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**Lee et al.** (43) **Pub. Date: Dec. 21, 2006**(54) **AORTIC CARBOXYPEPTIDASE-LIKE  
POLYPEPTIDE**(60) Provisional application No. 60/013,439, filed on Mar.  
15, 1996.(76) Inventors: **Mu-En Lee**, Newton, MA (US);  
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**G01N 33/53** (2006.01)(21) Appl. No.: **11/507,811**(52) **U.S. Cl.** ..... **435/6**; 435/69.1; 435/226;  
435/320.1; 435/325; 536/23.2;  
530/388.26; 435/7.2(22) Filed: **Aug. 21, 2006****Related U.S. Application Data**(63) Continuation of application No. 10/238,876, filed on  
Sep. 10, 2002, now Pat. No. 7,094,878, which is a  
continuation of application No. 09/060,482, filed on  
Apr. 15, 1998, now Pat. No. 6,468,766, which is a  
continuation-in-part of application No. 08/818,009,  
filed on Mar. 14, 1997, now abandoned.(57) **ABSTRACT**The invention features a aortic carboxypeptidase-like  
polypeptide (ACLP), DNA encoding ACLP, and methods of  
detecting genetic alterations associated with abdominal wall  
defects.

|       |                                                                                            |      |
|-------|--------------------------------------------------------------------------------------------|------|
| Human | <u>MAVRGAPILLSCLLALLALCPGGRPQTVLTDDEIEEFLEGLFLESELE..PREDDEVEAPPPEPTPRVRKAQAGGKPGKR</u>    | 78   |
| Mouse | <u>MAPVRTASLLCGLLALLTLCPGPNQTVLTDDEIEEFLEGLFLESELETSPPREDDEVEVQPLPEPTQRPRKSKAGGK...</u>    | 76   |
| Human | PGTAAEVPPEKTKDKGKKGKKDKGPKVPKESLEGSRRPPKKGKEKPPKATKKPKKEKPPKATKKPKKEPPKATKKPKKEPP          | 158  |
| Mouse | QRADVEVPPEKNKDKEKKGKKDKGPKATK.PLEGSTRPTKKPKKEKPPKATKKPKKEKPPKATKKPKKEKPPKATKKPKKEPP        | 155  |
| Human | <u>KATKKPPSGKRPPILAPSETLEWPLPPPPSPGPEELPQEGGAPLSNNWNQNPGEETHVEAQEHQPEPEEETEQTLDYNDQ</u>    | 238  |
| Mouse | <u>KATKRPSAGKKFSTVAPLETDLRLLPSPSNPSAQELPQKRDTFPFNWAGQGGEETQVEAKQPRPEPEEETEMPTLDYNDQ</u>    | 235  |
| Human | <u>IEREDYEDFEYIRRQKQPPPPSRRRRPERVWPEPPEEKAPAPAPEERIEPPVKPLLPPLPDYGDGYVIPNYDDMDYYF</u>      | 318  |
| Mouse | <u>IEKEDYEDFEYIRRQKQPRPTPSRR...RLWPERPEEKTEEPERKEVEPEPLKPL...LPDYGDGSYVIPNYDDLDDYYF</u>    | 308  |
| Human | GPPPPQKPDARQTDDEEKEELKKPKKEDSSPKEET.DKWAVEKGKDHPKRGKEELEEEWTPTEKVK <u>CPPIGMESHRIED</u>    | 397  |
| Mouse | PHPPPPQKPDVGQEVDEEKEEMKKPKKEGSSPKEDTEDKWTVEKNKDHKGPRKGEELEEEWAPVEKIK <u>CPPIGMESHRIED</u>  | 388  |
| Human | <u>NQIRASSMLRHGLGAQRGLNMOTGATEDDDYYDGAWCAEDDARTOWIEVDTRRTTRFTGVITQGRDSSIHDDFVTTFFVG</u>    | 477  |
| Mouse | <u>NQIRASSMLRHGLGAQRGLNMQAGANEDDDYYDGAWCAEDESQTOWIEVDTRRTTRFTGVITQGRDSSIHDDFVTTFFVG</u>    | 468  |
| Human | <u>FSNDSQTWVMYTYNGYEEMTFHGNVDKDTPLSELPEPVVARFIRIYPLTWNGSLCMRLEVLGCSVAPVYSYQAQNEVVAT</u>    | 557  |
| Mouse | <u>FSNDSQTWVMYTYNGYEEMTFYGNVDKDTPLSELPEPVVARFIRIYPLTWNGSLCMRLEVLGCFVTPVYSYQAQNEVVTT</u>    | 548  |
| Human | DDLDFRHHSYKDMRQLMKVVNEECPTITRTYSLGKSSRGLKIYAMEISDNPGEHELGEPEFRYTAGIHGNEVLGRELLLL           | 637  |
| Mouse | DSLDFRHHSYKDMRQLMKAVNEECPTITRTYSLGKSSRGLKIYAMEISDNPGDHELGEPEFRYTAGIHGNEVLGRELLLL           | 628  |
| Human | <u>LMQYLCREYRDGNPRVRSVLQDTRIHLVPSLNPDGYEVAQMGSEFGNWALGLWTEEGFDIFEDFPDLNSVLWGAERKWN</u>     | 717  |
| Mouse | <u>LMQYLCQEYRDGNPRVRNLVQDTRIHLVPSLNPDGYEVAQMGSEFGNWALGLWTEEGFDIFEDFPDLNSVLWAAEEKWN</u>     | 708  |
| Human | <u>VPYRVPNNNLPPIPERYLSPDATVSTEVRAIIAWMEKNPFVLGANLNGGERLVSYPYDMARTPTQEQLLAAAAAARGEDE</u>    | 797  |
| Mouse | <u>VPYRVPNNNLPPIPERYLSPDATVSTEVRAIISWMEKNPFVLGANLNGGERLVSYPYDMARTPSQEQLLAAEALAAARGEDD</u>  | 788  |
| Human | <u>DEVSEAQETPDHAIFRWLAISFASAHLTLTEPYRGGCQAQDYTGGMGIVNGAKWNPRTGTINDFSYLHTNCLELSFYLG</u>     | 877  |
| Mouse | <u>DGVSEAQETPDHAIFRWLAISFASAHLTMTPEYRGGCQAQDYTGSMGIVNGAKWNPRTGTINDFSYLHTNCLELSVYLG</u>     | 868  |
| Human | <u>DKFPHESELPREWENNK EALLTFMEQVHRGIKGVVTDQEQGPIANATISVSGINHGVKTASGGDYWRILNPGEYRVTAHA</u>   | 957  |
| Mouse | <u>DKFPHESELPREWENNK EALLTFMEQVHRGIKGVVTDQEQGPIANATISVSGINHGVKTASGGDYWRILNPGEYRVTAHA</u>   | 948  |
| Human | <u>EGYTPSAKTCNVDDYDIGATQCNFILARSNWKRIREIMAMNGNRPIPHIDPSRPMTPQORRLQORRLQHRRLRAQMRLRR</u>    | 1037 |
| Mouse | <u>EGYTSSAKICNVDDYDIGATQCNFILARSNWKRIREILAMNGNRPI LGVDPSPRPMTPQORRMQORRLQYRLRMREQMRLRR</u> | 1028 |
| Human | <u>LNATTTLGPHTPV.PTLPPAPATTLSTTIEPWGLIPPTTAGWEESETETYTEVVTEFGTEVEPEFGTKVEPEFETQLEPE</u>    | 1116 |
| Mouse | <u>LNSTA..GPATSPTPALMPPSPPTPAITLRPWVLPPTTAGWEESETETYTEVVTEFET.....E</u>                    | 1086 |
| Human | FETQLEPEFEFEFEFEFEFEFEIATGQAFPTTIVTYTVNFGDF (SEQ ID NO:2)                                  | 1158 |
| Mouse | YGTDLVEEIEEIEEIEEIEEEMDTGLTFLPTTIVTYTVNFGDF (SEQ ID NO:8)                                  | 1128 |

Fig. 1A

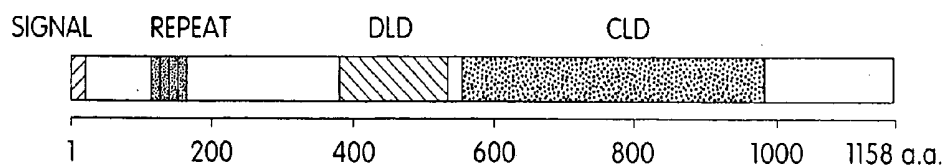


Fig. 1B

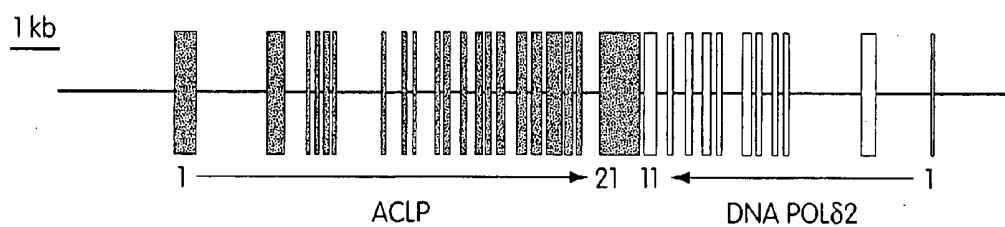


Fig. 2A

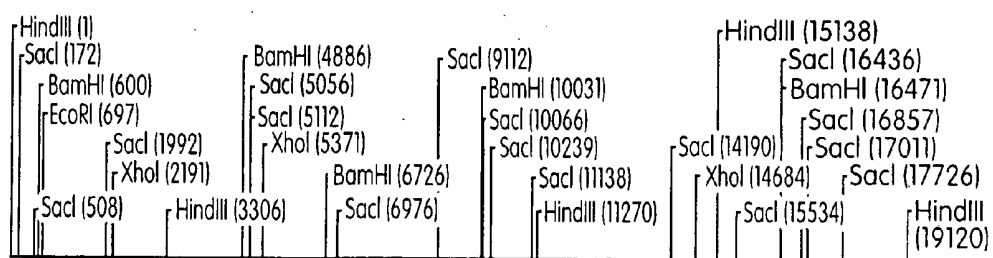


Fig. 2B

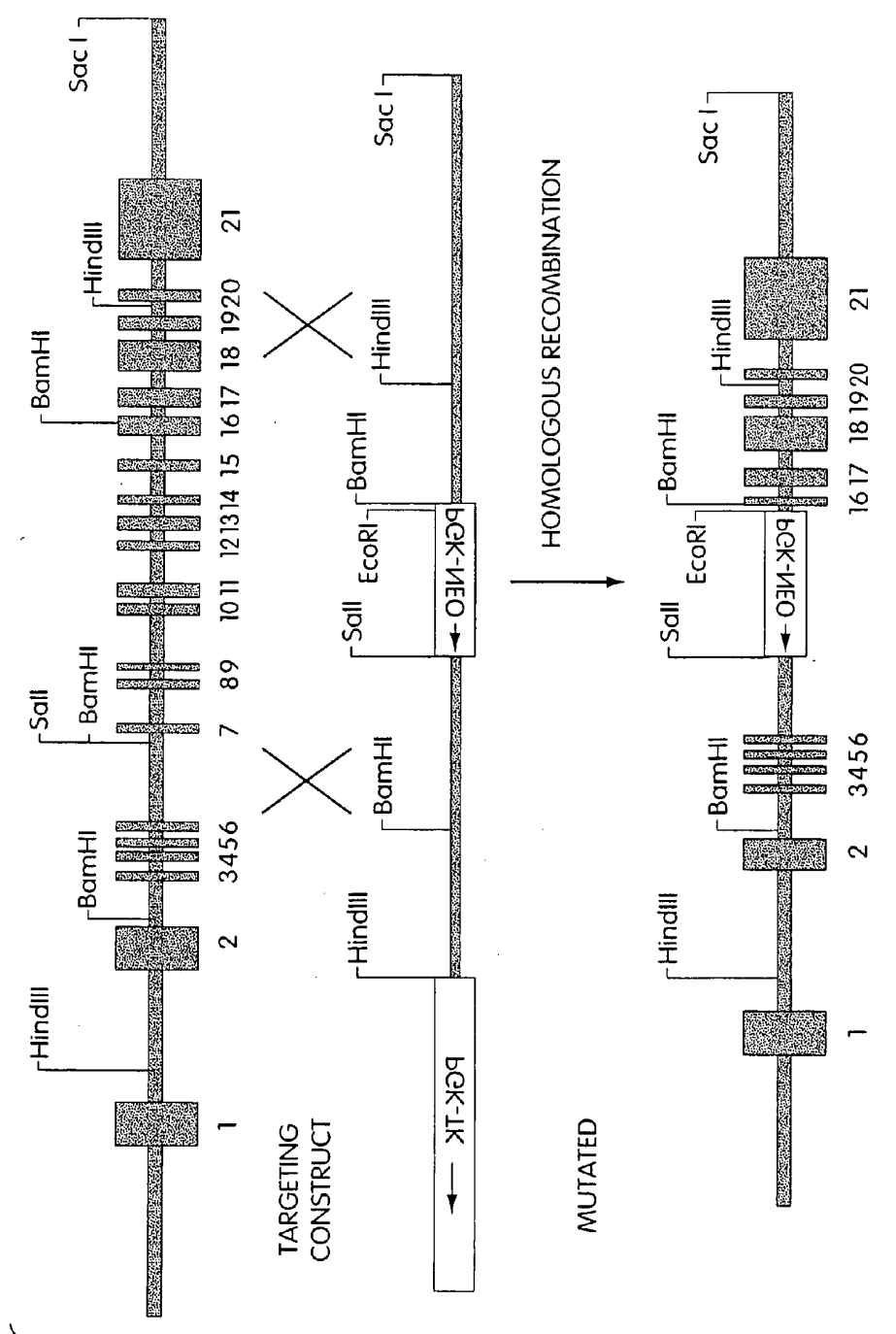


Fig. 3

## AORTIC CARBOXYPEPTIDASE-LIKE POLYPEPTIDE

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from application U.S. Ser. No. 08/818,009 filed on Mar. 14, 1997, which claims priority from provisional application U.S. Ser. No. 60/013,439, filed on Mar. 15, 1996, both of which are hereby incorporated by reference.

### STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was funded in part by the U.S. Government under grant numbers RO1GM awarded by the National Institutes of Health. The Government has certain rights in the invention.

### BACKGROUND OF THE INVENTION

[0003] The invention relates to gastrointestinal abnormalities.

[0004] Gastroschisis is a life-threatening abdominal wall defect that occurs in approximately 1-7 of every 10,000 human births. The defect is thought to originate on the right side of the umbilical cord and may involve the formation of the omphalomesenteric artery. Infants with gastroschisis can be born with abdominal organs outside the body cavity, i.e., protruding through the defect. Factors associated with an increased risk for gastroschisis include a maternal age below 20 years, ingestion of aspirin, and ingestion of pseudoephedrine. The cause of gastroschisis has not been identified.

