cDNA cloning and mRNA distribution of a mouse very long-chain acyl-CoA synthetase

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Abstract The interaction of the adrenoleukodystrophy protein (ALDP), mutated in the peroxisomal disorder X-linked adrenoleukodystrophy, and the very long-chain acyl-CoA synthetase (VLACS), the enzyme whose function is missing in this disease, remains obscure. As a first step to studying this interaction in wild type versus ALDP-deficient mice, we have cloned a VLACS cDNA from mouse liver. The 1860 bp open reading frame encodes a 620 amino acid protein with a predicted molecular mass of 70.3 kDa. By Northern blot analysis, a 2.6 kbp VLACS mRNA was highly abundant in liver and kidney and present at low levels in brain and testes. By RT-PCR VLACS mRNA was also detected in heart and lung but remained undetectable in skeletal muscle and spleen. In contrast to the peroxisomal β-oxidation marker acyl-CoA oxidase, whose mRNA level steadily increases during brain development, the VLACS transcript was found at a constant low level from embryo through adulthood, suggesting that additional isoforms may exist in brain.

Key words: Very long-chain acyl-CoA synthetase; Adrenoleukodystrophy; Peroxisome

1. Introduction

The principal biochemical abnormality of X-linked adrenoleukodystrophy (ALD) is the accumulation of saturated and unbranched very long-chain fatty acids (VLCFA; $>C_{22}$) in nervous system white matter, adrenal cortex, and other tissues, as well as in plasma and red blood cells [1]. This is due to deficient activation of VLCFAs, i.e. coupling of coenzyme A to the fatty acid, necessary for entering the peroxisomal β-oxidation pathway [2–5]. The enzyme responsible for VLCFA activation, very long-chain acyl-CoA synthetase (VLACS; also termed VLCFA-CoA ligase), has been described as a peroxisomal transmembrane protein with the active site either on the luminal [6] or the cytoplasmic [7] surface of the peroxisomal membrane. A VLACS cDNA was recently cloned from rat liver [8]. The predicted amino acid sequence of VLACS showed high sequence similarity to fatty acid transport protein, but was distinctly different from the long-chain acyl-CoA synthetase. However, the VLACS gene seems to be unaffected in X-ALD patients, as the strategy of positional cloning disclosed inherited defects in another gene, the *ald* gene, which is located on the X chromosome (Xq28) and encodes a different peroxisomal membrane protein [9,10]. More than 120 *ald* mutations have been detected in ALD patients so far (reviewed in [11]). Transfection of cultured fibroblasts from ALD patients with cDNA encoding the ALD protein (ALDP) can correct the deficient VLCFA metabolism in these cells [12,13]. ALDP-deficient cells are also capable of β-oxidation when already activated VLCFA are used as substrate [2], suggesting that an interaction between ALDP and VLACS is essential. However, neither the mechanism of interaction, e.g. ALDP as a transporter of or anchor for VLCFA-CoA synthetase in the peroxisomal membrane [14], nor the pathobiochemical role of ALDP in adrenoleukodystrophy has been clarified. Several groups have recently generated ALDP-deficient mice, which mirror the biochemical defect of human ALD [15–17]. In order to compare the status of VLACS in wild type and ALDP-deficient mice, we have now cloned and characterized the mouse VLACS cDNA. Here we describe the normal tissue distribution of the VLACS mRNA and, while the nervous system is the most severely affected site in adrenoleukodystrophy, we also compared the developmental time course of VLACS mRNA expression in the brain to the expression profiles of the ALD mRNA and the acyl-CoA oxidase mRNA, a marker for peroxisomal β-oxidation.

