (2) late prenatal, (3) infancy, (4) childhood, (5) adolescence, and (6) young adult (shown as age periods #1–6. The expression of each of the fifteen cathepsins relative to total expression of the fifteen cathepsin genes is shown in color coded fashion; the color key is shown on the right-hand side of the figure. Bar graphs show the total cathepsin gene expression as the average RPKM ± s.e.m., with statistical significance (*p < 0.05, **p<0.01, ***p<0.001) assessed by Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison post hoc test. Cathepsins B, F, and D (red, pink, and dark blue, respectively) were the most abundantly expressing cathepsins in panels (a) to (p). Cathepsins L, O, and A

(medium blue, green, and yellow, respectively) represented moderately expressing cathepsins. Lower expressing cathepsins are shown in Figure 3.

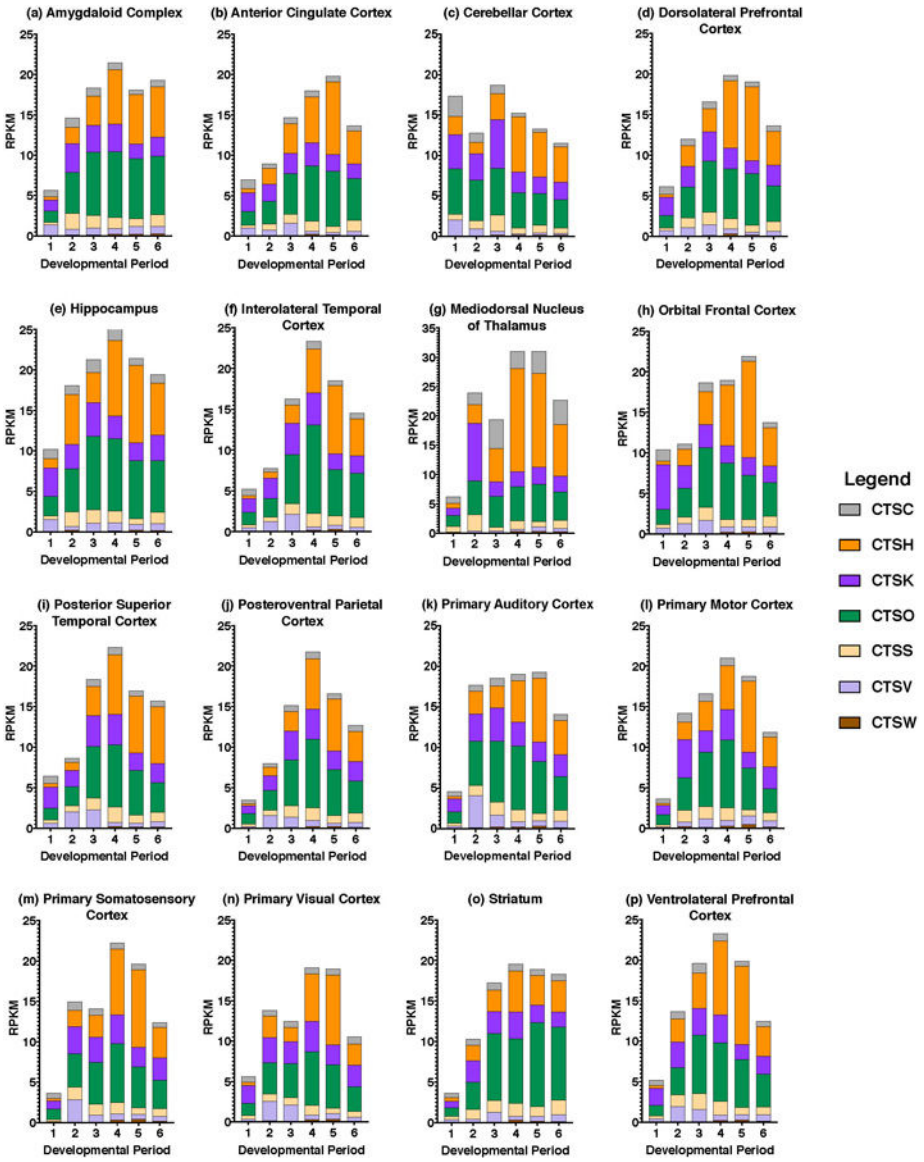

Figure 3. Low Expressing Cathepsins in Human Brain During Development from Prenatal to Young Adult.