### SUMMARY OF THE INVENTION

[0005] A novel human gene encoding aortic carboxypeptidase-like polypeptide (ACLP) has been discovered. A mutation in an ACLP gene has now been shown to be associated with the development of gastroschisis. Thus, a mutation in an ACLP gene is indicative of gastroschisis or a predisposition to develop the condition. Accordingly, the invention provides an isolated nucleic acid (e.g., genomic DNA, cDNA, or synthetic DNA) encoding an ACLP. By the term "human ACLP" is meant a polypeptide having the amino acid sequence of a naturally-occurring human ACLP. For example, the invention encompasses an ACLP with the amino acid sequence of SEQ ID NO:2 as well as naturally-occurring variants thereof such as mutant forms associated with gastroschisis or isoforms resulting from alternative splicing of exons of the ACLP gene.

[0006] The invention includes a nucleic acid molecule which contains the nucleotide sequence of human ACLP cDNA (SEQ ID NO:1). A nucleic acid molecule which contains nucleotides 140-3613 (ACLP coding sequence), inclusive, of SEQ ID NO:1 or a degenerate variant thereof, is also within the invention. Nucleotides 214-3613 encode an ACLP which lacks the first 25 residues (a putative signal peptide). Preferably, the nucleic acid molecule contains a nucleotide sequence encoding a polypeptide having an amino acid sequence that is at least 87% identical to the sequence of SEQ ID NO:2. More preferably, the sequence is at least 90% identical to SEQ ID NO:2, more preferably at least 95%, more preferably at least 98%, more preferably at least 99%, and most preferably, the nucleotide sequence encodes a polypeptide the amino acid sequence of which is SEQ ID NO:2.

[0007] An isolated nucleic acid molecule containing a strand which hybridizes at high stringency to a DNA having the sequence of SEQ ID NO:1, or the complement thereof is also within the invention. The nucleic acid molecule may be a primer useful to amplify ACLP DNA in a polymerase chain reaction (PCR). For example, the nucleic acid is at least 5 nucleotides but less than 50 nucleotides in length. Alternatively, the nucleic acid molecule may encompass the entire coding sequence of ACLP cDNA, i.e., nucleotides 140-3613, inclusive, of SEQ ID No:1. Preferably, the nucleic acid molecule spans a gastroschisis-associated mutation in an ACLP gene. Such a molecule is useful as a hybridization probe to identify a genetic alteration, e.g., a deletion, duplication, point mutation, or translocation, that indicates that an individual has gastroschisis, is predisposed to developing gastroschisis, or is a heterozygous carrier of a genetic alteration associated with gastroschisis.

[0008] By "isolated nucleic acid molecule" is meant a nucleic acid molecule that is free of the genes which, in the naturally-occurring genome of the organism, flank an ACLP gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a procaryote or eucaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. The term excludes large segments of genomic DNA, e.g., such as those present in cosmid clones, which contain an ACLP gene flanked by one or more other genes which naturally flank it in a naturally-occurring genome.

[0009] Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a procaryote or eucaryote at a site other than its natural site; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

[0010] Hybridization is carried out using standard techniques such as those described in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, (1989). "High stringency" refers to DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., hybridization and wash conditions of 65° C. at a salt concentration of 0.1×SSC. "Low" to "moderate" stringency refers to DNA hybridization and wash conditions characterized by low temperature and high salt concentration, e.g. wash conditions of less than 60° C. at a salt concentration of at least 1.0×SSC. For example, high stringency conditions may include hybridization at 42° C. in a solution containing 50% formamide; a first wash at 65° C.

using a solution of 2×SSC and 1% SDS; followed by a second wash at 65° C. using a solution of 0.1×SSC. Lower stringency conditions suitable for detecting DNA sequences having about 50% sequence identity to an ACLP gene are

detected by, for example, hybridization at 42° C. in the absence of formamide; a first wash at 2° C. in a solution of 6×SSC and 1% SDS; and a second wash at 50° C. in a solution of 6×SSC and 1% SDS.

TABLE 1

| Human ACLP cDNA |                                                                               |
|-----------------|-------------------------------------------------------------------------------|
| 1               | tccctcgtc accccatcct ctctcccgcc cttcctgga ttccctcacc cgtctcgatc (SEQ ID NO:1) |
| 61              | cctctccgc ctttccag agaccagag cccctgaccc ccgcgcctc ccccgagcc                   |
| 121             | ccccgcgct gccgcggcca tggcgccgt gcgcggggcg cccctgctca gctgcctcct               |
| 181             | ggcgttgctg gccctgtgcc ctggaggcg ccgcagacg gtgctgaccg acgacgagat               |
| 241             | cgaggagttc ctcgagggt tcctgtcaga gctagaacct gagccccggg aggacgacgt              |
| 301             | ggaggcccc ccgctcccc agcccacccc gcgggtccga aaagcccagg cggggggcaa               |
| 361             | gccagggaag cggccaggga cggccgcaga agtgccctcg gaaaagacca aagacaaagg             |
| 421             | gaagaaaggc aagaaagaca aagccccaa ggtgccaag gagtccctgg aggggtcccc               |
| 481             | caggccgccc aagaaggga aggagaagcc acccaaggcc accaagaagc ccaaggagaa              |
| 541             | gccacctaat gccaccaaga agcccaagga ggagccaccc aaggccacca agaagcccaa             |
| 601             | agagaagcca ccaaggcca ccaagaagcc ccgctcaggg aagaggcccc ccattctggc              |
| 661             | tccctcagaa accctggagt ggccactgcc cccaccccc agccttgccc ccgaggagct              |
| 721             | accccaggag ggaggggcg ccctctcaaa taactggcag aatccaggag aggagaccca              |
| 781             | tgtggaggca caggagcacc agcctgagcc ggaggaggag accgagcaac ccacactgga             |
| 841             | ctacaatgac cagatcgaga gggaggacta tggagacttt gagtacattc ggcgccagaa             |
| 901             | gcaaccagg ccacccccaa gcagaaggag gagggccgag cgggtctggc cagagcccc               |
| 961             | tgaggagaag gcccggccc cagccccga ggaggagatt gaggctcctg tgaagcctct               |
| 1021            | gctgcccccg ctgccccctg actatggtga tggttactgt atccccaaact acgatgacat            |
| 1081            | ggactattac ttggggcctc ctccgcccc gaagcccgat gctgagcgcc agacggacga              |
| 1141            | agagaaggag gactgaaga aacccaaaaa ggaggacagc agccccaagg aggagaccga              |
| 1201            | caagtggca gtggagaag gcaaggacca caaagagccc cgaaggggcg aggagtggga               |
| 1261            | ggaggagtgg agcctacgg agaaagtcaa gtgtccccc attgggatgg agtcacaccg               |
| 1321            | tattgaggac aaccagatcc gagcctcctc catgctgcgc caggcctgg gggcacagcg              |
| 1381            | cggccggctc aacatgcaga ccggtgccac tgaggacgac tactatgatg gtgcgtgggt             |
| 1441            | tgccgaggac gatgccagga ccagtggtat agagggtgac accaggagga ctaccgggtt             |
| 1501            | cacaggcgtc atcacccagg gcagagactc cagcatccat gacgattttg tgaccacctt             |
| 1561            | cttcgtgggc ttacgaatg acagccagac atgggtgatg tacaccaacg gctatgagga              |
| 1621            | aatgaccttt catgggaacg tggacaagga cacaccctg ctgagtgagc tcccagagcc              |
| 1681            | ggtggtggct cgtttcatcc gcactaccc actcacctgg aatggcagcc tgtgcatgcg              |
| 1741            | cctggagggt ctgggggtgt ctgtggcccc tgtctacagc tactacgcac agaatgaggt             |
| 1801            | ggtggccacc gatgacctg atttccggca ccacagctac aaggacatgc gccagctcat              |
| 1861            | gaaggtgggt aacgaggagt gcccacat caccgcact tacagcctgg gcaagagctc                |
| 1921            | acgaggcctc aagatctatg ccattggagat ctcagacaac cctggggagc atgaactggg            |
| 1981            | ggagcccgag ttccgtaca ctgctgggat ccatggcaac gaggtgctgg gccgagagct              |

TABLE 1-continued

| Human ACLP cDNA |                                                                    |
|-----------------|--------------------------------------------------------------------|
| 2041            | gttgctgctg ctcatgcagt acctgtgccg agagtaccgc gatgggaacc cacgtgtgctg |
| 2101            | cagcctgggtg caggacacac gcatccacct ggtgccctca ctgaaccctg atggctacga |
| 2161            | ggtggcagcg cagatgggct cagagtttgg gaactgggcg ctgggactgt ggactgagga  |
| 2221            | gggctttgac atctttgaag atttcccga tctcaactct gtgctctggg gagctgagga   |
| 2281            | gaggaaatgg gtccccacc gggccccaa caataacttg cccatccctg aacgtacct     |
| 2341            | ttcgccagat gccacggtat ccacggaggt cggggccatc attgcctgga tggagaagaa  |
| 2401            | ccccttctgt ctgggagcaa atctgaacgg cggcgagcgg ctagtatcct acccctacga  |
| 2461            | tatggccgc acgcctaccc aggagcagct gctggccgca gccatggcag cagcccgggg   |
| 2521            | ggaggatgag gacgaggtct ccgaggccca ggagactcca gaccacgcca tcttccggtg  |
| 2581            | gcttgccatc tccttcgct cgcacacct cacctgacc gagccctacc gcggaggtg      |
| 2641            | ccaagcccag gactacaccg gcggcatggg catcgtaac ggggccaagt ggaacccccg   |
| 2701            | gaccgggact atcaatgact tcagttacct gcataccaac tgcctggagc tctccttcta  |
| 2761            | cctgggctgt gacaagtcc ctcatgagag tgagctgccc cgcgagtggg agaacaacaa   |
| 2821            | ggaggcgctg ctacacctca tggagcaggt gcaccgcggc attaaggggg tggtagcgga  |
| 2881            | cgagcaaggc atccccattg ccaacgccac catctctgtg agtggcatta atcacggcgt  |
| 2941            | gaagacagcc agtgggtgtg attactggcg aatcttgaac ccgggtgagt accgcgtgac  |
| 3001            | agcccacgcg gagggctaca ccccgagcgc caagacctgc aatgttgact atgacatcgg  |
| 3061            | ggccactcag tgcaacttca tcctggctcg ctccaactgg aagcgcatcc gggagatcat  |
| 3121            | ggccatgaac ggaaccggc ctatcccaca catagacca tcgcgcccta tgacccccca    |
| 3181            | acagcgacgc ctgcagcagc gacgcctaca acaccgctg cggtctcggg cacagatgcg   |
| 3241            | gctgcggcgc ctcaacgcca ccaccacct agggcccccac actgtgcctc ccacgctgcc  |
| 3301            | ccctgcccct gccaccacco tgagcactac catagagccc tggggcctca taccgccaac  |
| 3361            | caccgctggc tgggaggagt cggagactga gacctacaca gaggtggtga cagagtttgg  |
| 3421            | gaccgaggtg gagcccgagt ttgggaccaa ggtggagccc gagtttgaga ccagttgga   |
| 3481            | gcctgagttc gagaccagc tggaaaccga gtttgaggaa gaggaggagg aggagaaaga   |
| 3541            | ggaggagata gccactggcc aggcattccc cttcacaaca gtagagacct acacagtga   |
| 3601            | ctttggggac tcttgagatc agcgtcctac caagaccca gcccaactca agctacagca   |
| 3661            | gcagcacttc ccaagcctgc tgaccacagt cacatcacco atcagcacat ggaaggcccc  |
| 3721            | tggatatggc actgaaagga agggctggtc ctgccccttt gagggggtgc aaacatgact  |
| 3781            | gggacctaa agccagaggc tgtgtagagg ctctgtctcc acctgccagt ctcgtaagag   |
| 3841            | atgggggttg tgcaagtgtg gagtaggggc agagggagg agccaagtc actccaataa    |
| 3901            | aacaagctca tggcaaaaaa aaaaaaaaaa aaaaa                             |

[0011] The invention also includes a substantially pure human ACLP polypeptide. A substantially pure ACLP polypeptide may be obtained, for example, by extraction from a natural source (e.g., a vascular smooth muscle cell); by expression of a recombinant nucleic acid encoding an ACLP; or by chemically synthesizing the protein. A polypeptide or protein is substantially pure when it is

separated from those contaminants which accompany it in its natural state (proteins and other naturally-occurring organic molecules). Typically, the polypeptide is substantially pure when it constitutes at least 60%, by weight, of the protein in the preparation. Preferably, the protein in the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, ACLP. A

substantially pure ACLP may be obtained, for example, by extraction from a natural source (e.g., a vascular smooth muscle cell); by expression of a recombinant nucleic acid encoding an ACLP; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. Accordingly, substantially pure polypeptides include recombinant polypeptides derived from a eucaryote but produced in *E. coli* or another prokaryote, or in a eucaryote other than that from which the polypeptide was originally derived.

[0012] For expression of recombinant ACLP, an ACLP-encoding nucleic acid is operably linked to a regulatory sequence, e.g., a promoter. By “promoter” is meant a minimal DNA sequence sufficient to direct transcription. Promoters may be constitutive or inducible, and may be coupled to other regulatory sequences or “elements” which render promoter-dependent gene expression cell-type specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5' or 3' region of the native gene, or within an intron. DNA encoding an ACLP may be operably linked to such regulatory sequences for expression of the polypeptide in prokaryotic or eucaryotic cells. By “operably linked” is meant that a coding sequence and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

[0013] To produce recombinant ACLP, a cell containing an ACLP-encoding sequence operably linked to appropriate regulatory sequences is cultured under conditions permitting expression of a nucleic acid molecule. The cell may be a prokaryotic cell or a eucaryotic cell. To obtain post-translationally modified, e.g., glycosylated recombinant ACLP, the recombinant polypeptide is produced in a eucaryotic cell, e.g., a yeast or mammalian cell.

[0014] An ACLP preferably contains an amino acid sequence that is at least 87% identical to the amino acid sequence of SEQ ID NO:2. More preferably, the amino acid sequence is at least 90% (more preferably at least 95%, more preferably at least 98%, more preferably at least 99%) identical to SEQ ID NO:2. Most preferably, the polypeptide contains the amino acid sequence of SEQ ID NO:2.

[0015] The invention also includes polypeptides which contain a portion of naturally-occurring ACLP, e.g., an

ACLP fragment containing a lysine-rich/proline rich domain (amino acids 117-164 of SEQ ID NO:2), an ACLP fragment containing a discoidin-like domain (amino acids 385-540 of SEQ ID NO:2), or an ACLP fragment containing a carboxypeptidase-like domain (amino acids 562-969 of SEQ ID NO:2)

[0016] Where a particular polypeptide or nucleic acid molecule is said to have a specific percent identity to a reference polypeptide or nucleic acid molecule of a defined length, the percent identity is relative to the reference polypeptide or nucleic acid molecule. Thus, a peptide that is 50% identical to a reference polypeptide that is 100 amino acids long can be a 50 amino acid polypeptide that is completely identical to a 50 amino acid long portion of the reference polypeptide. It might also be a 100 amino acid long polypeptide which is 50% identical to the reference polypeptide over its entire length. Of course, many other polypeptides will meet the same criteria. The same rule applies for nucleic acid molecules.