2. Materials and methods

2.1. Cloning of mouse VLACS cDNA

We searched the dbEST (non-redundant database of GenBank + EMBL-DDBJ EST Division [18]) with the rat VLACS cDNA sequence (dbj D85100) and identified several mouse expressed sequence tag (EST) clones. EST clones AA537712 and AA275019 were used for the design of a mouse-specific upstream PCR primer (Oli. 178: 5′-GCCGCCGCGCTGCACGCCTCG-3′) and downstream PCR primer (Oli. 180: 5′-GTCTATCGAGTTTCTTTCTGG-3′), respectively (positions 725–746 and 2680–2701 of the rat cDNA [8]). Poly(A)$^+$ mRNA was extracted from adult BALB/c mouse liver using Dynabeads Oligo $(dT)_{25}$ (DYNAL), according to the manufacturer's instructions, reverse transcribed using random hexamer primers and PCR amplified on a thermal cycler (Perkin Elmer, GeneAmp 2400) by 32 cycles of PCR (10 s at 94°C, 30 s at 59°C, and 4 min at 72°C) using 25 pmol of each primer and 2.5 units of Taq DNA polymerase (Advanced Biotechnologies). The PCR products were cloned into pCR II (Invitrogen) and sequenced. Mouse liver adaptor-ligated cDNA (Marathon PCR-Ready cDNA, Clontech) was used as a template in nested PCR reactions. The PCR product resulting from Oli. 205 (5′-CCAGTTACGCGAGGCCTCGGTTCCTGAG-3′) and adaptor primer AP1 (Clontech) was boosted in a 1:100 dilution with primers Oli. 190 (5′-ATTGATAAAACTCTGAAGCTC-3′) and AP2 (Clontech). The PCR products were cloned into pCR 2.1 (Invitrogen) and sequenced mainly on an automated fluorescence sequencing device (ABI-373A Sequencer) using fluorescent dye dideoxy terminator sequencing chemistry (ABI) according to the manufacturer's recommendations. The VLACS cDNA sequence has been deposited in the EMBL database (accession number AJ223958).

2.2. Northern blot analysis

Fresh mouse tissues or embryos (C57BL/6 or hybrid strains backcrossed to C57BL/6) were dissected, and total cellular RNA was isolated from frozen material as previously described [19]. For Northern blot analyses 10 μg aliquots of total RNA or 1–2 μg of poly(A)$^+$

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-49                                                                agcactccctcggccccggcgggaccggggtaccccgcagtctaccgcc   -1

  1 ATGCTGCCAGTGCTCTACACCGGCCTGGCGGGGCTGCTGCTGCTGCCTCTGCTGCTCACCTGCTGCTGCCCCCTACCTCCTCCAAGATGTG   90
    M  L  P  V  L  Y  T  G  L  A  G  L  L  L  L  P  L  L  L  T  C  C  C  P  Y  L  L  Q  D  V

 91 CGGTACTTCCTGCGGCTGGCCAACATGGCCCGGCGGGTGCGCAGCTACCGGCAGCGGCGACCCGTGCGTACCATCCTGCGGGCCTTCCTG  180
    R  Y  F  L  R  L  A  N  M  A  R  R  V  R  S  Y  R  Q  R  R  P  V  R  T  I  L  R  A  F  L

181 GAACAAGCGCGCAAGACCCCACACAAGCCCTTCCTGCTGTTCCGAGACGAGACGCTCACCTACGCCCAGGTGGACCGGCGCAGCAACCAA  270
    E  Q  A  R  K  T  P  H  K  P  F  L  L  F  R  D  E  T  L  T  Y  A  Q  V  D  R  R  S  N  Q

271 GTGGCGCGGGCGCTGCACGATCAACTGGGCCTACGACAGGGGGATTGCGTAGCCCTCTTCATGGGCAATGAGCCGGCCTACGTGTGGATC  360
    V  A  R  A  L  H  D  Q  L  G  L  R  Q  G  D  C  V  A  L  F  M  G  N  E  P  A  Y  V  W  I

361 TGGCTGGGACTGCTCAAACTGGGCTGTCCCATGGCGTGCCTCAACTACAACATTCGTGCCAAGTCTCTGCTGCACTGCTTTCAATGCTGC  450
    W  L  G  L  L  K  L  G  C  P  M  A  C  L  N  Y  N  I  R  A  K  S  L  L  H  C  F  Q  C  C