Gene expression levels of the nine lower expressing cathepsins O, K, H, S, C, V, W, G, and E are graphed together here, to visualize their RPKM expression levels (in the absence of the six most abundantly expressing cathepsins (B, F, D, L, O, and A) visible in Figure 2). Panels (a) to (p) represent sixteen brain areas, and brain area names are shown at the top of each panel. Each brain region displays expression during the six developmental age periods of (1) early prenatal, (2) late prenatal, (3) infancy, (4) childhood, (5) adolescence, and (6) young adult. The color key for the nine different cathepsins is shown on the right-hand side of the figure. (Note that this figure does not show total cathepsin expression, but shows only nine cathepsins.)

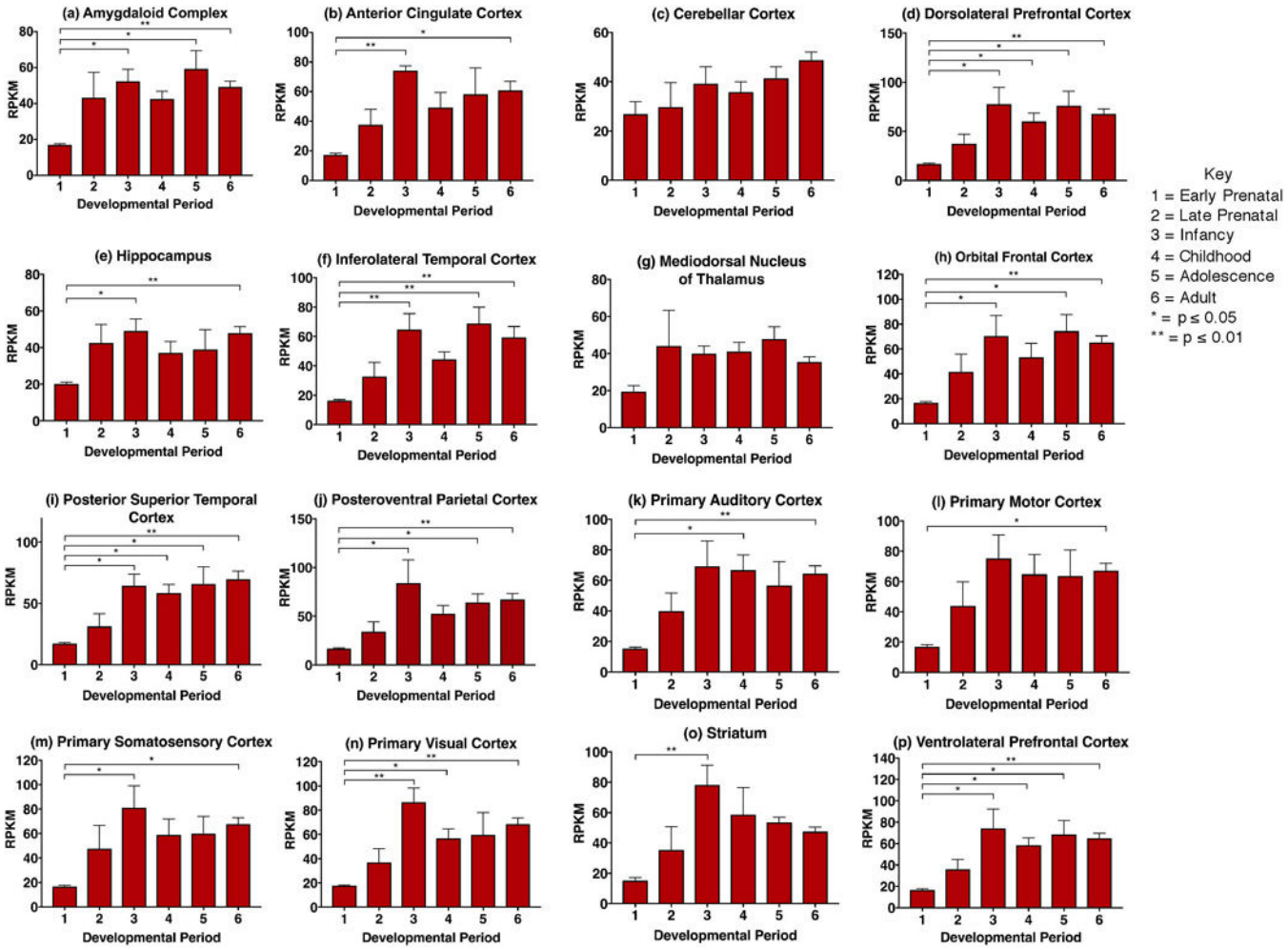

Figure 4. Cathepsin B Gene Expression in Human Brain During Development from Prenatal to Young Adult.

Expression levels of cathepsin B is illustrated for the sixteen brain regions, panels (a) to (p), during six age periods of (1) early prenatal, (2) late prenatal, (3) infancy, (4) childhood, (5) adolescence, and (6) young adult. Bars show the average RPKM ± s.e.m. for each cathepsin, with statistical significance of (*$p < 0.05$ and **$p<0.01$) assessed by Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison post hoc test.