[0017] For polypeptides, the length of the reference polypeptide sequence will generally be at least 10 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids, 50 amino acids, or 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least 25 nucleotides, preferably at least 50 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides or 300 nucleotides.

[0018] In the case of polypeptide sequences which are less than 100% identical to a reference sequence, the non-identical positions are preferably, but not necessarily, conservative substitutions for the reference sequence. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

[0019] Sequence identity can be measured using sequence analysis software (for example, the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705), with the default parameters as specified therein.

TABLE 2

| Human ACLP amino acid sequence                                            |  |
|---------------------------------------------------------------------------|--|
| MAAVRGAPLLSCLLALLALCPGGRPQTVLTDDIEEFLEGFLSELEPEPREDDVEAPPPP (SEQ ID NO:2) |  |
| EPTPRVRKAQAGKPGKRPGTAAEVPEKTKDKGKGGKDKGPKVPKESLEGSPPPPKKG                 |  |
| KEKPPKATKKPEKPPKATKKPEKPPKATKKPEKPPKATKKPPSGKRPPILAPSETLE                 |  |
| WPLPPPPSPGPEELPQGGAPLSNNWQNPGEETHVEAQEHQPEPEEETEQTLDYNDQIE                |  |
| REDYEDFEYIRRKQPRPPSRRRRPERVWPEPPEEKAPAPAPEERIEPPVKLLPPLPP                 |  |
| DYGDGYVIPNYDDMDYFYGPPPPQKPDARQTDDEEKEELKKPKKEDSSPKEETDKWAVEK              |  |
| GKDHKEPRKGELEEWEPTTEKVKCPPIGMESHRIEDNQIRASSMLRHGLGAQRGLNMQ                |  |
| TGATEDDYYDGAWCAEDDARTQWIEVDTRTRTFTGVTQGRDSSIHDHDFVTTFFVGFNS               |  |
| DSQTWVMYTYGEEMTFHGNVDKDTPVLSSELPEPVVARFIRIYPLTWNGSLCMRLEVLGC              |  |
| SVAPVYSYYAQNEVVATDDDLFRHHSYKDMRQLMKVVNEECPTITRTYSLGKSSRGLKIY              |  |
| AMEISDNPGHEHELGEPEFRYTAGIHNEVLGRELLLLLMQYLCREYRDGNPRVRSVLQDT              |  |
| RIHLVPSLNDGYEVAQMGSEFGNWALGLWTEEGFDIFEDFDLNSVLWGAEERKWPVY                 |  |
| RVPNNNLPPIPERYLSPDATVSTEVRAIIAWMEKNPFVLGANLNGGERLVSYPDMARTPT              |  |
| QEQLLAAMAAARGEDEDEVSEAQETPDHAIFRWLAISFASAHLTLETPEYRGGCQAQDYT              |  |

TABLE 2-continued

| Human ACLP amino acid sequence                                                                                                                                                                                                                                                                                                                    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GGMGIVNGAKWNPRTGTINDFSYLHTNCLLSFYLGCDKFPHESELPREWENNKALLTF<br>MEQVHRGIKGVVTDEQGIPIANATISVSGINHGVTASGGDYWRILNPGEYRVTAHAEGY<br>TPSAKTCNVDYDIGATQCNFILARSNWKRIEIMAMNGNRPIPHIDPSRPMTPQQRRLLQQ<br>RRLQHRLRLRAQMRLRLRNATTTLGPHTVPPTLPPAPATTLSTTIEPWGLIPPTTAGWEE<br>SETETYTEVVTEFGTEVEPEFGTKVEPEFETQLEPEFETQLEPEFEFEFEFEFEFEFEIATG<br>QAFPTTETVETVTNFGDF |

[0020] A substantially pure DNA containing an ACLP promoter/enhancer sequence (SEQ ID NO:3) is useful for directing transcription of DNA encoding all or part of ACLP or of DNA encoding a heterologous polypeptide (e.g., a polypeptide other than ACLP or an ACLP the sequence of which corresponds to a naturally-occurring ACLP of a species other than the species from which the promoter/enhancer sequence is derived). For example, a murine ACLP promoter/enhancer sequence may be operably linked to DNA encoding human ACLP for therapeutic expression of ACLP in human patients. To regulate transcription of the polypeptide-encoding sequence (e.g., developmental stage-specific transcription), the promoter/enhancer sequence is operably linked to a polypeptide-encoding sequence. The ACLP promoter/enhancer sequence directs transcription of a polypeptide-encoding sequence.

[0021] By “promoter/enhancer sequence” is meant a DNA sequence located 5' to the transcriptional start site of the ACLP gene and which contains one or more cis-acting elements which regulate transcription, e.g., cell specific transcription. The elements may be contiguous or separated by DNA not involved in the regulation of transcription, e.g., an enhancer element may be in a position immediately adjacent to the promoter element or up to several kilobases upstream or downstream of the transcriptional start site. The promoter/enhancer DNA is preferably derived from the 5' region of a mammalian ACLP gene, such as that of the mouse (SEQ ID NO:3), and regulates expression of a polypeptide-encoding DNA to which it is operably linked. The promoter/enhancer sequence regulates developmental stage-specific expression, e.g., expression in embryonic cells, of a polypeptide-encoding sequence.

TABLE 3

| Mouse ACLP promoter/enhancer                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AAGCTTAGTCTCCCTCTCTCTGGCTCTCTCTGGGGCTTCCCTATGGAGGTAGCACTT (SEQ ID NO:3)<br>ACAGAAGATGCTTGTTCACAACTTCAGGGGTACAACTACACAGATATACTGAAGGACA<br>GGAGGCTGGGGCTCCCCACCCCAACAGCCACTGTTCTCTCAGGAGCTCTGCTTCTG<br>CTCTGCAGCATTGAAAACAACTGAAGGACACCTTCTCTCTCAGGCCAGCCAGTGC<br>TGTTGTGTGATCCTCGGGAAGACTCTAACGCATTACAGGGACACAGGAGTTGGGAGG<br>GAGAGGAGTTACAGAACTTTCCAGCAGGACCTCAGGAGAACGCCTGGACACGGACAGGAA<br>CCCCAACCCCTCAGGGACCCCTTGGACCTTTGAGTGCTCTGATCATGGAAGCCACC<br>AGCCTCCCGATTCTCAGCTGTGGCCTTGGCAGTGCCCTCTGGACATTGACTTAAACGC<br>TATGCTCTTCAGCAGAGTGGAGAGCTCTCCTCACAGGCTCTGGCTTCTGGTTGCTCCTCTT<br>GCCCCAGCGCTGTGGGCCAGGTTAGAAAGACTTCTGAGGACAGGCTCCCTCAGGAGGA<br>TCCCCAGCGTACGACTGTGCTCCACGCACCTTTCGGATTCTCTGTGTGGAGGCTCAA<br>CCCCTCAGGCCTCTGGGCCAGTCTCTGCTCGAATTCTGTCCTGACTCATTGAGGC<br>TCAGGAAAAGGCTTTCTAGACCTTAGGTTCTTTGTTTTCCATTTTGAATGGCTCTG<br>TTTTCCCTGGCAGAGAAATCCAAACCAATTCAGTCCAAGTATGACCCATGCCTAGGGA<br>AGTGACATCCATGTCCCTCATGCACCTGTGGCATACCCAGCATGACACACTGGACCAG<br>ACTGGGGGCACGGAAGCCAATTCCAGAACTGACTTTGAGCACAATGATTCAGAGGTGA<br>CCATGAGTGAGACTTGCTTTACTTCTGCTCTGCGACAGGTTGAAGTCTCTCATGGGAG<br>GCCTAGCTGTGAGAGGATTGCTCTGGGATGGGGGAAGGGGAGCAAAGTGAGTGAAGACC<br>AACAGCCTGTGGGATGCAAGGGCTGATCGTGTGTGCTAGGCACAGCACAAAGTGGTCCAT<br>TTAGCCGGGAGTGGTGGTGCACACCTTTAATCCAGCACTTGGGAGGCAACAGCAGGTG<br>GGTTTCTGAGTTGAGGGCCAGCCTGGTCTACAGAGCAAGTTCCAGGACAGCCAGAGCTAC<br>ACAGAGAACTCTGTCTCAAAAAATCGAATAAACAGAAAGGTGGTCCATTAAATATGC<br>GTATAGTAAGTTGTGGACACGGGAGTTCCCTGCTGAGTCAGACAGCTAGGAGGGCTAAG<br>ATGGGTTAGACCTCCCCCCCCCACACACACACACACTCACACACATCAGTTT<br>TTGGCATAGTCTCCATGCTTCCCTCAAGGAGAGCCAGAAAGGAGACTGCCGGGAGGAGCTT<br>GCCTACTCCCTGAGAGCAGTGGGTTACAGAGCCAGTGGCCGAAAATTTCCCTTTTCT<br>CCCTGCTCATGCTGGACAGAGAGGGTGAGGGTGAGGGTGAAAGACTGAGGAGGTGGCATC<br>GTGTTGGTGTCTTCTGACCTGCTTTTCTTTTCTCTTCCAGCTGAGATGTAAACTTTT<br>CCATGTCAATCATCTGGGGTGCTATTCTTTTATCAGAGTGCTCCCACTTGGTT<br>GAAAGCTGCCTGCCACTACCCTGGACCTATGGCTGTACAAGCCACGTTCACTCTTTA<br>ATCCTTCATGGGTAAATGCTCTGGCATTCTGGGCTTAGCTATGATGGCCATTATGAGCC<br>AGCCAACGTTTGTATTCTAGAAGCCATAGCTGAAGCTGTTGTAACAATTTGTTGTTT<br>ACCGCTTCTGGTCAGAGGAAGGAGAGAATAGCTATTACTCCACATTGGGACCTGAGCCCT<br>GAGCTCTGAAGTGGGGCTCTATCTCCATAAGGACAGCAGCTTGCTGAGAACAGCTTTT<br>ACAGCCTTCTCTCAAAAATTTGGCTCCAAAGACCTGGGATGTTGGTGATACTGGACAAA<br>GGTGACACCTGTGCAAGCACACAGCAGGTGACACTTTGAAGAGCTAACCTCCAGAAAGTG<br>GAAAGAGGTGATCGCCAGTACCCTCGAGGGCCCTACTCCCTCCCTCCCTAGCAATCTC<br>CCTGGGCTCAGAGCAAAGGGCACAGCGGTTAGAGCACAGGTCTCCTTAGACTCCGCACA<br>CTCCTCTCCCATAACTGTTGCATTCTTTTCTCCAGGCTTCTCCCGCTAGCGCGCC |

TABLE 3-continued

| Mouse ACLP promoter/enhancer                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| TGCACCCAGACCCCTCTAAACTGGCGCGTGACGCTGCTATTAGTCTGGGCTCCGTGCTGTC<br>CGCCTCCCTCCCCCGCAGCCCCCGGTCCAAGGCCGGCTCCTCCTCCCTCCCGGAAA<br>CCCGAAGCCCCCGCCCGGCGAGCCGTCGCAAGCGCTCTGGAGGGCGGTCCGCGTGAGA<br>GCCAGCCACGCGGGGAGGAGCGCCAGTTGCTGCCGGAGCTGGGCCCGCCAGAACCTCT<br>CCTGGAGCCCCCTTGCTCTCCTTGAATCTCCCTTTCCACCGCTTTCTGGATACCTTGAC<br>GCCCAGTTCTTCGCGCCCTTTCCCGCCCTACGCGGGGCGCTGCCCTGCCACCCAAGT<br>CCTGCTCAAGCCCGCCCGGTCCCGCGCGTGCCAGAGCCATG |

[0022] The invention also includes a vector containing the promoter/enhancer DNA of the invention (operably linked to a polypeptide-encoding DNA sequence), and a vascular smooth muscle cell containing the vector. Also within the invention is a method of directing vascular smooth cell-specific expression of the polypeptide by introducing the vector into a vascular smooth muscle cell and maintaining the cell under conditions which permit expression of the polypeptide, e.g., introducing the vector into a human patient for gene therapy.

[0023] A method of detecting a gastroschisis-associated genetic alteration is carried out by providing a sample of DNA or RNA from a patient or fetus, and determining whether the DNA or RNA contains a mutation in a gene encoding an ACLP. Detection of such an ACLP mutation indicates that the patient or fetus has a genetic alteration that is associated with the development of gastroschisis. The presence of a gastroschisis-associated genetic alteration is diagnostic of gastroschisis or a predisposition to developing gastroschisis. The method can also be used to identify heterozygous carriers of a mutation associated with gastroschisis. Such individuals may be asymptomatic but are at risk of having children which are homozygous for an ACLP mutation (and therefore, likely to develop clinical gastroschisis). Tissue samples from adult patients are obtained by conventional means, e.g., biopsy or venipuncture. Prenatal testing is carried out by obtaining fetal tissue samples, e.g., by amniocentesis or chorionic villi sampling.

[0024] Patient-derived DNA is examined for genetic abnormalities in the ACLP gene, e.g., by detecting restriction fragment length polymorphisms (RFLPs), deletions, point mutations, or other defects. The diagnostic method includes the step of subjecting the sample to polymerase chain reaction (PCR), using a forward PCR primer complementary to a portion of the antisense strand of the gene, the portion being within (a) a first intron of the gene, or (b) the 5' untranslated region adjacent to the start codon of the gene; and a reverse PCR primer complementary to a fragment of the sense strand of the gene, this fragment being within (a) a second intron of the gene, or (b) the 3' untranslated region adjacent to the termination codon of the gene. PCR can also be used to detect mutations in an ACLP promoter or other regulatory sequences using primers that flank the mutation. ACLP mutations and/or aberrant ACLP expression can also be detected using standard hybridization techniques, such as Northern blotting.

[0025] Fragments of ACLP are useful to raise ACLP-specific antibodies. Accordingly, the invention includes an antibody, e.g., a polyclonal antiserum or a monoclonal antibody preparation, that selectively binds to an ACLP ACLP-specific antibodies are used to diagnose gastroschisis or a

predisposition thereto. For example, a diagnostic method is carried out by providing a tissue sample from a patient or fetus, and detecting expression of an ACLP gene in the tissue sample. Expression is measured by detecting the amount of ACLP-specific antibody that binds to the tissue sample, e.g., by ELISA assay, Western blot assay, or immunohistochemical staining of tissue sections. Expression of ACLP is also measured by detecting the level of ACLP transcript in the tissue sample. Regardless of the method of detection of ACLP expression, a reduction in the amount of expression in the patient-derived tissue sample compared to the level of expression in a normal control tissue sample indicates that the patient or fetus from which the sample was obtained has or is predisposed to developing gastroschisis.

[0026] Methods of treating or preventing the development of gastroschisis are also within the invention. For example, one treatment regimen includes the steps of identifying a patient with or at risk of developing gastroschisis, and introducing into cells of the patient an isolated nucleic acid encoding ACLP, e.g., a nucleic acid which contains the nucleotide sequence of 140 to 3613 of SEQ ID NO:1. The cells into which the DNA was introduced produce the recombinant ACLP to compensate for a gastroschisis-associated genetic alteration, e.g., a mutation resulting in reduced production of ACLP or a mutation resulting in the production of a defective ACLP. Rather than administering ACLP-encoding DNA to the patient, an ACLP (e.g., a polypeptide having the sequence of SEQ ID NO:2) or a fragment thereof may be introduced into the patient.