451 GGGGCGAAGGTGCTGCTGGCCTCCCCAGATCTACAAGAAGCTGTGGAGGAGGCTCTTCCAACCCTGAAAAAGGATGCCGTGTCCGTCTTT  540
    G  A  K  V  L  L  A  S  P  D  L  Q  E  A  V  E  E  A  L  P  T  L  K  K  D  A  V  S  V  F

541 TACGTAAGCAGAACTTCTAACACAAATGGTGTGGACACAATACTGGACAAAGTAGACGGAGTGTCGGCGGAACCCACCCCGGAGTCGTGG  630
    Y  V  S  R  T  S  N  T  N  G  V  D  T  I  L  D  K  V  D  G  V  S  A  E  P  T  P  E  S  W

631 AGGTCTGAAGTCACTTTTACCACGCCAGCAGTATACATTTATACTTCGGGAACCACAGGTCTTCCAAAAGCGGCAACCATCAATCATCAT  720
    R  S  E  V  T  F  T  T  P  A  V  Y  I  Y  T  S  G  T  T  G  L  P  K  A  A  T  I  N  H  H

721 CGCCTATGGTATGGGACAGGCCTTGCTATGTCGAGTGGAATCACGGCCCAGGATGTCATCTATACCACAATGCCCCTGTACCACAGTGCA  810
    R  L  W  Y  G  T  G  L  A  M  S  S  G  I  T  A  Q  D  V  I  Y  T  T  M  P  L  Y  H  S  A

811 GCGCTCATGATCGGCCTTCACGGATGCATCGTGGTTGGGGCTACTTTAGCTTTGCGGAGCAAATTCTCAGCCAGCCAGTTTTGGGACGAC  900
    A  L  M  I  G  L  H  G  C  I  V  V  G  A  T  L  A  L  R  S  K  F  S  A  S  Q  F  W  D  D

901 TGCAGGAAATACAACGTCACGGTCATTCAGTACATTGGTGAACTGCTTCGGTACCTGTGCAACACACCGCAGAAACCAAATGACCGGGAC  990
    C  R  K  Y  N  V  T  V  I  Q  Y  I  G  E  L  L  R  Y  L  C  N  T  P  Q  K  P  N  D  R  D

991 CACAAAGTGAAAAAAGCCCTGGGAAATGGCTTACGAGGAGATGTGTGGAGAGAGTTCATCAAGAGATTTGGGGACATCCACGTGTATGAG 1080
    H  K  V  K  K  A  L  G  N  G  L  R  G  D  V  W  R  E  F  I  K  R  F  G  D  I  H  V  Y  E

1081 TTCTACGCATCCACTGAAGGCAACATTGGATTTGTGAACTATCCAAGGAAAATCGGTGCTGTCGGGAGAGCAAACTACCTACAAAGAAAA 1170
     F  Y  A  S  T  E  G  N  I  G  F  V  N  Y  P  R  K  I  G  A  V  G  R  A  N  Y  L  Q  R  K

1171 GTTGCAAGGTATGAGCTGATCAAGTATGACGTGGAGAAGGACGAGCCGGTCCGTGACGCAAATGGATATTGCATCAAAGTCCCCAAAGGT 1260
     V  A  R  Y  E  L  I  K  Y  D  V  E  K  D  E  P  V  R  D  A  N  G  Y  C  I  K  V  P  K  G

1261 GAGGTTGGACTCTTGGTTTGCAAAATCACACAGCTCACACCATTTATTGGCTATGCTGGAGGAAAGACCCAGACAGAGAAGAAAAAACTC 1350
     E  V  G  L  L  V  C  K  I  T  Q  L  T  P  F  I  G  Y  A  G  G  K  T  Q  T  E  K  K  K  L

1351 AGAGATGTCTTTAAGAAAGGCGACATCTACTTCAACAGCGGAGACCTCCTGATGATCGACCGTGAGAACTTCGTCTACTTTCACGACAGG 1440
     R  D  V  F  K  K  G  D  I  Y  F  N  S  G  D  L  L  M  I  D  R  E  N  F  V  Y  F  H  D  R