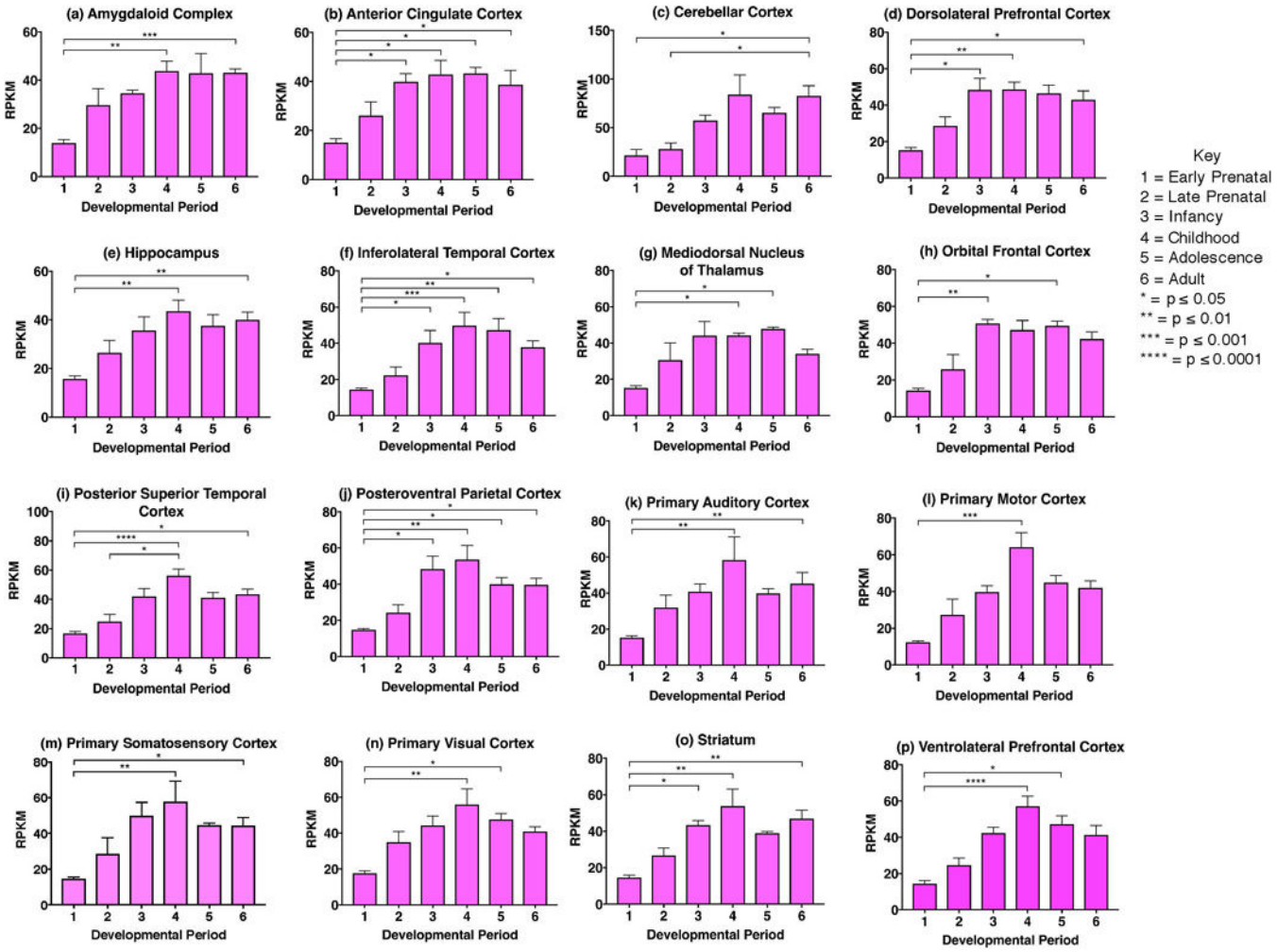

Figure 5. Cathepsin F Gene Expression in Human Brain During Development from Prenatal to Young Adult.

Expression levels of cathepsin F are illustrated for the sixteen brain regions, panels (a) to (p), during six age periods of (1) early prenatal, (2) late prenatal, (3) infancy, (4) childhood, (5) adolescence, and (6) young adult. Bars show the average RPKM ± s.e.m. for each cathepsin, with statistical significance of (*p < 0.05, **p<0.01, ***p<0.001, and ****p<0.0001) assessed by Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison post hoc test.