[0027] An animal model for gastroschisis is useful to study the development of the condition as well as to evaluate therapeutic approaches to treatment or prevention of gastroschisis. A genetically-altered non-human mammal, all diploid cells of which contain a mutation in an endogenous gene encoding an ACLP, is included in the invention. For example, a mammal with a homozygous null mutation in its ACLP gene(s) develops gastroschisis. Preferably, the mammal is a rodent such as a mouse. The genetically altered non-human mammal produces altered levels of ACLP or mutant forms of ACLP. The levels of ACLP gene product in the genetically altered mammal can be increased or decreased at different time periods during development. By "genetically altered mammal" is meant a mammal in which the genomic DNA sequence has been manipulated in some way. The genetically altered mammal may be a knockout in which the endogenous ACLP sequences have been deleted or otherwise altered to decrease or change the pattern of expression. Alternatively, the genetically altered mammal may be transgenic. For example, the transgenic mammal may express ACLP sequences from another species, may overexpress ACLP gene product, or may express ACLP in

tissues and at developmental stages other than those in which ACLP is expressed in a wild type animal.

[0028] The nucleated cells of a genetically altered mammal not producing a functional endogenous ACLP may be engineered to encode a human ACLP, and to express functional human ACLP, or, alternatively, ACLP from another heterologous species.

[0029] Preferably, the genetically altered non-human mammal is a rodent such as a mouse or a rat, the germ cells and somatic cells of which contain a mutation in DNA encoding ACLP. All diploid cells of such an animal contain a mutation in one or both alleles of the endogenous ACLP gene. The mutation can, for example, be a deletion, an insertion, or a nucleotide substitution. The mutation could be in the ACLP regulatory regions or in the coding sequence. It can, e.g., introduce a stop codon that results in production of a truncated, inactive gene product or it can be a deletion of all or a substantial portion of the coding sequence. For example, one or more exons, e.g., exons 7-15, of an ACLP gene may be deleted. By the term "null mutation" is meant a mutation that reduces the expression or activity level of the protein encoded by the mutated gene by more than 80% relative to the unmutated gene. A mouse harboring such a null mutation is a knockout mouse. An ACLP knockout mouse, i.e., one that harbors a homozygous ACLP null mutation, has been found to have an abdominal defect with an extrusion of abdominal organs i.e., gastroschisis.

[0030] The invention also includes a mammalian cell line, e.g., immortalized ACLP deficient cells, the genomic DNA of which contains a null mutation in DNA encoding ACLP. Such cells lack the ability to synthesize full length functional ACLP. The cells harboring the null mutation may be derived from a cell obtained from a ACLP deficient mammal, e.g., an ACLP knockout mouse.

[0031] Compounds capable of promoting expression or function of an ACLP may be therapeutically useful to treat gastroschisis. Accordingly, the invention includes a method of screening a candidate compound to identify a compound capable of stimulating expression of an ACLP, e.g., human ACLP, by (a) providing a cell or tissue expressing capable of expressing a ACLP, (b) contacting the cell or tissue with the candidate compound, and (c) determining the amount of expression of the ACLP by the cell. An increase in the amount of ACLP expression in the presence of the candidate compound compared to that in the absence of the candidate compound indicates that the compound stimulates expression of the ACLP.

[0032] In addition to diagnostic methods, such as described above, the present invention encompasses methods and compositions for evaluating appropriate treatment, and treatment effectiveness of pathological conditions associated with aberrant expression of ACLP. For example, the ACLP gene can be used as a probe to classify cells in terms of their level of ACLP expression, or as a source of primers for diagnostic PCR analysis in which mutations and allelic variation of ACLP can be detected.

[0033] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1A is a diagram showing a comparison of deduced open reading frames of human ACLP and mouse

ACLP. The human and mouse proteins contain 1158 and 1128 amino acids, respectively. Highlighted motifs include a signal peptide (bold, underline), a 4-fold lysine- and proline-rich repeating motif (bold, italic), a discoidin-like domain (bold, italic, underline), and a region with homology to the carboxypeptidases (bold).

[0035] FIG. 1B is a diagram showing the location of peptide domains of human ACLP. The signal peptide sequence at the N-terminus is designated "Signal"; the 4-fold repeating motif is designated "Repeat"; the discoidin-like domain is designated "DLD"; and the region with homology to carboxypeptidases is designated "CLD".

[0036] FIG. 2A is a diagram of mouse genomic DNA showing a map of the mouse ACLP gene and neighboring DNA polymerase delta small subunit gene.

[0037] FIG. 2B is a diagram of a restriction map of genomic DNA containing the mouse ACLP gene and neighboring DNA polymerase delta small subunit gene.

[0038] FIG. 3 is a diagram showing a map of the targeting construct used to make an ACLP knockout mouse.

#### DETAILED DESCRIPTION

[0039] A mutation in an ACLP-encoding nucleic acid resulting in a decrease in production of an ACLP compared to the level of ACLP production in an animal lacking the mutation has now been shown to result in the development of gastroschisis in newborn mice. The following examples describe the cloning and characterization of human ACLP and methods of diagnosing and treating gastroschisis the underlying defect of which is a genetic alteration in the ACLP genes.

#### EXAMPLE 1

##### Cloning of ACLP Genes

[0040] ACLP was identified in a screen for proteins interacting with the E47 product of the E2A gene. A recombinant E47 fusion protein (N3-SH[ALA]), containing the basic helix loop helix domain of hamster shPan-1 (amino acids 509-646, with mutations R551A, V552L, and R553A) with a heart muscle kinase recognition sequence and the FLAG epitope, was expressed and purified as described (Blanan et al., 1995, Proc. Natl. Acad. Sci. USA 92:5870-4; Blanan and Rutter, 1992, Science 256:1014-8). N3-SH[ALA] was phosphorylated by heart muscle kinase in the presence of  $\gamma$ -<sup>32</sup>P-ATP and used to screen a human aorta  $\lambda$ gt11 cDNA expression library (Clontech) by interaction cloning (Blanan et al., 1995, Proc. Natl. Acad. Sci. USA 92:5870-4; Blanan and Rutter, 1992, Science 256:1014-8). A 1450-bp cDNA clone ( $\Delta$ E2A-BP) obtained from interaction cloning was radiolabeled by random priming and used to isolate a 2786 bp cDNA clone from the same human aorta  $\lambda$ gt11 cDNA library. Data from Northern blotting experiments revealed that the ACLP-1 RNA was about 3.9 kb in size and suggested that the 2786 bp cDNA clone was a partial CDNA clone. Additional 5' sequences of the ACLP cDNA were isolated by 5' rapid amplification of cDNA ends from human aortic smooth muscle cell RNA (Gibco-BRL). The full length sequence of the human ACLP cDNA was found to be 3935 bp and is shown in Table 1 (SEQ ID NO:1). The full length human ACLP cDNA contains an open reading frame

(nucleotides 140-3613 of SEQ ID NO:1) encoding a polypeptide of 1158 amino acids. The open reading frame is preceded by a Kozak consensus translation initiation sequence, which in turn is preceded by an in frame stop codon.

[0041] The human ACLP protein has a calculated molecular mass of 130 kDa, an estimated pI of 4.8, and contains a putative signal peptide sequence. In addition, it contains an 11 amino acid lysine- and proline-rich motif repeated four times at the N-terminus, a domain with 30% amino acid identity to the slime mold adhesion protein discoidin I, and a C-terminal domain with 39% identity to carboxypeptidase E. The human ACLP gene maps to the short arm of chromosome 7 (between D7S478 and D7S519).

[0042] The sequence of the human ACLP cDNA (GENBANK™ accession number AF053944) was compared to sequences present in GENBANK™ databases. A 3' portion of ACLP cDNA was found to share homology with the sequence of a CDNA encoding mouse adipocyte enhancer binding protein 1 (AEBP1; He et al., 1995, *Nature* 378:92). AEBP1 was originally identified as a 2.5 kb cDNA that hybridized to a 4 kb band on Northern blot analysis, and was predicted to encode a 719 amino acid, 79 kDa protein.

[0043] To isolate mouse ACLP cDNA (GENBANK™ accession number AF053943), first strand cDNA from C2C12 mouse myoblast total RNA was synthesized by reverse transcription with the primer 5' ATCTGGTTGTC-CTCAAT 3' (SEQ ID NO:4). The nested primer 5'TGACTC-CATCCCAATAG 3' (SEQ ID NO:5) and the anchor primer included in the kit for 5' rapid amplification of cDNA ends was then amplified to produce a product of approximately 1400 bp in size. This product was sequenced using standard methods.

[0044] The entire open reading frame of mouse ACLP was then amplified from C2C12 RNA by reverse transcription PCR (EXPANDLONG™ Template PCR System, Boehringer Mannheim, Indianapolis, Ind.). The human and mouse clones were sequenced by the dideoxy nucleotide chain termination method using a combination of Sequenase Version 2.0 (Amersham, Arlington Heights, Ill.), the Thermo Sequenase <sup>33</sup>P terminator cycle sequencing kit (Amersham), and the Thermo Sequenase fluorescent-labeled cycle sequencing kit with 7-deaza-GTP (Amersham) on a Licor (Lincoln, Nebr.) apparatus.

[0045] Sequencing of the 3633 bp mouse ACLP cDNA fragment, revealed that it encoded an open reading frame (1128 amino acids) similar to that of the full-length human ACLP cDNA, indicating that it is the mouse ACLP homologue. A comparison of the human and mouse ACLP amino acid sequences is shown in **FIG. 1A**. Overall, the two proteins are 85% identical and 90% similar.

#### EXAMPLE 2

##### Identification of a Promoter-Enhancer Sequence Associated with the ACLP Gene

[0046] To identify genomic sequences that mediate tissue specific and developmental expression pattern of the ACLP gene, a region of genomic DNA adjoining the 5' end of the mouse ACLP coding sequences was isolated (**FIGS. 2A and 2B**). Portions of this genomic DNA were then used in

reporter transfection assays to determine their ability to direct expression of a reporter gene in transfection assays. ACLP promoter/enhancer DNA was cloned into the pGL2 Basic vector (Stratagene) and transfected into rat aortic smooth muscle cells (RASMC) to measure promoter activity. Using this assay, a region containing an ACLP promoter/enhancer sequence was identified and is shown in Table 3 (SEQ ID NO:3). ACLP promoter/enhancer DNA was found to have transcriptional activity both in vitro (using cultured cells) and in vivo (in a transgenic mouse).

#### EXAMPLE 3

##### Generation and Characterization of Antibodies to ACLP Peptides

[0047] A carboxy terminal fragment of mouse ACLP was expressed in bacteria, purified, and used as an immunogen to raise antibodies in rabbits.

[0048] To produce a polyclonal anti-ACLP antibody, a BamHI-EcoRI fragment of mouse ACLP (encoding amino acids 615-1128) was subcloned into the pRSET C bacterial expression vector (Invitrogen), and the resulting plasmid was transformed into BL21(DE3)pLysS-competent bacteria (Stratagene). Protein expression was induced with 1 mM isopropyl β-D-thiogalactopyranoside for 3 h. Bacteria were sonicated in lysis buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM Tris, pH 8, 100 mM NaCl) containing the protease inhibitors aprotinin, leupeptin, and phenylmethylsulfonyl fluoride. Lysates were clarified by centrifugation at 10,000 g for 15 min, and the pellet was resuspended in lysis buffer supplemented with 8 M urea. His-tagged proteins were purified with Talon resin (Clontech) and eluted in lysis buffer containing 8 M urea and 100 mM ethylene diamine tetraacetic acid. Proteins were dialyzed against water and measured with the Bio-Rad (Hercules, Calif.) protein assay reagent. 100 μg of the purified protein was used to immunize New Zealand white rabbits. Antiserum was collected, titered against the recombinant protein, and used for immunoblot analysis. Specificity of the antiserum was determined by using preimmune serum and by competition with a recombinant protein. The same methods are used to raise antibodies to human ACLP. The rabbit antisera raised against a portion of mouse ACLP was found to crossreact with human ACLP.

[0049] Protein extracts from cultured cells were prepared for Western blotting in extraction buffer (25 mM Tris, pH 7.4, 50 mM NaCl, 0.5% sodium deoxycholate, 2% Nonidet P-40, and 0.2% sodium dodecyl sulfate) containing the protease inhibitors aprotinin, leupeptin, and phenylmethylsulfonyl fluoride. To obtain proteins from mouse tissues, individual organs were homogenized in 25 mM Tris, pH 7.5, 50 mM NaCl, and 10 mM ethylene diamine tetraacetic acid containing protease inhibitors (Complete, Boehringer Mannheim). Proteins were measured with the BCA protein assay kit (Pierce, Rockford, Ill.). After 50 μg aliquots had been resolved on 6% sodium dodecyl sulfate-polyacrylamide gels (18), proteins were transferred electrophoretically to nitrocellulose membranes (Schleicher and Schuell, Keene, N.H.) in 48 mM Tris, pH 8.3, 39 mM glycine, 0.037% sodium dodecyl sulfate, and 20% methanol transfer buffer. Blots were equilibrated with 25 mM Tris, pH 8, 125 mM NaCl, and 0.1% Tween 20 and blocked in the same solution containing 4% nonfat dry milk. Blots were incubated with anti-ACLP serum diluted 1:1000 and then horseradish per-

oxidase-conjugated goat anti-rabbit serum diluted 1:4000. Membranes were processed with an enhanced chemiluminescence reagent (ECL reagent, NEN, Boston, Mass.) and exposed to film.

[0050] By Western blot analysis, this antibody detected a single band corresponding to a protein with an apparent mobility of approximately 175 kDa in mouse aortic smooth muscle cells (MASMC) extracts. This protein showed a similar migration to a protein generated by transcription and translation in vitro of a mouse ACLP cDNA clone, providing additional evidence that the isolated human and mouse cDNA clones encode full-length ACLP.

[0051] Monoclonal antibodies can be obtained using full-length human or mouse ACLP or fragments thereof using standard methods, e.g., the process described by Milstein and Kohler, 1975, *Nature* 256:495-97, or as modified by Gerhard, 1980, *Monoclonal Antibodies*, Plenum Press, pages 370-371. Hybridomas are screened to identify those producing antibodies that are specific for an ACLP. Preferably, the antibody will have an affinity of at least about  $10^8$  liters/mole and more preferably, an affinity of at least about  $10^9$  liters/mole.

