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# Molecular cloning of cDNA encoding mitochondrial very-long-chain acyl-CoA dehydrogenase from bovine heart[1,2]

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**KEY WORDS** fatty acid desaturases; fatty acids; heart mitochondria; molecular cloning; complementary DNA; molecular sequence data; amino acid sequence

**AIM**: To clone the cDNA encoding an isoenzyme of mitochondrial very-long-chain acyl-CoA dehydrogenase (VLCAD) from bovine heart λ*gt*11 and λ*gt*10 cDNA libraries. **METHODS**: The clone was isolated with immunoscreening technique and validated by (1) the microsequences of the N-terminus and three internal proteolytic fragments from the purified enzyme; (2) identification of the acyl-CoA dehydrogenase (AD) signature sequence; and (3) high homology of the deduced peptide sequences, as expected, with those of rat liver mitochondrial VLCAD. **RESULTS**: The cDNA (2203 bp) corresponds to a ~2.4-kb mRNA band from the same tissue source revealed by a Northern blotting. The deduced peptide sequence of 655 amino acids (70 537 Da) is composed of a 40-amino acid mitochondrial leader peptide moiety (4 346 Da) and a 615-amino acid peptide as a mature protein (66 191 Da). A comparison of the peptide sequences in the AD family shows the major diversity in their signal sequences, suggesting a structural basis for their different mitochondrial locations. The catalytic sites are all highly conserved among VLCAD. Ser-251 analogous to and Cys-215 diversified to other family members. A pseudo-consensus sequence of leucine zipper was found in the C-terminal region from Leu-568 to Leu-589, implying a mechanism whereby the dimer of this protein is formed by zipping these leucine residues from the α-helixes of 2 monomers. **CONCLUSION**: The isolated cDNA clone encodes an isoenzyme of mitochondrial VLCAD in bovine heart.

It is proposed that acyl-CoA dehydrogenases (AD) have evolved from a common ancestral gene and acquired their distinctive substrate specificities in the course of evolution[1]. Short-(SCAD), medium-(MCAD), and long-chain acyl-CoA dehydrogenases (LCAD)[2,3] located in the mitochondrial matrix are characterized. Very-long-chain acyl-CoA dehydrogenase (VLCAD), a new member of the AD superfamily, is located in the mitochondrial inner membrane and catalyzes the first, possibly rate-limiting, step of β-oxidation. The reaction involves the 1, 2-dehydrogenation of acyl-CoA thioesters >16 carbons in length and leads to the formation of *trans*-2-enoyl-CoA products[4,5].

Genetic defects of AD have been found in infants and children and commonly present with fasting coma and hypoglycemia[6]. Prominent symptomatology also includes muscle weakness and cardiomyopathy, reflecting the major role of fatty acids as a source of energy for skeletal muscle and heart. These genetic defects have also been implicated in the etiology of Sudden Infant Death. Two patients with VLCAD deficiency have been described[7].

Rat liver VLCAD (RLVLCAD) was purified and characterized[4], and its cDNA has been cloned and expressed in hepatoma cell lines[5]. Human placenta VLCAD (HPVLCAD) was also cloned[8]. We now report isolation and sequencing of the cDNA encoding the precursor of bovine heart VLCAD (BHVLCAD), and compare this sequence with those of other AD.

## MATERIALS AND METHODS

**Materials** Bovine heart mitochondrial preparations were obtained from Dr Keith D GARLID's laboratory at the Oregon Graduate Institute. The polyclonal antibodies against BHVLCAD were prepared in our Lab using purified

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[2] The nucleotide sequence data reported have been submitted to GenBank[TM] (Accession number BVLCAD U30817).
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BHVLCAD (The purification of BHVLCAD and the preparation of the polyclonal antibodies will be published separately). Bovine heart λ*gt*11 and λ*gt*10 cDNA libraries were obtained from Clontech Laboratories. Alkaline phosphatase-conjugated goat anti-rabbit IgG antibody and other reagents used for Western blotting were purchased from Sigma, and polyvinylidene difluoride membrane was obtained from Millipore. [$\alpha$-$^{32}$P] dCTP, [$\gamma$-$^{32}$P] dATP, and [$\alpha$-$^{35}$S]dATP were from Dupont/NEN. *Escherichia coli* strains Y1090 and XL1-Blue were from Stratagen; TA cloning vector was from InvetroGen; oligo (dT) column was from Gibco BRL; Gene Clean kit was from Bio-101; nylon membranes were from S&S Nytran; and the RNA size markers were from Boehringer Mannheim. All restriction enzymes and $T_4$ DNA ligase were obtained from BRL. Reagents used for DNA sequencing (Sequenase Ⅱ) were from United States Biochemical Corp.