1441 GTTGGAGATACTTTCCGGTGGAAAGGAGAGAACGTAGCTACCACAGAAGTCGCTGACATCGTGGGACTGGTAGATTTTGTTGAAGAAGTG 1530
     V  G  D  T  F  R  W  K  G  E  N  V  A  T  T  E  V  A  D  I  V  G  L  V  D  F  V  E  E  V

1531 AATGTGTATGGCGTGCCTGTGCCAGGTCATGAGGGTCGAATTGGGATGGCCTCCCTCAAGATCAAAGAAAACTACGAGTTCAATGGAAAG 1620
     N  V  Y  G  V  P  V  P  G  H  E  G  R  I  G  M  A  S  L  K  I  K  E  N  Y  E  F  N  G  K

1621 AAACTCTTTCAACACATCGCGGAGTACCTGCCCAGTTACGCGAGGCCTCGGTTCCTGAGGATACAAGATACCATTGAGATCACTGGGACT 1710
     K  L  F  Q  H  I  A  E  Y  L  P  S  Y  A  R  P  R  F  L  R  I  Q  D  T  I  E  I  T  G  T

1711 TTTAAACACCGCAAAGTGACCCTGATGGAAGAGGGCTTCAATCCCACAGTCATCAAAGATACCTTGTATTTCATGGATGATGCAGAGAAA 1800
     F  K  H  R  K  V  T  L  M  E  E  G  F  N  P  T  V  I  K  D  T  L  Y  F  M  D  D  A  E  K

1801 ACATTTGTGCCCATGACTGAGAACATTTATAATGCCATAATTGATAAAACTCTGAAGCTCTGAatattccctggtggtttagctcatgac 1890
     T  F  V  P  M  T  E  N  I  Y  N  A  I  I  D  K  T  L  K  L  *

1891 atttccagaaagaaactcgatagacctcgcagagccacttcatacgtagaatccaactttaacttgattgaaggctataaggtgcgattt 1980
1981 tatttttaggaaattattcattaaaaggatagtttgtttgtttttttttttaaattacacctgaacctttgcaagtaaaaagatttagaaa 2070
2071 caattatttttcaatgtgcacctgccatttgtccttgcaaactaagcttcttggagagagggccttatttttttaaagacataataaact 2160
2161 atattaacagtaaaaaaaaaaaaaaaaaaaa
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Fig. 1. cDNA and deduced amino acid sequence of the mouse VLACS. The A of the translation initiation codon is number 1. Two utilized polyadenylation signals are underlined.
←

RNA (oligo-dT-selected fraction from 100 μg total RNA samples), and a size marker (RNA ladder, BRL) were fractionated on 1.2% agarose-formaldehyde gels [20] and transferred to nitrocellulose membranes (Schleicher and Schuell). As a control of equal loading and transfer, all blots were probed with a mouse cDNA [21] of the ubiquitously expressed cyclophilin mRNA [22]. A 543 bp VLACS cDNA fragment (mapping position −70 to 473 of the mouse cDNA sequence in Fig. 1) was used for detection of the VLACS mRNA, after radioactive labelling by random priming using [α-^{32}P]dCTP (Amersham). For detection of the acyl-CoA oxidase mRNA, a cDNA probe corresponding to nucleotides 174–1834 of the mouse peroxisomal acyl-CoA oxidase mRNA (GenBank accession number AF006688) was kindly provided by C.H. Noehammer (University of Graz, Austria). All blots were washed to high stringency (68°C, 0.2×SSC/0.2% SDS) before autoradiographic exposure to X-OMAT AR (Kodak) film and intensifying screens.

2.3. RT-PCR analysis

Approximately 2 μg of total cellular RNA (extracted as described in Section 2.2) was reverse transcribed and PCR amplified using the Titan One Tube RT-PCR System (Boehringer Mannheim) with Oli. 205 and Oli. 180 (sequences listed above). This primer combination was selected such that a 261 bp VLACS-specific product is amplified from cDNA, but an intron in the VLACS gene (J. Berger, unpublished data) discriminates products arising from genomic DNA potentially contaminating the RNA extracts. RT-PCR was performed under conditions not allowing quantitative analysis. As a positive control, RT-PCR of an ALD-specific fragment of similar size (which should be obtained from all tissues) was performed on the same RNA preparations.