#### EXAMPLE 4

##### Subcellular Localization and Tissue Localization of ACLP Proteins

[0052] To assess the subcellular localization of ACLP, a mouse ACLP expression construct was generated with a c-myc epitope at the C-terminus. The myc epitope was placed at the C-terminus to avoid interference with signal peptide-mediated processes, e.g., ACLP secretion mechanisms. To construct a c-myc-tagged ACLP expression plasmid (pcDNA3.1/ACLP-Myc-His), the open reading frame of mouse ACLP was amplified with the Expand Long Template PCR System (Boehringer Mannheim). A 5' primer containing an EcoRI site (5' CGGAATTCAGTCCCTGCTCAAGCCCG 3'; SEQ ID NO:6) and a 3' primer containing a HindIII site (5' CGAAGCTTGAAGTCCCCAAAGT-TCACTG 3'; SEQ ID NO:7) was used, which resulted in the deletion of the endogenous termination codon in the PCR product. The PCR product was then digested with EcoRI and HindIII restriction enzymes and ligated into the EcoRI and HindIII sites of pcDNA3.1(-)/Myc-His A (Invitrogen). Cells were transfected transiently with pcDNA3.1/ACLP-Myc-His by the DEAE-dextran method with minor modifications (Tan et al., *Kidney International* 46:690, 1994). Twenty-four hours after transfection, cells were trypsinized and plated onto chamber slides (Nunc, Naperville, Ill.) and grown for an additional 24 h. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline and immunostained using standard methods. A monoclonal anti-c-myc primary antibody (9E10 Ab-1, Oncogene Research Products, Cambridge, Mass.) and a rhodamine-conjugated goat anti-mouse IgG secondary antibody were used to immunostain the cells. Nuclei were counterstained with Hoechst 33258 (1  $\mu$ g/ml) and visualized with a fluorescence microscope.

[0053] RASMC and A7r5 cells both exhibited strong membrane-associated or cytoplasmic staining. Staining was most intense in the perinuclear region and was not observed in the nucleus. Various other tissues were examined for the presence of ACLP mRNA and protein. Gene expression

studies confirmed expression in aortic smooth muscle cells, and levels of ACLP mRNA were found to be high in the whole aorta (including adventitia) compared to most other tissue types tested, e.g., heart, brain, stomach, thymus, and liver. ACLP message was also detectable in colon and kidney tissue.

[0054] To examine expression of ACLP, extracts from mouse tissues were subjected to Western blot analysis using anti-ACLP sera. ACLP was strongly expressed in the mouse aorta (without adventitia) but not in the adventitia, heart, liver, skeletal muscle, or kidney. The presence of ACLP mRNA in the kidney (but absence of protein) indicates that the level of ACLP in the cells is regulated at the level of translation of ACLP mRNA into polypeptide.

[0055] To identify cell types expressing ACLP in an adult animal, in situ hybridization was performed on adult rat aorta and skeletal muscle using known methods. Adult male Sprague-Dawley rats were perfused with 4% paraformaldehyde and their organs were removed and sectioned. ACLP mRNA was detected with a [ $^{35}$ S]UTP-labeled antisense riboprobe synthesized with SP6 RNA polymerase from a linearized 0.7 kb fragment of ACLP cDNA. As a control, a sense RNA probe was synthesized with T7 RNA polymerase from a linearized ACLP cDNA fragment. The antisense riboprobe detected specific ACLP expression in the smooth muscle cells of the aorta, whereas the control (sense) probe did not. Neither the sense nor the antisense probe hybridized to skeletal muscle cells.

#### EXAMPLE 5

##### ACLP Expression in Smooth Muscle Cell Differentiation

[0056] ACLP protein expression was examined during vascular smooth muscle cell growth and differentiation. RASMC and MASMC were isolated from the thoracic aortas of adult male Sprague-Dawley rats and C57Bl/6 mice using standard methods. Human aortic smooth muscle cells (HASMC) were purchased from Clonetics (San Diego, Calif.), and rat A7r5 smooth muscle cells and C2C12 mouse myoblasts were purchased from the American Type Culture Collection (Rockville, Md.). Mouse neural crest cells (Monc-1 cells) were cultured on fibronectin-coated plates. RASMC, MASMC, and A7r5 cells were cultured in Dulbecco's modified Eagle's medium with 3.7 g/liter glucose (Gibco-BRL, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah), 4 mM L-glutamine, 100  $\mu$ g/ml streptomycin, 100 units/ml penicillin, and 10 mM HEPES (pH 7.4). C2C12 cells were grown in Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum, 4 mM L-glutamine, 100  $\mu$ g/ml streptomycin, and 100 units/ml penicillin. HASMC were cultured in M199 medium (Gibco) supplemented with 20% fetal bovine serum, 4 mM L-glutamine, 100  $\mu$ g/ml streptomycin, and 100 units/ml penicillin. Cells were grown at 37° C. in a humidified incubator containing 5% CO<sub>2</sub>. MASMC were cultured for 3 days in 0.4% calf serum containing medium that induces quiescence. RNA and protein extracts were then prepared from the cells and analyzed.

[0057] The amount of ACLP mRNA was higher (about 2-fold) in serum-starved (quiescent) MASMC than in control cells (normal proliferating MASMC). In RASMC,

ACLP mRNA was approximately 3-fold more abundant in quiescent cells than in their actively proliferating counterparts. ACLP protein was also elevated in quiescent MASM. C.

[0058] ACLP expression was examined in an in vitro system for differentiating smooth muscle cells from a Monc-1 cell line, a mouse line derived from the neural crest. Monc-1 cells differentiate into smooth muscle cells when tissue culture medium supplemented with chick embryo extract is replaced with differentiation medium. To examine ACLP expression during the transition of undifferentiated Monc-1 cells to smooth muscle, the time course of ACLP expression was measured. ACLP mRNA was nearly undetectable in undifferentiated Monc-1 cells. As the cells differentiated, however, ACLP expression increased until it became marked at days 4 and 6 after the start of differentiation. Under these conditions, induction of ACLP appeared to lag behind that of smooth muscle  $\alpha$ -actin, a marker for smooth muscle cells. To compare the level of ACLP protein in cells treated similarly, protein extracts were prepared from undifferentiated Monc-1 cells and from cells allowed to differentiate for 6 days. ACLP protein was not detectable in undifferentiated Monc-1 cells but was expressed highly (day 6) under conditions that promote Monc-1 cell differentiation into smooth muscle cells. The abundance of ACLP protein in these cells was similar to that in MASM. C.

[0059] As is described below, the Monc-1 cells (and other cells expressing ACLP) can be used to screen for compounds that stimulate a therapeutic increase in ACLP production (e.g., during cell differentiation and/or fetal development).

#### EXAMPLE 6

##### Genetically-Altered Animals

[0060] An ACLP deficient animal, e.g., an ACLP knockout mouse, is produced as follows.

[0061] The targeting construct was made by deleting exons, e.g., 7-15 of the mouse ACLP gene (see FIG. 3). A Sall-BamHI fragment of the ACLP gene was replaced with pPGK-neo to generate the targeting construct.

[0062] The linearized targeting construct (shown in FIG. 3) was transfected into murine D3 embryonic stem (ES) cells, and a clone with the correct homologous recombination (yielding the appropriately disrupted ACLP gene shown in FIG. 3) injected into blastocysts and used to generate ACLP chimeric mice using standard methods. The chimeric mice were bred with wild type mice to generate ACLP-mutated heterozygous mice. ACLP-mutated heterozygous mice were born normal. To generate an ACLP knockout mouse (i.e., homozygous for the ACLP mutation), the heterozygous mice were mated. The genotype of newborn mice was evaluated at 3 weeks. Out of 205 live pups, 74 were found to have the wild type ACLP gene, 113 were found to be heterozygotes, and 18 were found to be homozygous for the ACLP null mutation, i.e., ACLP knockout mice. These data indicate that many of the knockout mice died before or immediately after birth.

[0063] The phenotype of ACLP knockout mice was evaluated during development. Cesarean sections were performed to obtain embryos at 18.5 embryonic days (E18.5). ACLP-

knockout mice were found to have an abdominal defect with extrusion of abdominal organs, whereas the wild type mice were normal.

[0064] The progress of the development of gastroschisis is evaluated by mating heterozygous ACLP-mutant mice and obtaining embryos at various time points, e.g., E18.5, E16.5, E12.5, E10.5, and E8.5. Embryos are examined at both gross and microscopic levels. Histological evaluation of embryonic tissue, e.g., to follow the formation of the omphalomesenteric arteries, is used to determine the incidence and time of development of gastroschisis.

[0065] ACLP-deficient animals can be used to screen for compounds to treat or prevent the development of gastroschisis. To determine whether a given compound prevents or reduces the development of gastroschisis in developing embryos, the compound is administered to the pregnant animal (e.g., systemically, in utero, or directly to an embryo itself) and the embryos examined as described above. For example, a nucleic acid encoding a full length wild type ACLP gene (or an ACLP gene which may differ from the wild type sequence but still retains ACLP function) can be tested to evaluate the effect of such gene therapy on the development of gastroschisis. A reduction in the severity of gastroschisis in treated embryos compared to untreated embryos indicates that the compound or gene therapy approach to treatment of gastroschisis is clinically beneficial.

[0066] ACLP deficient mice and ACLP deficient cell lines derived from such mice are useful in determining the etiology of by astroschisis and screening for therapeutic compositions.

#### EXAMPLE 7

##### Diagnosis of Disorders Associated with Altered Levels of ACLP Expression or Activity

[0067] The data described herein indicates that an ACLP mutation (e.g., in ACLP coding or regulatory sequences) is involved in the development of gastroschisis. Thus, individuals (e.g., those with a family history of the disease) can be tested for the presence of a mutated ACLP gene which may contribute to the development of gastroschisis in children of an individual harboring a mutated gene. Detection of such a mutation will permit appropriate genetic counseling of those individuals regarding the risks associated with pregnancy. In addition, such testing can be used to identify individuals with subclinical gastroschisis or other related gastrointestinal abnormalities. Prenatal testing may be carried out to determine whether a developing fetus is at risk of developing gastroschisis. Although gastroschisis may be detected at approximately the second trimester of pregnancy by conventional prenatal ultrasound testing, early detection of a genetic abnormality permits early intervention, including genetic therapy, which may prevent the development of the condition or reduce its severity.

[0068] Analysis can be carried out on any suitable genomic DNA sample (e.g., maternal tissue and/or fetal tissue) to be tested. Typically, a blood sample or a sample of placental or umbilical cord cells is tested. A sample of fetal cells can be obtained by amniocentesis or chorionic villi sampling.

[0069] Standard genetic diagnostic methods are used to detect a mutation in the ACLP gene. For example, PCR (polymerase chain reaction) is used to identify the presence of a deletion, addition, or substitution of one or more nucleotides within any one of the exons of ACLP. Following the PCR reaction, the PCR product can be analyzed by methods as described above, such as the heteroduplex detection technique based upon that of White et al., 1992, *Genomics* 12:301-306, or by techniques such as cleavage of RNA-DNA hybrids using RNase A (Myers et al., 1985, *Science* 230:1242-1246); single-stranded conformation polymorphism (SSCP) analysis (Orita et al., 1989, *Genomics* 10:298-299); and denaturing gradient gel electrophoresis (DGGE; Myers et al., 1987, *Methods Enzymol.* 155:501-527). PCR may be carried out using a primer which adds a G+C-rich sequence (termed a "GC-clamp") to one end of the PCR product, thus improving the sensitivity of the subsequent DGGE procedure (Sheffield et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:232-236). If the particular mutation present in the patient's family is known to have removed or added a restriction site, or to have significantly increased or decreased the length of a particular restriction fragment, a protocol based upon restriction fragment length polymorphism (RFLP) analysis (perhaps combined with PCR) can be used to identify the genetic defect.

[0070] In addition to evaluating genomic DNA of a patient, an ACLP defect can be detected by evaluating an ACLP gene product. Unlike genomic DNA-based diagnostic methods, this approach permits detection of defects resulting in a decrease in the level of expression of an ACLP gene (i.e., a defect which does not involve mutations in the coding sequence itself). In addition to detection of a gene product, gene expression is also measured using mRNA-based methods, such as Northern blots and in situ hybridization (using a nucleic acid probe derived from the relevant cDNA), and quantitative PCR.

[0071] An ACLP gene product can be tested for abnormalities, e.g., differences in the level of expression compared to wild type ACLP, truncation of an ACLP gene product, or deletion of a portion of an ACLP gene product. Deletion ACLP mutants, e.g., those characterized by the loss of an ACLP epitope, can be detected using an ACLP-specific antibody. Western blotting and Northern blotting techniques are used to quantitate the amount of expression of a ACLP in the tissue of interest. For example, an individual who is heterozygous for a genetic defect affecting level of expression of ACLP may be diagnosed by detecting reduction in the level of expression of this gene in such a hybridization or antibody-based assay, and an individual who is homozygous may be identified by detection of a comparatively lower level of expression.

[0072] The diagnostic method of the invention is carried out by measuring ACLP gene expression in a tissue, e.g., a biopsy, or in a bodily fluid, e.g., blood or plasma. Detection of expression and determination of the level of gene expression is measured using methods known in the art, e.g., in situ hybridization, Northern blot analysis, or Western blot analysis using ACLP-specific monoclonal or polyclonal antibodies. An decrease in the level of ACLP expression per cell in the test sample of tissue compared to the level per cell in control tissue indicates that the patient has gastroschisis, is predisposed to developing gastroschisis, or is a carrier of a genetic defect associated with gastroschisis.

[0073] The diagnostic procedures described above are useful to identify patients in need of therapeutic intervention to reduce the severity of or prevent the development of gastroschisis.

#### EXAMPLE 8

##### Treatment of Disorders Associated with Altered Levels of ACLP Expression or Activity

[0074] Gene therapy may be carried out by administering to a patient a nucleic acid encoding a therapeutic polypeptide, e.g., an ACLP or fragment thereof, by standard vectors and/or gene delivery systems. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses and adeno-associated viruses, among others.

[0075] In addition to a gene delivery system as described above, the therapeutic composition may include a pharmaceutically acceptable carrier, e.g., a biologically compatible vehicle such as physiological saline, suitable for administration to an animal. A therapeutically effective amount of a compound is an amount which is capable of producing a medically desirable result in a treated animal, e.g., a reduction in the severity of gastroschisis or the prevention of the development of gastroschisis (e.g., in a fetus).

[0076] Parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal delivery routes, may be used to deliver the compound. Dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosage of the compound to be administered will vary. A preferred dosage for intravenous administration of nucleic acids is from approximately 106 to 1022 copies of the nucleic acid molecule. Compounds, including therapeutic nucleic acids, may be administered locally through the uterine wall to the developing fetus using known methods.

[0077] ACLPs may be similarly administered, e.g., locally or systemically, e.g., intravenously, in a pharmaceutically acceptable carrier such as physiological saline. Standard methods for intracellular delivery of peptides can be used, e.g. packaged in liposomes. Such methods are well known to those of ordinary skill in the art. It is expected that an intravenous dosage of approximately 1 to 100  $\mu$ moles of the polypeptide of the invention would be administered per kg of body weight per day. The compositions of the invention are useful for parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal.

[0078] ACLP encoding DNA is be introduced into target cells of the patient by standard vectors, e.g., a vector which contains DNA encoding an ACLP operably linked to an ACLP promoter/enhancer sequence. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, and adenoviruses, among others. ACLP DNA under the control of a strong constitutive promoter may be administered locally using an adenovirus delivery system.

[0079] Drugs which stimulate an endogenous ACLP promoter may also be administered as described above to

increase the level of expression ACLP in patients in which the underlying clinical defect is a pathologically low level of ACLP production.