**Determination of partial amino acid sequences of BHVLCAD** The purified BHVLCAD was electrophoresed on a 10 % polyacrylamide gel containing 0.2 % sodium dodecylsulfate and then electrically transferred to a polyvinylidene difluoride membrane. Western blots with the polyclonal antibodies to BHVLCAD were used to monitor the purification. The protein bands were cut from the membrane for N-terminal microsequencing. The remaining protein was eluted from the membrane and subjected to trypsin digestion, followed by HPLC. Three proteolytic polypeptides were obtained and microsequenced by Edman degradation using an automated gas-phase protein sequencer, Model 470A, equipped with a Model 120A PTH analyzer (Applied Biosystems).

**Immunoscreening of cDNA libraries** The bovine heart λ*gt*11 cDNA library was screened using polyclonal antibodies to BHVLCAD as probe, according to Clontech's protocols. Briefly, the phage from the bovine heart λ*gt*11 cDNA expression library were plated, employing *E coli* strain Y1090 as host, at a density of $10^5$ PFU/plate. The λ*gt*11 phage without insert were used as negative controls. After 42 ℃ incubation for 3 h, nitrocellulose filters that had been presaturated with isopropylthio-β-*D*-galactoside 10 mmol·L$^{-1}$ were applied on top of the plates and 37 ℃ incubation continued for 3.5 h. Following a typical immunostaining with the polyclonal antibodies, the positive clones were picked out for rescreening and isolation.

**Further cDNA library screening with hybridization probe** Inserts from the positive clones obtained from the immunoscreening were cleaved using *Eco*RI and random labeled[9] with [$\alpha$-$^{32}$P] dCTP as hybridization probes for screening the bovine heart λ*gt*10 cDNA library for longer cDNA fragments according to Clontech's procedures. Briefly, the phage were plated, employing *E coli* strain C600 hfl as host at the titer of $3 \times 10^4$/plate and incubated for 6 – 8 h. After cooling down at 4 ℃, the plaques were transferred onto duplicate nitrocelulose filters which were then denatured, neutralized, rinsed, and baked at 80 ℃ under vacuum for 2 h. The prehybridization, with a buffer containing 6 × SSPE, 5 × Denhardt's, 0.1 % SDS, and 100 μg of denatured salmon sperm DNA · mL$^{-1}$, was carried out at 65 ℃ for 4 h. Hybridization was carried out in the same buffer at 65 ℃ overnight with the boiled probe of $10^6$ dpm · mL$^{-1}$. Filters were then washed four times: twice under the conditions of 2 × SSC, 0.5 % SDS for 15 min; once with the buffer containing 1 × SSC, 0.1 % SDS at 37 ℃ for 30 min; and once with the buffer containing 0.2 × SSC, 0.1 % SDS at 65 ℃ for 1 h. The filters were then dried on Watman paper and subjected to autoradiography at − 70 ℃ for 24 h. The resultant positive plaques were isolated, rescreened, and further purified.

**Subcloning and DNA sequencing** The cDNA inserts of an isolated clone from the λ phage were excised by *Eco*RI digestion, subcloned into a pUC19 plasmid vector, and then transformed into *E coli* strain XL1-Blue using the $Ca^{2+}$ method[9]. The recombinant plasmid DNA was isolated and purified on a large scale using a CsCl gradient. Both stains of the inserts were sequenced from both directions by the dideoxynucleotide chain termination method. A total of 24 oligos (18-to-22-mer each) spanning the length of the cDNA were utilized as primers for the sense or antisense cDNA strands (Fig 1).