3. Results

3.1. Cloning of mouse VLACS cDNA

Using a PCR-based approach, we have cloned and determined the sequence of a 2220 bp cDNA containing the complete coding region of a putative VLACS from mouse liver. Nucleotide sequence analysis (Fig. 1) revealed an open reading frame of 1860 bp encoding a 620 amino acid protein with a predicted molecular mass of 70.3 kDa. This protein can be aligned without gaps to rat liver VLACS [8] and a human liver VLACS sequence (dbj data base accession number D88308), displaying 93% and 82% amino acid identity, respectively (Fig. 2). Most of the amino acid substitutions are conservative, leaving no doubt that the mouse sequence represents an ortholog of the rat liver synthetase. Two polyadenylation signals are indicated in the sequence (underlined in Fig. 1), and cDNA clones originating from mRNAs polyadenylated at either one of these were obtained, demonstrating that both sites are used in vivo.

3.2. Tissue distribution of mouse VLACS mRNA

The size tissue distribution of the VLACS mRNA was analyzed by Northern blot hybridization, using total cellular RNA (Fig. 3) or poly(A)$^+$ RNA (Fig. 4). A VLACS

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mouse MLPVLYTGLAGLLLLPLLLTCCCPYLLQDVRYFLRLANMARRVRSYRQRRPVRTILRAFLEQARKTPHKPFLLFRDETLT   80
rat   ..............................F..Q......Q.............HV.....................
human ..SAI..V.....F....VNL....FF..IG...KV.AVG......G....A.........K..Q.............

mouse YAQVDRRSNQVARALHDQLGLRQGDCVALFMGNEPAYVWIWLGLLKLGCPMACLNYNIRAKSLLHCFQCCGAKVLLASPD  160
rat   ................H.....................L.................................E
human ................H..........L.........L....V....A.........................V..E

mouse LQEAVEEALPTLKKDAVSVFYVSRTSNTNGVDTILDKVDGVSAEPTPESWRSEVTFTTPAVYIYTSGTTGLPKAATINHH  240
rat   .H.....V......EG................V.........D.I.................................
human ..A....I..S....D..IY........D.I.SF.....E..T..I.........S...L.............M.T.Q

mouse RLWYGTGLAMSSGITAQDVIYTTMPLYHSAALMIGLHGCIVVGATLALRSKFSASQFWDDCRKYNVTVIQYIGELLRYLC  320
rat   ......S..LR...K.H.............................F.....................A.............
human .I......TFV..LK.D....I.L.F......L..I.....A.......T.............................

mouse NTPQKPNDRDHKVKKALGNGLRGDVWREFIKRFGDIHVYEFYASTEGNIGFVNYPRKIGAVGRANYLQRKVARYELIKYD  400
rat   ..............I.....................I.............M...........E....K..V.H......
human .S...........RL............Q.V......CI.....A.......M..A..V.....V....K.IIT.D.....

mouse VEKDEPVRDANGYCIKVPKGEVGLLVCKITQLTPFIGYAGGKTQTEKKKLRDVFKKGDIYFNSGDLLMIDRENFVYFHDR  480
rat   ........................I....E....F.....................V.................I.....
human .........E....VR.....................N....A.A.................L.........V.H...I.....

mouse VGDTFRWKGENVATTEVADIVGLVDFVEEVNVYGVPVPGHEGRIGMASLKIKENYEFNGKKLFQHIAEYLPSYARPRFLR  560
rat   ..................................................I.M..............S......S......
human ...................T.......Q.......H..D.........I.M...H..D.........D............