## EXAMPLE 9

Identification of Compounds that Alter ACLP  
Expression or Activity

[0080] ACLP knockout mice have the clinical manifestations of gastroschisis. Compositions that ameliorate the symptoms of gastroschisis or prevent the development of gastroschisis in a developing fetus can be identified using ACLP knockout mice. A test compound is administered to an ACLP knockout mouse. As a control, the compound is administered to a normal wild type mouse (preferably with the same genetic background as the ACLP knockout mouse). A reduction in the severity of gastroschisis in ACLP knockout mice treated with the test compound compared to control

ACLP mice which have not been exposed to the test compound is an indication that the test compound is capable of ameliorating the symptoms of or preventing the development of gastroschisis.

[0081] Compounds can also be screened by contacting cells in vitro, e.g., VASMC, MASMC, RASMC, Monc-1 cells, or cells derived from an ACLP knockout mouse or from an animal or patient with gastroschisis, with a candidate compound and measuring the level of ACLP expression (or activity) in the cells. An increase in cellular ACLP expression (compared to the level of expression in the absence of a test compound) indicates that the compound is clinically useful to prevent or treat gastroschisis in which the underlying defect is pathological reduction in the level of ACLP production.

[0082] Other embodiments are within the following claims.

## SEQUENCE LISTING

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<210> SEQ ID NO 1

<211> LENGTH: 3935

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (140)...(3613)

<400> SEQUENCE: 1

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ccccgcgcgt gccgcggcc atg gcg gcc gtg cgc ggg gcg ccc ctg ctc agc      172
                Met Ala Ala Val Arg Gly Ala Pro Leu Leu Ser
                1             5             10
tgc ctc ctg gcg ttg ctg gcc ctg tgc cct gga ggg cgc ccg cag acg      220
Cys Leu Leu Ala Leu Leu Ala Leu Cys Pro Gly Gly Arg Pro Gln Thr
                15             20             25
gtg ctg acc gac gac gag atc gag gag ttc ctc gag ggc ttc ctg tca      268
Val Leu Thr Asp Asp Glu Ile Glu Glu Phe Leu Glu Gly Phe Leu Ser
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Glu Leu Glu Pro Glu Pro Arg Glu Asp Asp Val Glu Ala Pro Pro Pro
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Pro Glu Pro Thr Pro Arg Val Arg Lys Ala Gln Ala Gly Gly Lys Pro
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Asp Lys Gly Lys Lys Gly Lys Lys Asp Lys Gly Pro Lys Val Pro Lys
                95             100             105
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cgg cgc ctc aac gcc acc acc acc cta ggc ccc cac act gtg cct ccc 3292
Arg Arg Leu Asn Ala Thr Thr Thr Leu Gly Pro His Thr Val Pro Pro
      1040                1045                1050

acg ctg ccc cct gcc cct gcc acc acc ctg agc act acc ata gag ccc 3340
Thr Leu Pro Pro Ala Pro Ala Thr Thr Leu Ser Thr Thr Ile Glu Pro
      1055                1060                1065

tgg ggc ctc ata ccg cca acc acc gct ggc tgg gag gag tcg gag act 3388
Trp Gly Leu Ile Pro Pro Thr Thr Ala Gly Trp Glu Glu Ser Glu Thr
      1070                1075                1080

gag acc tac aca gag gtg gtg aca gag ttt ggg acc gag gtg gag ccc 3436
Glu Thr Tyr Thr Glu Val Val Thr Glu Phe Gly Thr Glu Val Glu Pro
      1085                1090                1095

gag ttt ggg acc aag gtg gag ccc gag ttt gag acc cag ttg gag cct 3484
Glu Phe Gly Thr Lys Val Glu Pro Glu Phe Glu Thr Gln Leu Glu Pro
      1100                1105                1110                1115

gag ttc gag acc cag ctg gaa ccc gag ttt gag gaa gag gag gag gag 3532
Glu Phe Glu Thr Gln Leu Glu Pro Glu Phe Glu Glu Glu Glu Glu
      1120                1125                1130

gag aaa gag gag gag ata gcc act ggc cag gca ttc ccc ttc aca aca 3580
Glu Lys Glu Glu Glu Ile Ala Thr Gly Gln Ala Phe Pro Phe Thr Thr
      1135                1140                1145

gta gag acc tac aca gtg aac ttt ggg gac ttc tgagatcagc gtcctaccaa 3633
Val Glu Thr Tyr Thr Val Asn Phe Gly Asp Phe
      1150                1155

gacccagcc caactcaagc tacagcagca gcacttccca agcctgctga ccacagtcac 3693

atcacccatc agcacatgga aggcccttg tatggacact gaaaggaagg gctggtcctg 3753

cccctttgag ggggtgcaaa catgactggg acctaagagc cagaggctgt gtagaggctc 3813

ctgctccacc tgccagtctc gtaagagatg ggggtgctgc agtgttgag taggggcaga 3873

gggagggagc caaggtcact ccaataaaac aagctcatgg caaaaaaaaa aaaaaaaaaa 3933

aa 3935

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<210> SEQ ID NO 2
<211> LENGTH: 1158
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 2

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Met Ala Ala Val Arg Gly Ala Pro Leu Leu Ser Cys Leu Leu Ala Leu
  1             5             10             15

Leu Ala Leu Cys Pro Gly Gly Arg Pro Gln Thr Val Leu Thr Asp Asp
      20             25             30

Glu Ile Glu Glu Phe Leu Glu Gly Phe Leu Ser Glu Leu Glu Pro Glu
      35             40             45

Pro Arg Glu Asp Asp Val Glu Ala Pro Pro Pro Pro Glu Pro Thr Pro
      50             55             60

Arg Val Arg Lys Ala Gln Ala Gly Gly Lys Pro Gly Lys Arg Pro Gly
      65             70             75             80

Thr Ala Ala Glu Val Pro Pro Glu Lys Thr Lys Asp Lys Gly Lys Lys
      85             90             95

Gly Lys Lys Asp Lys Gly Pro Lys Val Pro Lys Glu Ser Leu Glu Gly
      100            105            110

Ser Pro Arg Pro Pro Lys Lys Gly Lys Glu Lys Pro Pro Lys Ala Thr
      115            120            125

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Lys | Pro | Lys | Glu | Lys | Pro | Pro | Lys | Ala | Thr | Lys | Lys | Pro | Lys | Glu | 130 | 135 | 140 |
| Glu | Pro | Pro | Lys | Ala | Thr | Lys | Lys | Pro | Lys | Glu | Lys | Pro | Pro | Lys | Ala | 145 | 150 | 155 |
| Thr | Lys | Lys | Pro | Pro | Ser | Gly | Lys | Arg | Pro | Pro | Ile | Leu | Ala | Pro | Ser | 165 | 170 | 175 |
| Glu | Thr | Leu | Glu | Trp | Pro | Leu | Pro | Pro | Pro | Pro | Ser | Pro | Gly | Pro | Glu | 180 | 185 | 190 |
| Glu | Leu | Pro | Gln | Glu | Gly | Gly | Ala | Pro | Leu | Ser | Asn | Asn | Trp | Gln | Asn | 195 | 200 | 205 |
| Pro | Gly | Glu | Glu | Thr | His | Val | Glu | Ala | Gln | Glu | His | Gln | Pro | Glu | Pro | 210 | 215 | 220 |
| Glu | Glu | Glu | Thr | Glu | Gln | Pro | Thr | Leu | Asp | Tyr | Asn | Asp | Gln | Ile | Glu | 225 | 230 | 235 |
| Arg | Glu | Asp | Tyr | Glu | Asp | Phe | Glu | Tyr | Ile | Arg | Arg | Gln | Lys | Gln | Pro | 245 | 250 | 255 |
| Arg | Pro | Pro | Pro | Ser | Arg | Arg | Arg | Arg | Pro | Glu | Arg | Val | Trp | Pro | Glu | 260 | 265 | 270 |
| Pro | Pro | Glu | Glu | Lys | Ala | Pro | Ala | Pro | Ala | Pro | Glu | Glu | Arg | Ile | Glu | 275 | 280 | 285 |
| Pro | Pro | Val | Lys | Pro | Leu | Leu | Pro | Pro | Leu | Pro | Pro | Asp | Tyr | Gly | Asp | 290 | 295 | 300 |
| Gly | Tyr | Val | Ile | Pro | Asn | Tyr | Asp | Asp | Met | Asp | Tyr | Tyr | Phe | Gly | Pro | 305 | 310 | 315 |
| Pro | Pro | Pro | Gln | Lys | Pro | Asp | Ala | Glu | Arg | Gln | Thr | Asp | Glu | Glu | Lys | 325 | 330 | 335 |
| Glu | Glu | Leu | Lys | Lys | Pro | Lys | Lys | Glu | Asp | Ser | Ser | Pro | Lys | Glu | Glu | 340 | 345 | 350 |
| Thr | Asp | Lys | Trp | Ala | Val | Glu | Lys | Gly | Lys | Asp | His | Lys | Glu | Pro | Arg | 355 | 360 | 365 |
| Lys | Gly | Glu | Glu | Leu | Glu | Glu | Glu | Trp | Thr | Pro | Thr | Glu | Lys | Val | Lys | 370 | 375 | 380 |
| Cys | Pro | Pro | Ile | Gly | Met | Glu | Ser | His | Arg | Ile | Glu | Asp | Asn | Gln | Ile | 385 | 390 | 395 |
| Arg | Ala | Ser | Ser | Met | Leu | Arg | His | Gly | Leu | Gly | Ala | Gln | Arg | Gly | Arg | 405 | 410 | 415 |
| Leu | Asn | Met | Gln | Thr | Gly | Ala | Thr | Glu | Asp | Asp | Tyr | Tyr | Asp | Gly | Ala | 420 | 425 | 430 |
| Trp | Cys | Ala | Glu | Asp | Asp | Ala | Arg | Thr | Gln | Trp | Ile | Glu | Val | Asp | Thr | 435 | 440 | 445 |
| Arg | Arg | Thr | Thr | Arg | Phe | Thr | Gly | Val | Ile | Thr | Gln | Gly | Arg | Asp | Ser | 450 | 455 | 460 |
| Ser | Ile | His | Asp | Asp | Phe | Val | Thr | Thr | Phe | Phe | Val | Gly | Phe | Ser | Asn | 465 | 470 | 475 |
| Asp | Ser | Gln | Thr | Trp | Val | Met | Tyr | Thr | Asn | Gly | Tyr | Glu | Glu | Met | Thr | 485 | 490 | 495 |
| Phe | His | Gly | Asn | Val | Asp | Lys | Asp | Thr | Pro | Val | Leu | Ser | Glu | Leu | Pro | 500 | 505 | 510 |
| Glu | Pro | Val | Val | Ala | Arg | Phe | Ile | Arg | Ile | Tyr | Pro | Leu | Thr | Trp | Asn | 515 | 520 | 525 |

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Ser | Leu | Cys | Met | Arg | Leu | Glu | Val | Leu | Gly | Cys | Ser | Val | Ala | Pro |
| 530 |     |     |     |     |     | 535 |     |     |     | 540 |     |     |     |     |     |
| Val | Tyr | Ser | Tyr | Tyr | Ala | Gln | Asn | Glu | Val | Val | Ala | Thr | Asp | Asp | Leu |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
| Asp | Phe | Arg | His | His | Ser | Tyr | Lys | Asp | Met | Arg | Gln | Leu | Met | Lys | Val |
|     |     |     | 565 |     |     |     |     | 570 |     |     |     |     |     | 575 |     |
| Val | Asn | Glu | Glu | Cys | Pro | Thr | Ile | Thr | Arg | Thr | Tyr | Ser | Leu | Gly | Lys |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |
| Ser | Ser | Arg | Gly | Leu | Lys | Ile | Tyr | Ala | Met | Glu | Ile | Ser | Asp | Asn | Pro |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |
| Gly | Glu | His | Glu | Leu | Gly | Glu | Pro | Glu | Phe | Arg | Tyr | Thr | Ala | Gly | Ile |
| 610 |     |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |
| His | Gly | Asn | Glu | Val | Leu | Gly | Arg | Glu | Leu | Leu | Leu | Leu | Leu | Met | Gln |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |
| Tyr | Leu | Cys | Arg | Glu | Tyr | Arg | Asp | Gly | Asn | Pro | Arg | Val | Arg | Ser | Leu |
|     |     |     | 645 |     |     |     |     | 650 |     |     |     |     |     | 655 |     |
| Val | Gln | Asp | Thr | Arg | Ile | His | Leu | Val | Pro | Ser | Leu | Asn | Pro | Asp | Gly |
|     |     |     | 660 |     |     |     |     | 665 |     |     |     | 670 |     |     |     |
| Tyr | Glu | Val | Ala | Ala | Gln | Met | Gly | Ser | Glu | Phe | Gly | Asn | Trp | Ala | Leu |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |
| Gly | Leu | Trp | Thr | Glu | Glu | Gly | Phe | Asp | Ile | Phe | Glu | Asp | Phe | Pro | Asp |
| 690 |     |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
| Leu | Asn | Ser | Val | Leu | Trp | Gly | Ala | Glu | Glu | Arg | Lys | Trp | Val | Pro | Tyr |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |
| Arg | Val | Pro | Asn | Asn | Asn | Leu | Pro | Ile | Pro | Glu | Arg | Tyr | Leu | Ser | Pro |
|     |     |     | 725 |     |     |     |     |     | 730 |     |     |     |     | 735 |     |
| Asp | Ala | Thr | Val | Ser | Thr | Glu | Val | Arg | Ala | Ile | Ile | Ala | Trp | Met | Glu |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
| Lys | Asn | Pro | Phe | Val | Leu | Gly | Ala | Asn | Leu | Asn | Gly | Gly | Glu | Arg | Leu |
|     |     | 755 |     |     |     |     | 760 |     |     |     | 765 |     |     |     |     |
| Val | Ser | Tyr | Pro | Tyr | Asp | Met | Ala | Arg | Thr | Pro | Thr | Gln | Glu | Gln | Leu |
|     |     | 770 |     |     |     | 775 |     |     |     |     | 780 |     |     |     |     |
| Leu | Ala | Ala | Ala | Met | Ala | Ala | Ala | Arg | Gly | Glu | Asp | Glu | Asp | Glu | Val |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
| Ser | Glu | Ala | Gln | Glu | Thr | Pro | Asp | His | Ala | Ile | Phe | Arg | Trp | Leu | Ala |
|     |     |     |     | 805 |     |     |     |     | 810 |     |     |     |     | 815 |     |
| Ile | Ser | Phe | Ala | Ser | Ala | His | Leu | Thr | Leu | Thr | Glu | Pro | Tyr | Arg | Gly |
|     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |     |
| Gly | Cys | Gln | Ala | Gln | Asp | Tyr | Thr | Gly | Gly | Met | Gly | Ile | Val | Asn | Gly |
|     |     | 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |
| Ala | Lys | Trp | Asn | Pro | Arg | Thr | Gly | Thr | Ile | Asn | Asp | Phe | Ser | Tyr | Leu |
|     | 850 |     |     |     |     | 855 |     |     |     |     | 860 |     |     |     |     |
| His | Thr | Asn | Cys | Leu | Glu | Leu | Ser | Phe | Tyr | Leu | Gly | Cys | Asp | Lys | Phe |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     |     | 880 |
| Pro | His | Glu | Ser | Glu | Leu | Pro | Arg | Glu | Trp | Glu | Asn | Asn | Lys | Glu | Ala |
|     |     |     |     | 885 |     |     |     |     | 890 |     |     |     |     | 895 |     |
| Leu | Leu | Thr | Phe | Met | Glu | Gln | Val | His | Arg | Gly | Ile | Lys | Gly | Val | Val |
|     |     |     |     | 900 |     |     |     | 905 |     |     |     |     | 910 |     |     |
| Thr | Asp | Glu | Gln | Gly | Ile | Pro | Ile | Ala | Asn | Ala | Thr | Ile | Ser | Val | Ser |
|     |     | 915 |     |     |     |     | 920 |     |     |     |     |     | 925 |     |     |