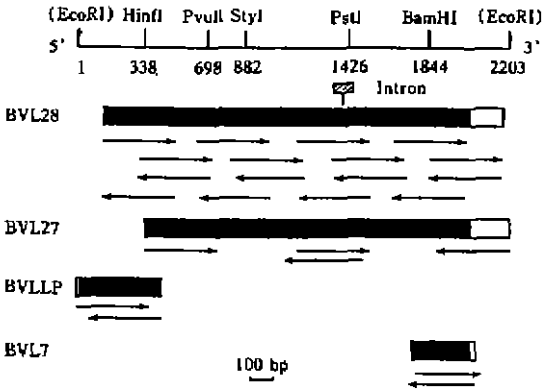


**Fig 1. Partial restriction map and sequencing strategy of BHVLCAD cDNA clones. Representative restriction sites are shown on the top. Sequencing strategy is summarized on the bottom. Solid and blank rectangular bars indicate the coding and noncoding regions, respectively. Horizontal arrows indicate the direction and length of the cDNA sequenced from each fragment. Hatched bar presents an intron of the BVL-28 fragment.**

**Northern blotting** Total RNA fractions were prepared from bovine heart[10]. Briefly, fresh tissue was homogenized with guanidine isothiocyanate and then centrifuged through a CsCl cushion. About 5 μg poly-A RNA, selected with an

oligo (dT) column, was electrophoresed at 3 V/cm on a 1.2 % agarose/2.2 mol · L$^{-1}$ formaldehyde gel and then transferred to a nylon membrane in 10 × SSC overnight by capillary action. The poly-A RNA was crosslinked to the membrane with a UV-crosslinker (254 nm) followed by rinsing in 6 × SSC. The membrane was prehybridized in a buffer containing 6 × SSC, 0.5 % SDS, 5 × Denhardt's, and 100 μg of denatured salmon sperm DNA/ml at 65 ℃ for 4 h. Hybridization was performed in the same buffer at 68 ℃ for 16 h with the [$\alpha$-$^{32}$P] dCTP labeled insert of the positive clone, BVL27. ($10^6$ dpm · mL$^{-1}$) by the random labeling method[15]. The filter was washed twice with 6 × SSC, 0.5 % SDS for 15 min, twice with 1 × SSC, 0.1 % SDS at 37 ℃ for 30 min × 2, and once with 0.2 × SSC, 0.1 % SDS at 65 ℃ for 1 h. The result was visualized by autoradiography at −70 ℃ for 16 h.

**Obtaining the 5′ end of the cDNA fragment** For rapid amplification of cDNA ends (RACE)[11], 5 μg of mRNA were reverse-transcripted using gene-specific primer GP1 with the sequence of 5′-CTCCCCAGATGCCAGTTTG corresponding to nucleotide 633 to nucleotide 615 of the cDNA (Fig 2).

The mRNA/cDNA hybrids were tailed with poly (A) using terminal deoxynucleotidyl transferase. PCR amplification was first performed with 3 primers: GP2 (5′-TCTCCTCCACTCTTTCCAG) corresponding to nucleotide 397 to nucleotide 379 of the cDNA; at the 5′ end, a poly (A) primer [5′-AAGGATCCGTCGACATCGATAATACGACTCACTATAGGGA(T)$_{17}$] plus a primer with the 5′ region of the poly (T) primer (5′-AGGATCCGTCGACATCGATAATAC). A 2nd run of PCR was performed with 1 μL of product (1:20 diluted) from the 1st run and another pair of primers: GP3 (5′-TTGAGCACAGACGGGTATGGAAACA) corresponding to nucleotide 290 to nucleotide 266 and a primer with the 3′ region of the poly (T) primer (5′-ATAATACGACTCACTATAGGGATTTT). The resultant products were separated on 1 % agarose gel, cut out, purified with a Gene Clean Kit, ligated into the TA cloning vector, transformed into the supplied competent cells, and sequenced (3 different colonies simultaneously to avoid mutated errors).

**Computer analysis of DNA and protein sequence** Nucleotide and protein sequences were analyzed using PC/GENE (IntelliGenetics) Version 6.85 software. GenBank™ DNA and protein database searches were conducted using the BLAST Server from the National Center for Biotechnology Information.

## RESULTS AND DISCUSSION

**Isolation of bovine heart VLCAD cDNA clones** About $10^7$ independent recombinants were screened with the anti-BHVLCAD antibody. Two positive clones (BVL-7 and BVL-24), each 353-bp long, were determined to be identical by subcloning and sequencing. The cDNA fragment of BVL-7 was used as a probe to rescreen a bovine heart λgt10 cDNA library. A screening of $7 \cdot 10^6$ plaques yielded 24 positive plaques. Restriction mapping of the inserts (Fig 1) indicated that one clone, BVL-28 (2125 bp), extended further than any other inserts at the 5′ end, while another clone, BVL-27 (1886 bp), extended at the 3′ end. These clones were subcloned into the pUC19 plasmid and sequenced in their entirety on both strands (Fig 1). The BVL-28 cDNA contained an 89-bp non-coding insertion. Since the insertion was absent in the BVL-27 cDNA and since the intron consensus sequences [$\begin{matrix}C\\A\end{matrix}$AG/GT $\begin{matrix}A\\G\end{matrix}$AGT of donor and $(\begin{matrix}T\\C\end{matrix})_{11}$N $\begin{matrix}C\\T\end{matrix}$AG/G of acceptor][12] are present at the 3′ and 5′ ends of the insertion, respectively, this insertion is likely an unspliced intron. The total length of 2 overlapped cDNA clones is 2077 bp, including 1845 bp for the coding region and 232 bp at the 3′-noncoding region, leaving the 5′ end open.