mouse IQDTIEITGTFKHRKVTLMEEGFNPTVIKDTLYFMDDAEKTFVPMTENIYNAIIDKTLKL*                    621
rat   ............................S............T...Y.....D............*
human ...............M..V......A....A...L..TA.MY.....D.....SA.....*
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Fig. 2. Alignment of the amino acid sequences of the mouse, rat, and human ortholog VLACS proteins. Standard one-letter amino acid symbols are used. Dots represent identical amino acids at the apropriate position.

mRNA of 2.6 kbp was expressed at high levels in samples from liver and kidney, and at low levels in brain and testes. The cloned cDNA was slightly shorter than the estimated 2.6 kbp mRNA size; adding an average size poly(A) tail still leaves 100–200 nucleotides unaccounted for. Preliminary data suggest that this is also the extent of missing 5′ untranslated sequence. In testes an additional, smaller (2.0 kbp), slightly more abundant transcript was present. However, in this tissue additional transcripts without functional relevance are often seen. Heart, skeletal muscle, spleen, and lung revealed no VLACS mRNA at the level of sensitivity of our Northern blot analysis. However, by reverse transcription-coupled PCR (a more sensitive assay), PCR products indicative of VLACS mRNA expression (see Section 2) were obtained from heart and lung, in addition to the sites of expression detected by Northern blot. In skeletal muscle and spleen VLACS expression remained undetectable.

To evaluate the possibility of a coordinated temporal regulation of the ALD and VLACS genes during mouse brain differentiation, the developmental time course of VLACS mRNA expression was investigated. By Northern blot hybridization, the 2.6 kbp VLACS transcript was found in the CNS at a constant, low level from the earliest time point (embryonic day 12) through adulthood (Fig. 4). This is in contrast to the ALD mRNA, whose expression is the highest in the immature mouse brain and gradually decreases during differentiation (Berger et al., manuscript in preparation). Since the exact role of the ALD protein in β-oxidation has not been established, we also investigated the mRNA expression profile of mouse peroxisomal acyl-CoA-oxidase (ACOX), the first enzyme of the peroxisomal β-oxidation system (Fig. 4). The ACOX mRNA was relatively abundant in all tissues analyzed, with the highest levels in liver and kidney. During brain development the ACOX mRNA was easily detected at embryonic day 12 (at least 30-fold more abundant than VLACS) and the expression steadily increased to the adult level, which was estimated to be about 100-fold higher than the VLACS level.

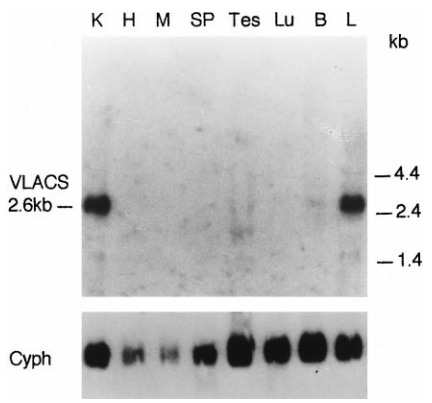

Fig. 3. Northern blot analysis of VLACS mRNA expression in various mouse tissues. Total RNA (10 μg per lane) from kidney (K), heart (H), skeletal muscle (M), spleen (SP), testes (Tes), lung (Lu), brain (B), and liver (L) of a 2 month old male was hybridized with ^{32}P-labeled cDNA probes. Top panel: VLACS; bottom panel: cyclophilin (Cyph). The positions of RNA size standards are indicated on the right.