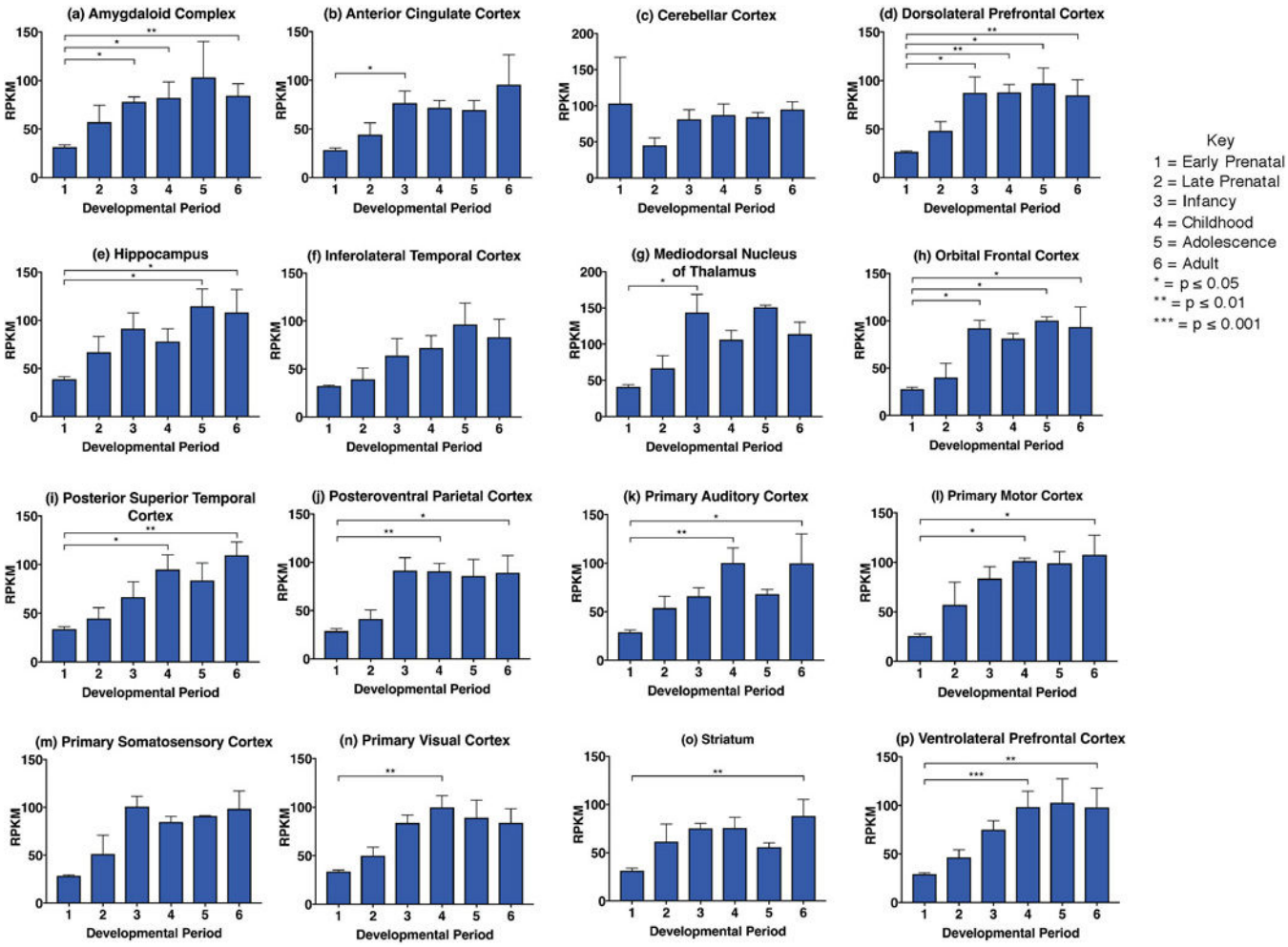

Figure 6. Cathepsin D Gene Expression in Human Brain During Development from Prenatal to Young Adult.

Expression levels of cathepsin D are illustrated for the sixteen brain regions, panels (a) to (p), during six age periods of (1) early prenatal, (2) late prenatal, (3) infancy, (4) childhood, (5) adolescence, and (6) young adult. Bars show the average RPKM ± s.e.m. for each cathepsin, with statistical significance of (*$p < 0.05$, **$p<0.01$, and ***$p<0.001$) assessed by Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison post hoc test.

Table 1.

Development Periods Evaluated for Cathepsin Gene Expression in Human Brain

Developmental Stage	Age Period	# of Brain Samples (genders)
Early Prenatal	8–18 weeks post-conception	12 (7 males, 4 females)
Late Prenatal	19–38 weeks post-conception	8 (3 males, 5 females)
Infancy	8–18 months	5 (4 males, 1 female)
Childhood	19 months to 11 years	7 (4 males, 3 females)
Adolescence	12–19 years	4 (2 males, 2 females)
Young Adult	20–40 years	6 (3 males, 3 females)