**Northern blot revealed a 2.4-kb mRNA band** Total RNA was isolated from bovine heart and poly-A RNA was isolated by affinity chromatography. A cDNA insert of BVL-27 (1886 bp) was used as a hybridization probe. The Northern blot (Fig 3) showed a clear, single band at ~2.4 kb.

**Obtaining and amplifying 5′ end of cDNA** The obtained cDNA, in comparison with the 2.4-kb mRNA band, appeared to lack ~300 bp at the 5′-end region. We applied the standard RACE method of PCR amplification[11] to obtain a single cDNA fragment (BVLLP) of ~0.4 kb. This fragment was confirmed by hybridization to be a part of the BHVLCAD (data not shown), subcloned into a TA-cloning vector, and sequenced. The additional sequence (126 bp) revealed an in-frame ATG translation initiation codon. The flanking sequences G(−3) and C(+4) of ATG are consistent with Kozak's consensus sequence for a eukaryotic transcription initiation site[13].

**Nucleotide sequence of BHVLCAD** Combining BVLLP, BVL-28, and BVL-27, we obtained a cDNA fragment with a total length of 2203

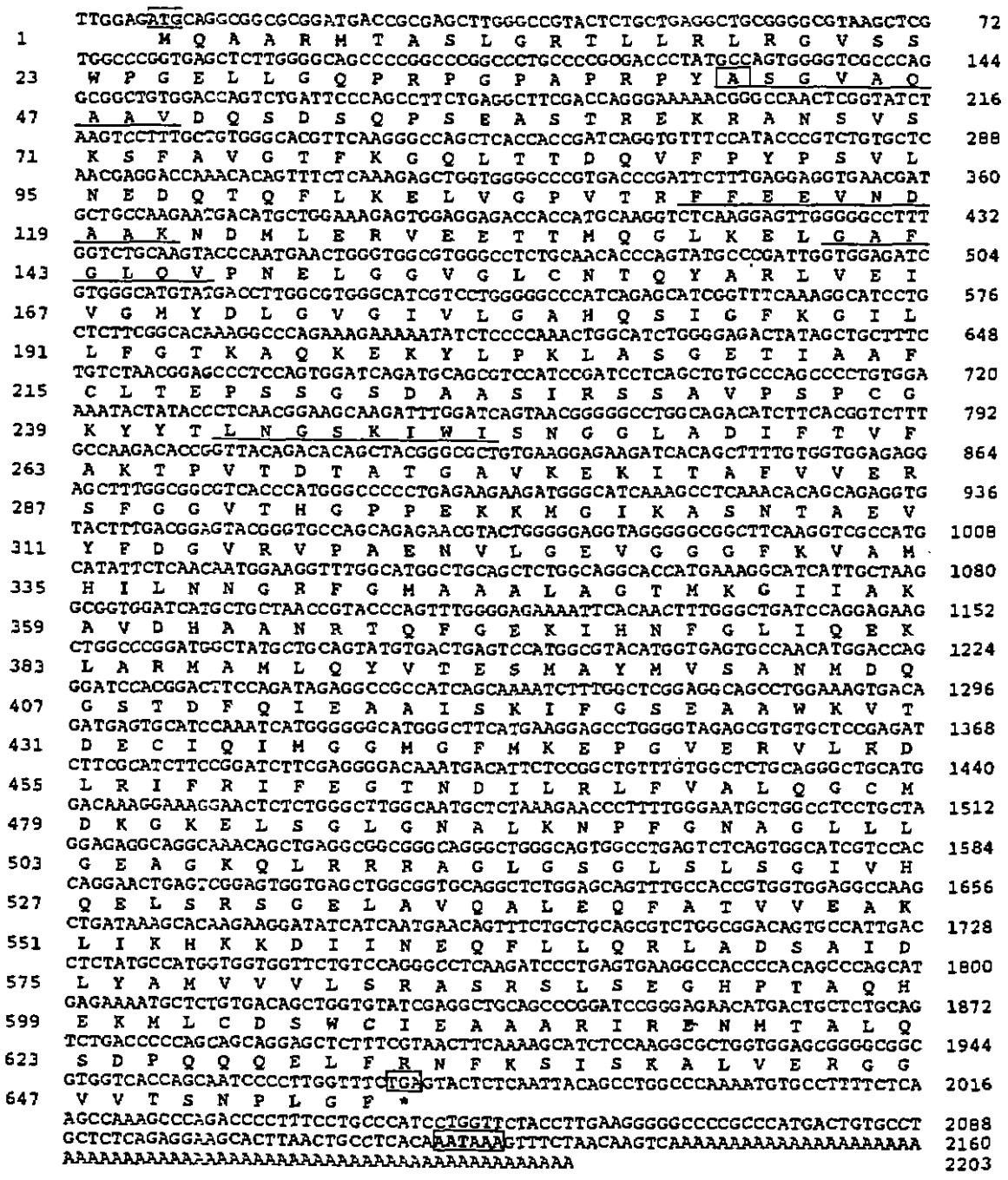

**Fig 2. Nucleotide and deduced amino-acid sequence of the BHVLCAD cDNA. The numbering of amino acids starts at the 1st residue of the coding sequence. The initiation codon, the 1st amino acid residue of the mature BHVLCAD, termination codon, and a possible polyadenylation signal in the 3′-untranslated region are boxed. The start Met, Ala at N-terminus of the mature protein, and the polyadenylation signal are in bold. The amino acid sequences matching those obtained from the microsequences are underlined.