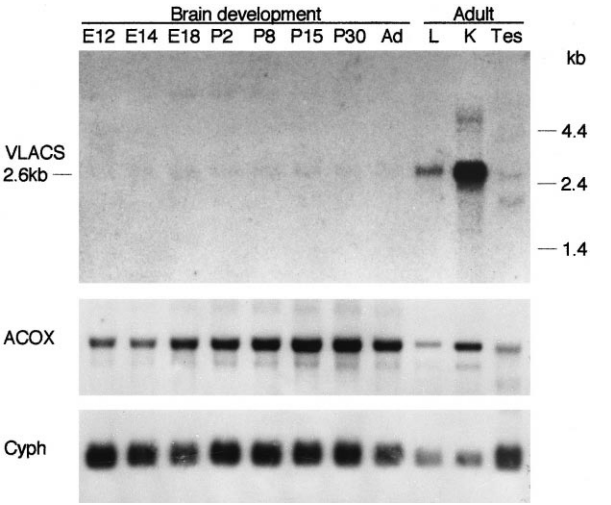

Fig. 4. Northern blot analysis of VLACS mRNA distribution during brain development and in adult tissues. Poly(A)$^+$ RNA (1–2 μg per lane) was extracted from dissected brain or whole head (lanes E12 and E14) at the indicated developmental stages and hybridized with ^{32}P-labeled cDNA probes. Top panel: VLACS; middle panel: acyl-CoA oxidase (ACOX); bottom panel: cyclophilin (Cyph) as loading control. The positions of RNA size standards are indicated on the right. The lanes are labeled: E12–E18, 12.5–18.5 days of gestation; P2–P30, days of postnatal development; adult brain (Ad), liver (L), kidney (K), and testes (Tes).

4. Discussion

We have cloned and sequenced a cDNA of the mouse VLACS. Protein alignments demonstrate 93% amino acid identity to rat liver VLACS [8], for which also VLCFA-CoA synthetase activity and a peroxisomal and microsomal subcellular localization were demonstrated. The amino acid sequences of both species lack consensus motifs corresponding to known peroxisomal targeting signals [23,24], as is the case for ALDP and a number of other peroxisomal membrane proteins including all peroxisomal half-ABC transporters, suggesting that a different targeting mechanism operates on this group of peroxisomal proteins. Although VLACS is thought to be a peroxisomal membrane protein [6,7], hydropathy analysis [25] indicated no extensive hydrophobic domains other than the amino-terminus, unlike peroxisomal membrane protein 70 kDa and 69 kDa [26–28], adrenoleukodystrophy protein [9] and adrenoleukodystrophy related protein [29], which all have six transmembrane regions.

A strong expression of VLACS mRNA was observed in liver and kidney, in good agreement with the overall high level of β-oxidation in these tissues and the distribution of the rat VLACS mRNA [8]. In addition, the mouse mRNA was found at a much lower abundance, but still detectable by Northern blot analysis, in testes and brain, which was not reported in the rat VLACS study [8], possibly due to differences in the sensitivity of detection. Only by RT-PCR were VLACS transcripts detectable also in heart and lung.

Brain contains quite high amounts of VLCFAs, such as lignoceric acid [30]. VLACS activity, as well as acyl-CoA oxidase activity, was shown to be present in peroxisomes and microsomes of postnatal brain homogenates from 1–6 week old rats, with a maximum around day 10–16 [31]. Furthermore, VLACS activity was also demonstrated within the myelin membrane [32]. Thus, the low level of VLACS mRNA

expression in mouse brain and the lack of upregulation during postnatal differentiation were unexpected. The acyl-CoA oxidase mRNA, in contrast, displayed an appropriate expression profile (Fig. 4) fitting the biochemical findings. The mRNA expression patterns obtained for two other peroxisomal membrane proteins, PMP70 and PMP35, were almost identical to the ACOX data (Berger et al., manuscript in preparation). Interestingly, in rat at least two different long chain acyl-CoA synthetases exist; the mRNA for the brain-enriched isozyme is upregulated during brain development, while the liver-enriched isozyme remains at a constant, low level in brain during differentiation [33]. Taken together, these data suggest that additional isoforms of VLACS, with a different regulatory pattern, may exist in brain and possibly in other tissues.

Many VLACS-related sequences can be found in the EST database and most of these were from kidney or liver-derived cDNA libraries. Only a few were amplified from other tissues, like diaphragm and embryonic heart, probably representing mRNAs expressed at very low copy numbers. Other EST clones display similar, but not identical cDNA sequences, and may be candidates for other isoforms of VLACS. Future analyses of such related sequences will show if some of these represent transcripts of novel VLACS genes.

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