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Gly Ile Asn His Gly Val Lys Thr Ala Ser Gly Gly Asp Tyr Trp Arg  
 930 935 940  
 Ile Leu Asn Pro Gly Glu Tyr Arg Val Thr Ala His Ala Glu Gly Tyr  
 945 950 955 960  
 Thr Pro Ser Ala Lys Thr Cys Asn Val Asp Tyr Asp Ile Gly Ala Thr  
 965 970 975  
 Gln Cys Asn Phe Ile Leu Ala Arg Ser Asn Trp Lys Arg Ile Arg Glu  
 980 985 990  
 Ile Met Ala Met Asn Gly Asn Arg Pro Ile Pro His Ile Asp Pro Ser  
 995 1000 1005  
 Arg Pro Met Thr Pro Gln Gln Arg Arg Leu Gln Gln Arg Arg Leu Gln  
 1010 1015 1020  
 His Arg Leu Arg Leu Arg Ala Gln Met Arg Leu Arg Arg Leu Asn Ala  
 1025 1030 1035 1040  
 Thr Thr Thr Leu Gly Pro His Thr Val Pro Pro Thr Leu Pro Pro Ala  
 1045 1050 1055  
 Pro Ala Thr Thr Leu Ser Thr Thr Ile Glu Pro Trp Gly Leu Ile Pro  
 1060 1065 1070  
 Pro Thr Thr Ala Gly Trp Glu Glu Ser Glu Thr Glu Thr Tyr Thr Glu  
 1075 1080 1085  
 Val Val Thr Glu Phe Gly Thr Glu Val Glu Pro Glu Phe Gly Thr Lys  
 1090 1095 1100  
 Val Glu Pro Glu Phe Glu Thr Gln Leu Glu Pro Glu Phe Glu Thr Gln  
 1105 1110 1115 1120  
 Leu Glu Pro Glu Phe Glu Glu Glu Glu Glu Glu Lys Glu Glu Glu  
 1125 1130 1135  
 Ile Ala Thr Gly Gln Ala Phe Pro Phe Thr Thr Val Glu Thr Tyr Thr  
 1140 1145 1150  
 Val Asn Phe Gly Asp Phe  
 1155

<210> SEQ ID NO 3  
 <211> LENGTH: 2743  
 <212> TYPE: DNA  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

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ggaggctggg gcctcccccc accccaaca gccactgttc tctcaggagc tctgcttctg    180
ctctgcagca ttgaaacaa aactgaagga caccttcctt ctctcaggcc agcccagtgc    240
tgttgtgtga tccctcgggg agactctaac gcattcacag ggacaacagg agttggggagg    300
gagaggagtt acagaacttt ccagcaggac ctcaggagaa cgcctggaca cggacaggaa    360
cccccaacc ctcagggacc cccttgacc ctttgagtgc tcctgatcat ggaagccacc    420
agcctcccga ttctcagct gtggccttg cagtgccctc tggacatttg acttaaacgc    480
tatgtctctc agcagagtgg agagctctcc tcacaggctc tggtctcttg ttgtcctctt    540
gccccagcgc tgtgggcca ggttagaaag acttcctgag gacaggctcc ctcaggagga    600
tccccagcgt acgactgtgc tcccacgcac ctttcggat tttctgtgtg gaggcctcaa    660
  
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|                                                                    |      |
|--------------------------------------------------------------------|------|
| cccctcaggc ctctctgggcc agctcctctg ctccaattcc tgtccgtgac tcattgaggc | 720  |
| tcaggaaaag gctttctaga ccttaggttt ctttgtttcc catttttgaa atggcttctg  | 780  |
| ttttccctgg cagagaatat ccaacccaaa ttcagtccaa gtatgaccca tgcctagga   | 840  |
| agtacatcc atgtcccctc atgcaccctg tggcataccc agcatgacac actggaccag   | 900  |
| actgggggca cgaagaccaa tccccagaac tgactttgag cacaatgatt cagaggggta  | 960  |
| ccatgagtga gacttgcttt actcttgctc tgcgaccagg ttgaagtctc tcatggggag  | 1020 |
| gcctagctgt gagaggattg tcctgggatg ggggaagggg gagcaaatg gatgaggacc   | 1080 |
| aacagcctgt gggatgcaag ggctgatcgt gtgtgctagg cacagcaca agtggtccat   | 1140 |
| ttagccgggc agtgggtgtg cacaccttta atcccagcac ttgggaggca gcagcagggtg | 1200 |
| ggtttctgag ttccaggcca gcctgggtcta cagagcaagt tccaggacag ccagagctac | 1260 |
| acagagaaac tctgtctcaa aaaaatcgaa taaaccagaa agtggtgtcca tttaatatgc | 1320 |
| gtatagtaag ttgtggacac gggagttccc ctgctgagtc agacagctag gagggctaag  | 1380 |
| atgggttaga cctcccccc cccacacac acacacacac actcacacac acatcagttc    | 1440 |
| ttggcatagt ctccatgctt cctcaaggag agccagaaag gagactgccg ggaggagctt  | 1500 |
| gcctactccc tgagagcagt gggttacaga gccagtgcc cgaattttc cctttttct     | 1560 |
| ccctgctcat gctggacaga gaggggtgagg gtgaggggta aagactgagg aggtggcctc | 1620 |
| gtgttggtgt ttcttgacct gctttttct ttttctctc cagctgagat gtaactttc     | 1680 |
| ccatgtcaat catctggggg tcgtattctt tttttatcag agtgccctcc caccttggtt  | 1740 |
| gaaagctgcc tgcactacc ctggacctat ggctgtaca agcccacgtt cacatcttta    | 1800 |
| atccttcatg ggtaaatgct ctggcattcc tgggcttagc tatgatggcc attatgagcc  | 1860 |
| agccaacgtt tgtattctag aagccatagc tgaagctgtt gtaacaatt tgtgtttta    | 1920 |
| accgcttctg gtcagaggaa ggagagaata gctattactc cacattggga cctgagccct  | 1980 |
| gagctctgaa gtggggctcc tatctccata aggacagcag cttgctgaga acagcttttc  | 2040 |
| acagccttcc tcgcaaaaat tggctccaaa gacctgggat gttggtgata actggacaaa  | 2100 |
| ggtgacacct gtgcaagcac acagcagggt acactttgaa gagctaacct ccagaaagt   | 2160 |
| gaaaggaggt gatcgccagt accctcgagg gccctactcc ctccctccc tagcaatctc   | 2220 |
| cctgggctca gagcaagggt cacagcgggt tagagcacag gtctccttag actccgcaca  | 2280 |
| ctcccttccc cataactgtt gcattctttt ctcccaggcc ttcctccccg ctaggcgccc  | 2340 |
| tgcaccacga ccctctaaac tggcgctga cgctgctatt agtctgggct ccgtgctgtc   | 2400 |
| cgctctccc ccccgagcc cccgggtccaa ggccggctcc tctctctccc cctccgaaa    | 2460 |
| cccgaagccc ccgcccggc caggccgtcg caagcgctct ggaggcggt ccgctgaga     | 2520 |
| gccagccacg cggggcagga gcgcccagtt gctgccggag ctgggcccgc cagaacctct  | 2580 |
| cctggagccc cttgctcttc ttgaatctcc ctttcccacc gotttctgga tacccttgac  | 2640 |
| gcccacgttc ctgcgcctt ttcccgcctc tacgcggggc gctgcccctg ccaccaagt    | 2700 |
| ccctgctcaa gcccgccggg tcccgcgct gccagagcc atg                      | 2743 |

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 17

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Mus musculus

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&lt;400&gt; SEQUENCE: 4

atctggttgt cctcaat

17

&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 17

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Mus musculus

&lt;400&gt; SEQUENCE: 5

tgactccatc ccaatag

17

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 26

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Mus musculus

&lt;400&gt; SEQUENCE: 6

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26

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 28

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Mus musculus

&lt;400&gt; SEQUENCE: 7

cgaagcttga agtccccaaa gttcactg

28

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 1128

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Mus musculus

&lt;400&gt; SEQUENCE: 8

Met Ala Pro Val Arg Thr Ala Ser Leu Leu Cys Gly Leu Leu Ala Leu  
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20 25 30Glu Ile Glu Glu Phe Leu Glu Gly Phe Leu Ser Glu Leu Glu Thr Gln  
35 40 45Ser Pro Pro Arg Glu Asp Asp Val Glu Val Gln Pro Leu Pro Glu Pro  
50 55 60Thr Gln Arg Pro Arg Lys Ser Lys Ala Gly Gly Lys Gln Arg Ala Asp  
65 70 75 80Val Glu Val Pro Pro Glu Lys Asn Lys Asp Lys Glu Lys Lys Gly Lys  
85 90 95Lys Asp Lys Gly Pro Lys Ala Thr Lys Pro Leu Glu Gly Ser Thr Arg  
100 105 110Pro Thr Lys Lys Pro Lys Glu Lys Pro Pro Lys Ala Thr Lys Lys Pro  
115 120 125Lys Glu Lys Pro Pro Lys Ala Thr Lys Lys Pro Lys Glu Lys Pro Pro  
130 135 140Lys Ala Thr Lys Lys Pro Lys Glu Lys Pro Pro Lys Ala Thr Lys Arg  
145 150 155 160Pro Ser Ala Gly Lys Lys Phe Ser Thr Val Ala Pro Leu Glu Thr Leu  
165 170 175

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Arg | Leu | Leu | Pro | Ser | Pro | Ser | Asn | Pro | Ser | Ala | Gln | Glu | Leu | Pro | 180 | 185 | 190 |     |
| Gln | Lys | Arg | Asp | Thr | Pro | Phe | Pro | Asn | Ala | Trp | Gln | Gly | Gln | Gly | Glu | 195 | 200 | 205 |     |
| Glu | Thr | Gln | Val | Glu | Ala | Lys | Gln | Pro | Arg | Pro | Glu | Pro | Glu | Glu | Glu | 210 | 215 | 220 |     |
| Thr | Glu | Met | Pro | Thr | Leu | Asp | Tyr | Asn | Asp | Gln | Ile | Glu | Lys | Glu | Asp | 225 | 230 | 235 | 240 |
| Tyr | Glu | Asp | Phe | Glu | Tyr | Ile | Arg | Arg | Gln | Lys | Gln | Pro | Arg | Pro | Thr | 245 | 250 | 255 |     |
| Pro | Ser | Arg | Arg | Arg | Leu | Trp | Pro | Glu | Arg | Pro | Glu | Glu | Lys | Thr | Glu | 260 | 265 | 270 |     |
| Glu | Pro | Glu | Glu | Arg | Lys | Glu | Val | Glu | Pro | Pro | Leu | Lys | Pro | Leu | Leu | 275 | 280 | 285 |     |
| Pro | Pro | Asp | Tyr | Gly | Asp | Ser | Tyr | Val | Ile | Pro | Asn | Tyr | Asp | Asp | Leu | 290 | 295 | 300 |     |
| Asp | Tyr | Tyr | Phe | Pro | His | Pro | Pro | Pro | Gln | Lys | Pro | Asp | Val | Gly | Gln | 305 | 310 | 315 | 320 |
| Glu | Val | Asp | Glu | Glu | Lys | Glu | Glu | Met | Lys | Lys | Pro | Lys | Lys | Glu | Gly | 325 | 330 | 335 |     |
| Ser | Ser | Pro | Lys | Glu | Asp | Thr | Glu | Asp | Lys | Trp | Thr | Val | Glu | Lys | Asn | 340 | 345 | 350 |     |
| Lys | Asp | His | Lys | Gly | Pro | Arg | Lys | Gly | Glu | Glu | Leu | Glu | Glu | Glu | Trp | 355 | 360 | 365 |     |
| Ala | Pro | Val | Glu | Lys | Ile | Lys | Cys | Pro | Pro | Ile | Gly | Met | Glu | Ser | His | 370 | 375 | 380 |     |
| Arg | Ile | Glu | Asp | Asn | Gln | Ile | Arg | Ala | Ser | Ser | Met | Leu | Arg | His | Gly | 385 | 390 | 395 | 400 |
| Leu | Gly | Ala | Gln | Arg | Gly | Arg | Leu | Asn | Met | Gln | Ala | Gly | Ala | Asn | Glu | 405 | 410 | 415 |     |
| Asp | Asp | Tyr | Tyr | Asp | Gly | Ala | Trp | Cys | Ala | Glu | Asp | Glu | Ser | Gln | Thr | 420 | 425 | 430 |     |
| Gln | Trp | Ile | Glu | Val | Asp | Thr | Arg | Arg | Thr | Thr | Arg | Phe | Thr | Gly | Val | 435 | 440 | 445 |     |
| Ile | Thr | Gln | Gly | Arg | Asp | Ser | Ser | Ile | His | Asp | Asp | Phe | Val | Thr | Thr | 450 | 455 | 460 |     |
| Phe | Phe | Val | Gly | Phe | Ser | Asn | Asp | Ser | Gln | Thr | Trp | Val | Met | Tyr | Thr | 465 | 470 | 475 | 480 |
| Asn | Gly | Tyr | Glu | Glu | Met | Thr | Phe | Tyr | Gly | Asn | Val | Asp | Lys | Asp | Thr | 485 | 490 | 495 |     |
| Pro | Val | Leu | Ser | Glu | Leu | Pro | Glu | Pro | Val | Val | Ala | Arg | Phe | Ile | Arg | 500 | 505 | 510 |     |
| Ile | Tyr | Pro | Leu | Thr | Trp | Asn | Gly | Ser | Leu | Cys | Met | Arg | Leu | Glu | Val | 515 | 520 | 525 |     |
| Leu | Gly | Cys | Pro | Val | Thr | Pro | Val | Tyr | Ser | Tyr | Tyr | Ala | Gln | Asn | Glu | 530 | 535 | 540 |     |
| Val | Val | Thr | Thr | Asp | Ser | Leu | Asp | Phe | Arg | His | His | Ser | Tyr | Lys | Asp | 545 | 550 | 555 | 560 |
| Met | Arg | Gln | Leu | Met | Lys | Ala | Val | Asn | Glu | Glu | Cys | Pro | Thr | Ile | Thr | 565 | 570 | 575 |     |