**

nucleotides. Fig 2 shows the nucleotide sequence of BHVLCAD along with the deduced amino acid sequence. The total length is comparable to the observed length of the mRNA (~2.4 kb) as determined by a Northern blot. The difference of ~0.2 kb might be the measurement error or some missing noncoding region. Thus, the complete cDNA sequence (2203 nt) includes 6 nt beyond the initiation codon, an encoding region of 1965 nt, and a noncoding region of 232 nt at the 3′ end. The

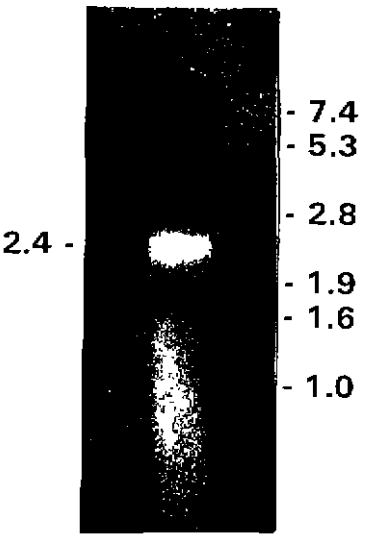


**Fig 3. Northern blot analysis of BVLCAD in bovine heart. A [$^{32}$P]-labeled BHVLCAD cDNA fragment of BVL-7 was hybridized with 5 μg bovine heart poly-A RNA. A single transcript of about 2400 nucleotides was detected. RNA sized markers (kbp) are indicated on the right.**

polyadenylation signal (AATAAA) is located at position 2120, and a poly (A) tail is found 15 nt downstream from this signal.

**Validation and amino-acid sequence** The microsequences of the N-terminal and 3 internal-peptide fragments from the purified BHVLCAD match exactly the deduced peptide sequences underlined in Fig 2 (Ala$^{41}$-Ser-Gly-Val-Q-Ala-Ala-Val, Phe$^{112}$-Phe-Glu-Glu-Val-Asn-Asp-Ala-Ala-Lys, Gly$^{140}$-Ala-Phe-Gly-Lys-Gln-Val, Lys$^{243}$-Asn-Gly-Ser-Lys-Ile-Trp-Ile), confirming that the cDNA clones were authentic. Thus, Ala-41 is the N terminus of the mature protein, and the first 40 residues from Met-1 to Tyr-40 constitute the leading sequence. The calculated molecular mass of the mature BHVLCAD, the leader peptide moiety, and total length were 66 191 Da, 4346 Da, and 70 537 Da, respectively.