The ages for each development stage are indicated. For each age period, sixteen brain regions were analyzed for cathepsin gene expression levels by RNA-Seq, as described in the experimental procedures.

Table 2.
Cathepsin Gene Expression Levels during Human Brain Development

Expression Level	Gene	% Total Cathepsin Expression
High:	CTSB	12 – 32 %
	CTSD	25 – 49 %
	CTSF	25 – 49 %
Moderate:	CTSA	5 – 9 %
	CTSL	4 – 9 %
	CTSZ	3 – 12%
Low:		
Low Expression:	CTSH	0.3 – 6.4 %
	CTSK	0.5 – 6.2 %
	CTSO	1.5 – 3.9 %
Lower Expression:	CTSC	0.15 – 1.7%
	CTSS	0.22 – 1.3%
	CTSV	0.05 – 2.2 %
Very Low:	CTSE	0.001 – 0.020%
	CTSG	0.003 – 0.24 %
	CTSW	0.01 – 0.17 %

The profiles of cathepsin gene expression among brain regions at all age periods are illustrated as percent of total cathepsin expression. The developmental stages assessed were early prenatal, late prenatal, infancy, childhood, adolescence, and young adult. This table is based on the compiled gene expression data for the cathepsins in the sixteen human brain regions at the different age periods, shown in supplemental Table 2.