**Sequence analysis** The calculated half life (see manu) of mature BHVLCAD is 4.4 h according to the N-terminal rule, and the instability index is 35.88[14]. Hence it is classified as a stable protein, which is consistent with the observation of a stable over-expression for RLVLCAD[5].

VLCAD is a mitochondrial inner membrane protein based on the detergent extraction experiments[4]. However, how the protein is associated with the membrane still remains unclear. The protein can be extracted from the membrane only with a detergent like 1 % Triton X-100 but not with alkaline extraction in our lab (unpublished data). The latter is commonly used to remove peripheral protein from the membrane. This observation suggests a tighter association. Hydropathy analysis using Kyte and Doolittle's method revealed significant hydrophobic regions. This is consistent with another analysis using the method of Rao and Argos, revealing 2 potential transmembrane helices located at 166 – 182 and 342 – 357 (Fig 4).

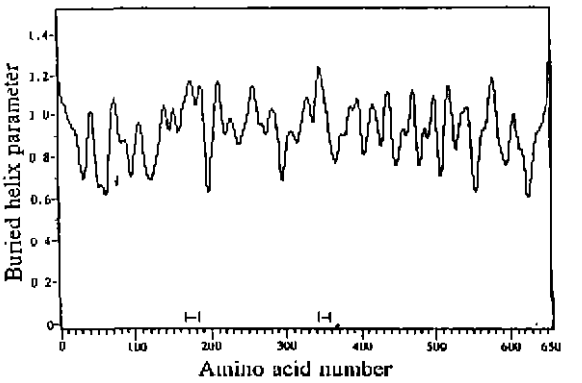


**Fig 4. Plot of the smoothed buried helix profile for amino acid sequence BHVLCAD obtained by the Rao and Argos' method. Two potential transmembrane domains (166 – 182 and 342 – 357) are marked.**

These regions correspond to those found in VLCADs from rat liver (RLVLCAD) and human placenta (HPVLCAD) at the same domain. But no such domains were found in LCAD, MCAD, or SCAD which are mitochondrial matrix proteins. There is low homology in these corresponding regions between VLCAD and other AD (Fig 3 of reference 2). This finding is at variance with the proposal by Aoyama *et al* that VLCAD is a membrane-binding protein[15]. However, these 2 potential transmembrane domains predicted by computer analyses are probably not sufficient to prove that it is an integral membrane protein. Further studies are needed to establish whether these regions function as hydrophobic cores of the enzyme, hydrophobic anchors in the mitochondrial inner membrane, or even transmembrane domains. The signal peptide fragment is located from Met-1 to Tyr-40 and is

consistent with the features of a mitochondrial signal peptide sequence: (1) the sequence is 15 – 70 amino acids long and rich in basic, hydrophobic, and hydroxylated residues with a few acidic residues (one in the case of BHVLCAD); (2) the N-terminal portion has the potential to form a positively-charged amphiphilic α helix; and (3) the C-terminal portion contains a proteolytic site. In case of BHVLCAD, the C-terminal portion of the leading peptide contains the R(-3) motif R-x-Y-A which serves as a cleavage site.

**Sequence comparisons** In an overall comparison of VLCAD including the signal sequences from bovine heart, human placenta, and rat liver (Fig 5), there is 81.8 % identity and 15.4 % similarity. The smaller diversity is mainly seen in the signal peptide. But there are striking differences between VLCAD and other AD in signal peptides, such as their lengths and cleavage sites[5]. This may be one of the structural bases by which VLCAD are led to the mitochondrial inner membrane and other AD to the mitochondrial matrix.

Comparison of the main bodies (from N-terminus Ala-41 to Ser-485) of three VLCAD showed an 82.5 % identity and 14.4 % similarity (Fig 4); consistent with them being isoenzymes. The consensus sequence of the AD signature II [PC/GENE Sequence Analysis Software Manual, Version 6.85 (1995) Appendix for Release 6.8, p 25, IntelliGenetics, Mountain View CA] was found in all three VLCAD. The catalytic site Glu-462 (arrow 5 in Fig 5), proposed by Kim and Wu, is highly conservative in all AD family members. Four other sites interacting with the flavin ring – Cys-215, Thr-217, Trp-249, and Ser-251 (arrows 1, 2, 3, 4 in Fig 4) – are all highly conservative among the three VLCAD. A comparison with MCAD shows that Thr-217 and Trp-249 are highly conservative, Ser-251 is analogous to other family members, and Cys-215 is diversified among the family members.

Although some key amino-acid residues are highly conservative, the VLCAD share only 20 – 30 % similarity, including identity with other family members of AD. This is a notable finding considering that the substrates that these emzymes catalize differ only in the lengths of carbon chains. The diversity in the primary sequence may imply a significant difference in the three-dimensional structure of VLCAD from other shorter chain AD.

The C-terminus region (~ 22 – 25 kDa) of VLCAD constitutes an extra peptide sequence, which has been proposed to be a modulator with substrate specificity, a catalytic activator, or an association site for homodimer formation[5]. We note that a pseudo-consensus sequence of the leucine zipper exits in the region from Leu-568 to Leu-589. Whereas this fragment is consistent with the leucine zipper pattern of L-$(X)_6$-L-$(X)_6$-L-$(X)_6$-L[15], only of leucines fall into a predicted α-helix structure. There is no evidence that VLCAD is a DNA-binding protein. Nevertheless, it still may provide a possible structural basis for the formation of a dimer by zipping these leucines from the α-helixes of two VLCAD monomers.

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BHVLCAD     MQAARMTASLGRTLLRLRGVSSWPGELLGQPRPGPAPRPYASGVAQAAVDQSDSQ    55
HPVLCAD     MQAARMAASLGRQLLRLGGGSSRLTALLGQPRPGPARRPYAGGAAQLALDKSDSH    55
RLVLCAD     MQSARMTPSVGRQLLRLGARSSRSAALQGQPRPTSAQRLYASEATQAVLEKPETL    55
            **.***..*.** **** . **. ..* *****..*.* **....* .......

BHVLCAD     PSEASTREKRANSVSKSFAVGTFKGQLTTDQVFPYPSVLNEDQTQFLKELVGPVT   110
HPVLCAD     PSDALTRKKPAKAESKSFAVGMFKGQLTTDQVFPYPSVLNEEQTQFLKELVEPVS   110
RLVLCAD     SSDASTREKPARAESKSFAVGMFKGQLTTDQVFPYPSVLNEGQTQFLKELVGPVA   110
            .*.* **.*.*.. ******* ********************.*********.**.

BHVLCAD     RFFEEVNDAAKNDMLERVEETTMQGLKELGAFGLQVPNELGGVGLCNTQYARLVE   165
HPVLCAD     RFFEEVNDPAKNDALEMVEETTWQGLKELGAFGLQVPSELGGVGLCNTQYARLVE   165
RLVLCAD     RFFEEVNDPAKNDSLEKVEEDTLQGLKELGAFGLQVPSELGGLGLSNTQYARLAE   165
            ********.**** **.***.* **************.****.**.*******.*
                                                  ↓1↓2
BHVLCAD     IVGMYDLGVGIVLGAHQSIGFKGILLFGTKAQKEKYLPKLASGETIAAFCLTEPS   220
HPVLCAD     IVGMHDLGVGITLGAHQSIGFKGILLFGTKAQKEKYLPKLASGETVAAFCLTEPS   220
RLVLCAD     IVGMHDLGVSVTLGAHQSIGFKGILLYGTKAQKEKYLPRVASGQALAAFCLTEPS   220
            ****.****...**************.***********..***...*********
                                      ↓3↓4
BHVLCAD     SGSDAASIRSSAVPSPCGKYYTLNGSKIWISNGGLADIFTVFAKTPVTDTATGAV   275
HPVLCAD     SGSDAASIRTSAVPSPCGKYYTLNGSKLWISNGGLADIFTVFAKTPVTDPATGAV   275
RLVLCAD     SGSDVASIRSSAVPSPCGKYYTLNGSKIWISNGGLADIFTVFAKTPIKDAATGAV   275
            ****.****.*****************.******************..*.******

BHVLCAD     KEKITAFVVERSFGGVTHGPPEKKMGIKASNTAEVYFDGVRVPAENVLGEVGGGF   330
HPVLCAD     KEKITAFVVERGFGGITHGPPEKKMGIKASNTAEVFFDGVRVPSENVLGEVGSGF   330
RLVLCAD     KEKITAFVVERSFGGVTHGLPEKKMGIKASNTSEVYFDGVKVPAENVLGEVGDGF   330
            ***********.***.*** ************.**.****.**.*******.**

BHVLCAD     KVAMHILNNGRFGMAAALAGTMKGIIAKAVDHAANRTQFGEKIHNFGLIQEKLAR   385
HPVLCAD     KVAMHILNNGRFGMAAALAGTMRGIIAKAVDHATNRTQFGEKIHNFGLIQEKLAR   385
RLVLCAD     KVAVNILNNGRFGMAATLAGTMKAIIAKAVDHATNRTQFGDKIHNFGVIQEKLAR   385
            ***..***********.*****..*********.******.******.*******

BHVLCAD     MAMLQYVTESMAYMVSANMDQGSTDFQIEAAISKIFGSEAAWKVTDECIQIMGGM   440
HPVLCAD     MVMLQYVTESMAYMVSANMDQGATDFQIEAAISKIFGSEAAWKVTDECIQIMGGM   440
RLVLCAD     MAILQYVTESMAYMLSANMDQGFKDFQIEAAISKIFGSEAAWKVTDECIQIMGGM   440
            *..***********.******* .**********************************
                                  ↓5
BHVLCAD     GFMKEPGVERVLRDLRIFRIFEGTNDILRLFVALQGCMDKGKELSGLGNALKNPF   495
HPVLCAD     GFMKEPGVERVLRDLRIFRIFEGTNDILRLFVALQGCMDKGKELSGLGSALKNPF   495
RLVLCAD     GFMKEPGVERVLRDIRIFRIFEGTNDILRLFVALQGCMDKGKELTGLGNALKNPL   495
            ***************.**************************.***.******.

BHVLCAD     GNAGLLLGEAGKQLRRRAGLGSGLSLSGIVHQELSRSGELAVQALEQFATVVEAK   550
HPVLCAD     GNAGLLLGEAGKQLRRRAGLGSGLSLSGLVHPELSRSGELAVRALEQFATVVEAK   550
RLVLCAD     GNVGLLIGEASKQLRRRTGIGSGLSLSGIVHPELSRSGELAVQALEQFATVVEAK   550
            **.***.***.******.*.********.**.**********.************

BHVLCAD     LIKHKKDIINEQFLLQRLADSAIDLYAMVVVLSRASRSLSEGHPTAQHEKMLCDS   605
HPVLCAD     LIKHKKGIVNEQFLLQRLADGAIDLYAMVVVLSRASRSLSEGHPTAQHEKMLCDT   605
RLVLCAD     LMKHKKGIVNEQFLLQRLADGAIDLYAMVVVLSRASRSLSEGYPTAQHEKMLCDS   605
            *.****.*.***********.**********************.***********.

BHVLCAD     WCIEAAARIRENMTALQSDPQQQELFRNFKSISKALVERGGVVTSNPLGF    655
HPVLCAD     WCIEAAARIREGMAALQSDPWQQELYRNFKSISKALVERGGVVTSNPLGF    655
RLVLCAD     WCIEAATRIRENMASLQSNPQQQELFRNFRSISKAMVENGGLVTSNPLRV    655
            ******.****.*..***.* ****.***.******.**.**.******
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**Fig 5.** Amino-acid sequences of BHVLCAD, RLVLCAD, and HPVLCAD. Identical residues are indicated with stars and conservative substitutions with a single dot. The N terminus is shown in bold letters. Arrows 1 – 5 are described in the text. The consensus sequences of AD signature 2 are boxed. The sequence data of RLVLCAD and HPVLCAD were taken from Ref 5 and 8, respectively.

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## 编码线粒体特长脂酰辅酶 A 脱氢酶牛心同功酶 cDNA 的分子克隆

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关键词 脂肪酸去饱和酶类；脂肪酸类；心线粒体；分子克隆；互补 DNA；分子序列结构；氨基酸序列

目的：分子克隆编码牛心线粒体特长脂酰辅酶 A 脱氢酶的 cDNA 基因. 方法：基因的分离是采用抗体筛选技术并采用已知的氨基末端和肽链内部的氨基酸序列等多种方法核对其可靠性. 结果：所获 cDNA 片段对应于 RNA 印迹法所示的约 2.4 kb 的 mRNA 片段，可翻译为 655 个氨基酸分子序列(70.5 kDa). 比较脂酰辅酶 A 脱氢酶家族的肽链序列显示主要不同在于它们的信号肽与其它脂酰辅酶 A 脱氢酶比较，其催化部位高度保守. 在羧基末端，存在一个和"亮氨基拉锁"特征序列类似的结构(亮 568 到亮 589)，为该蛋白四级结构的形成提供了一个潜在机制. 结论：我们分离的 cDNA 克隆编码牛心线粒体内膜特长脂酰辅酶 A 脱氢酶的同功酶.

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