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Thr | Tyr | Ser | Leu | Gly | Lys | Ser | Ser | Arg | Gly | Leu | Lys | Ile | Tyr | Ala |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |
| Met | Glu | Ile | Ser | Asp | Asn | Pro | Gly | Asp | His | Glu | Leu | Gly | Glu | Pro | Glu |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |
| Phe | Arg | Tyr | Thr | Ala | Gly | Ile | His | Gly | Asn | Glu | Val | Leu | Gly | Arg | Glu |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |
| Leu | Leu | Leu | Leu | Leu | Met | Gln | Tyr | Leu | Cys | Gln | Glu | Tyr | Arg | Asp | Gly |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |
| Asn | Pro | Arg | Val | Arg | Asn | Leu | Val | Gln | Asp | Thr | Arg | Ile | His | Leu | Val |
|     |     |     | 645 |     |     |     |     |     | 650 |     |     |     |     | 655 |     |
| Pro | Ser | Leu | Asn | Pro | Asp | Gly | Tyr | Glu | Val | Ala | Ala | Gln | Met | Gly | Ser |
|     |     |     | 660 |     |     |     |     | 665 |     |     |     |     |     | 670 |     |
| Glu | Phe | Gly | Asn | Trp | Ala | Leu | Gly | Leu | Trp | Thr | Glu | Glu | Gly | Phe | Asp |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |
| Ile | Phe | Glu | Asp | Phe | Pro | Asp | Leu | Asn | Ser | Val | Leu | Trp | Ala | Ala | Glu |
|     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
| Glu | Lys | Lys | Trp | Val | Pro | Tyr | Arg | Val | Pro | Asn | Asn | Asn | Leu | Pro | Ile |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |
| Pro | Glu | Arg | Tyr | Leu | Ser | Pro | Asp | Ala | Thr | Val | Ser | Thr | Glu | Val | Arg |
|     |     |     | 725 |     |     |     |     |     | 730 |     |     |     |     | 735 |     |
| Ala | Ile | Ile | Ser | Trp | Met | Glu | Lys | Asn | Pro | Phe | Val | Leu | Gly | Ala | Asn |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
| Leu | Asn | Gly | Gly | Glu | Arg | Leu | Val | Ser | Tyr | Pro | Tyr | Asp | Met | Ala | Arg |
|     |     | 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |
| Thr | Pro | Ser | Gln | Glu | Gln | Leu | Leu | Ala | Glu | Ala | Leu | Ala | Ala | Ala | Arg |
|     | 770 |     |     |     |     | 775 |     |     |     |     |     | 780 |     |     |     |
| Gly | Glu | Asp | Asp | Asp | Gly | Val | Ser | Glu | Ala | Gln | Glu | Thr | Pro | Asp | His |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
| Ala | Ile | Phe | Arg | Trp | Leu | Ala | Ile | Ser | Phe | Ala | Ser | Ala | His | Leu | Thr |
|     |     |     | 805 |     |     |     |     |     | 810 |     |     |     |     | 815 |     |
| Met | Thr | Glu | Pro | Tyr | Arg | Gly | Gly | Cys | Gln | Ala | Gln | Asp | Tyr | Thr | Ser |
|     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |     |
| Gly | Met | Gly | Ile | Val | Asn | Gly | Ala | Lys | Trp | Asn | Pro | Arg | Ser | Gly | Thr |
|     |     | 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |
| Phe | Asn | Arg | Phe | Ser | Tyr | Leu | His | Thr | Asn | Cys | Leu | Glu | Leu | Ser | Val |
|     | 850 |     |     |     |     |     | 855 |     |     |     |     | 860 |     |     |     |
| Tyr | Leu | Gly | Cys | Asp | Lys | Phe | Pro | His | Glu | Ser | Glu | Leu | Pro | Arg | Glu |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     |     | 880 |
| Trp | Glu | Asn | Asn | Lys | Glu | Ala | Leu | Leu | Thr | Phe | Met | Glu | Gln | Val | His |
|     |     |     |     | 885 |     |     |     |     | 890 |     |     |     |     | 895 |     |
| Arg | Gly | Ile | Lys | Gly | Val | Val | Thr | Asp | Glu | Gln | Gly | Ile | Pro | Ile | Ala |
|     |     |     | 900 |     |     |     |     | 905 |     |     |     |     | 910 |     |     |
| Asn | Ala | Thr | Ile | Ser | Val | Ser | Gly | Ile | Asn | His | Gly | Val | Lys | Thr | Ala |
|     |     | 915 |     |     |     |     | 920 |     |     |     |     | 925 |     |     |     |
| Ser | Gly | Gly | Asp | Tyr | Trp | Arg | Ile | Leu | Asn | Pro | Gly | Glu | Tyr | Arg | Val |
|     | 930 |     |     |     |     | 935 |     |     |     |     |     | 940 |     |     |     |
| Thr | Ala | His | Ala | Glu | Gly | Tyr | Thr | Ser | Ser | Ala | Lys | Ile | Cys | Asn | Val |
| 945 |     |     |     |     | 950 |     |     |     |     | 955 |     |     |     |     | 960 |
| Asp | Tyr | Asp | Ile | Gly | Ala | Thr | Gln | Cys | Asn | Phe | Ile | Leu | Ala | Arg | Ser |
|     |     |     | 965 |     |     |     |     |     | 970 |     |     |     |     | 975 |     |

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|                                                                 |      |      |      |
|-----------------------------------------------------------------|------|------|------|
| Asn Trp Lys Arg Ile Arg Glu Ile Leu Ala Met Asn Gly Asn Arg Pro | 980  | 985  | 990  |
| Ile Leu Gly Val Asp Pro Ser Arg Pro Met Thr Pro Gln Gln Arg Arg | 995  | 1000 | 1005 |
| Met Gln Gln Arg Arg Leu Gln Tyr Arg Leu Arg Met Arg Glu Gln Met | 1010 | 1015 | 1020 |
| Arg Leu Arg Arg Leu Asn Ser Thr Ala Gly Pro Ala Thr Ser Pro Thr | 1025 | 1030 | 1035 |
| Pro Ala Leu Met Pro Pro Pro Ser Pro Thr Pro Ala Ile Thr Leu Arg | 1045 | 1050 | 1055 |
| Pro Trp Glu Val Leu Pro Thr Thr Thr Ala Gly Trp Glu Glu Ser Glu | 1060 | 1065 | 1070 |
| Thr Glu Thr Tyr Thr Glu Val Val Thr Glu Phe Glu Thr Glu Tyr Gly | 1075 | 1080 | 1085 |
| Thr Asp Leu Glu Val Glu Glu Ile Glu Glu Glu Glu Glu Glu Glu     | 1090 | 1095 | 1100 |
| Glu Glu Met Asp Thr Gly Leu Thr Phe Pro Leu Thr Thr Val Glu Thr | 1105 | 1110 | 1115 |
| Tyr Thr Val Asn Phe Gly Asp Phe                                 | 1125 |      |      |

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1-25. (canceled)

26. A method of detecting a gastroschisis associated genetic alteration diagnosing gastroschisis or a predisposition thereto comprising

- (a) providing a sample of DNA from a patient or fetus; and
- (b) determining whether said DNA comprises a mutation in a gene encoding an aortic carboxypeptidase-like polypeptide (ACLP), said mutation being an indication that said patient or fetus has a gastroschisis-associated genetic alterations thereby diagnosing gastroschisis or a predisposition thereto.

27. The method of claim 26, wherein said sample is obtained by amniocentesis or chorionic villi sampling.

28. The method of claim 26, wherein said method includes the step of subjecting said sample to polymerase chain reaction (PCR), using a forward PCR primer complementary to a portion of the antisense strand of said gene, said portion being within (a) a first intron of said gene, or (b) the 5' untranslated region adjacent to the start codon of said gene; and a reverse PCR primer complementary to a fragment of the sense strand of said gene, said fragment being within (a) a second intron of said gene, or (b) the 3' untranslated region adjacent to the termination codon of said gene.

29-30. (canceled)

31. A method of diagnosing gastroschisis or a predisposition thereto comprising

- a) providing a tissue or fluid sample from a patient or fetus; and
- b) measuring expression of an ACLP gene in said tissue or fluid sample, wherein a reduction in said expression

in said tissue or fluid sample compared to that in a normal control tissue or fluid sample indicates that said patient or fetus has or is predisposed to developing gastroschisis.

32. The method of claim 31, wherein said expression is measured by contacting said tissue or fluid sample with an ACLP-specific antibody.

33-40. (canceled)

41. The method of claim 26, wherein said sample is obtained from maternal tissue.

42. The method of claim 26, wherein said sample is obtained from fetal tissue.

43. The method of claim 26, wherein said sample is obtained from blood, placenta, or umbilical cord.

44. The method of claim 26, wherein said mutation in said gene encoding an ACLP polypeptide is a mutation selected from a deletion, a restriction fragment length polymorphism (RLFP), a duplication, a point mutation, and a translocation.

45. The method of claim 26, wherein said mutation in said gene encoding an ACLP polypeptide occurs in a region selected from:

- (a) nucleotides 140-3613, inclusive, of SEQ ID NO:1; and
- (b) nucleotides 214-3613, inclusive, of SEQ ID NO:1.

46. The method of claim 32, wherein said ACLP-specific antibody is a monoclonal antibody.

47. The method of claim 32, wherein said ACLP-specific antibody is a polyclonal antibody.

48. The method of claim 32, wherein said ACLP-specific antibody binds to an ACLP polypeptide comprising the amino acid sequence of SEQ ID NO:2.

49. The method of claim 32, wherein said ACLP-specific antibody binds to a fragment of an ACLP polypeptide comprising residues 117-164, inclusive, of SEQ ID NO:2.

**50.** The method of claim 32, wherein said ACLP-specific antibody binds to a fragment of an ACLP polypeptide comprising residues 385-540, inclusive, of SEQ ID NO:2.

**51.** The method of claim 32, wherein said ACLP-specific antibody binds to a fragment of an ACLP polypeptide comprising residues 562-969, inclusive, of SEQ ID NO:2.

**52.** The method of claim 31, wherein said sample is a tissue sample.

**53.** The method of claim 31, wherein said sample is a fluid sample selected from blood or plasma.

**54.** The method of claim 31, wherein said expression is measured by detecting the level of ACLP transcript in said tissue or fluid sample.

\* \* \* \* \*

|                |                                                                                                                      |         |            |
|----------------|----------------------------------------------------------------------------------------------------------------------|---------|------------|
| 专利名称(译)        | 主动脉羧肽酶样多肽                                                                                                            |         |            |
| 公开(公告)号        | <a href="#">US20060286597A1</a>                                                                                      | 公开(公告)日 | 2006-12-21 |
| 申请号            | US11/507811                                                                                                          | 申请日     | 2006-08-21 |
| [标]申请(专利权)人(译) | LEE穆恩<br>LAYNE MATTHEWD<br>YET SHAW方                                                                                 |         |            |
| 申请(专利权)人(译)    | 李MU-EN<br>LAYNE MATTHEWD<br>YET SHAW-FANG                                                                            |         |            |
| 当前申请(专利权)人(译)  | 李MU-EN<br>LAYNE MATTHEWD<br>YET SHAW-FANG                                                                            |         |            |
| [标]发明人         | LEE MU EN<br>LAYNE MATTHEW D<br>YET SHAW FANG                                                                        |         |            |
| 发明人            | LEE, MU-EN<br>LAYNE, MATTHEW D.<br>YET, SHAW-FANG                                                                    |         |            |
| IPC分类号         | C12Q1/68 G01N33/567 C07H21/04 C12P21/06 C12N9/64 G01N33/53 A61K38/00 C07K14/47 C12N9/48                              |         |            |
| CPC分类号         | A01K2217/05 A01K2217/075 A61K38/00 C07K14/47 G01N2800/062 C12N9/6421 C12Y304/16005 G01N33/6893 G01N2333/948 C12N9/48 |         |            |
| 优先权            | 08/818009 1997-03-14 US<br>09/060482 1998-04-15 US<br>10/238876 2002-09-10 US<br>60/013439 1996-03-15 US             |         |            |
| 外部链接           | <a href="#">Espacenet</a> <a href="#">USPTO</a>                                                                      |         |            |

摘要(译)

本发明的特征在于主动脉羧肽酶样多肽 ( ACLP ) , 编码ACLP的DNA , 以及检测与腹壁缺陷相关的遗传改变的方法。

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Human  MAAYKQAPLLSCIALALLCFGGREFQVLTDDIEISFLGGFLSELEPE...PREDQVEAPPPPEPTPRVRKAQAGCKPKK 78
Mouse  MAAYRTASLLGGLALLTLCPGNGQVLTDDIEISFLGGFLSELETSPPREDQVEVQPLPEPTQRPKSKAGCK... 76
Human  PGTAAEVDPPEKTKDKGKKKKDKGKVKPKSELESPPPKKGKKEPPKATKKPKKEPPKATKKPKKEPPKATKKPKKEPP 158
Mouse  QRADVEVDPKDKKDKKKDKGKPKTK...PLEGSTRTKPKPKPKPKATKKPKKEPPKATKKPKKEPPKATKKPKKEPP 155
Human  KATKKPKSKKPPILAPSETLMLPLPPPGPGDEELPGKGGAPLSNNWNPGEETHVEAQHQPEPESETSQPTLDVNDQ 238
Mouse  KATKRPKADKKFETVAPLETDLLELSPENISAGELPKGRDTFFPHAGQGQGETOVKAKQRPFESEETEMPTLDVNDQ 235
Human  LKEDVDEPTFKKKGKPPPPSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS 318
Mouse  LKEDVDEPTFKKKGKPPPPSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS 308
Human  GPPPPQKPDAREOTDREKELAKPKKDESSPKET...DKNAVKGKDKKPKPKKSELEENTTEKVKCPKQNGSSSHLED 397
Mouse  PHPPKQFVQGVDEKESKQVQKSSSPKQRTEDVQTLKAGKKGKPKKSELEENTTEKVKCPKQNGSSSHLED 388
Human  NQTRASGKLNGLGAQSGELNQTGATEDDYDQANCAEDDARTONIEVDRTTETFTQVITQGRDSSINDDEVTTFV 477
Mouse  NQTRASGKLNGLGAQSGELNQTGATEDDYDQANCAEDDARTONIEVDRTTETFTQVITQGRDSSINDDEVTTFV 468
Human  FENSGTQVNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNT 557
Mouse  FENSGTQVNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNTQNT 548
Human  DDLQFHHHYKDMQLKQVWRECPITITATYSLGKSSGKLIYAMISDNPGHELEGEPEFTTAGIHGNEVLGRLELL 637
Mouse  DDLQFHHHYKDMQLKQVWRECPITITATYSLGKSSGKLIYAMISDNPGHELEGEPEFTTAGIHGNEVLGRLELL 628
Human  LMYLCRFYEDQDQRYSELQDTRILHLYPGLNDQGVAAQGGSPQNALGLWTERGDFIDPPDGLNGLVMAAREK 717
Mouse  LMYLCRFYEDQDQRYSELQDTRILHLYPGLNDQGVAAQGGSPQNALGLWTERGDFIDPPDGLNGLVMAAREK 708
Human  VYFVYVWNLFFPFRYLSTPAFVSTVVALEAWHWPVYLAHNGSELVSTPDMATTFQDGLAAMAARAGDE 797
Mouse  VYFVYVWNLFFPFRYLSTPAFVSTVVALEAWHWPVYLAHNGSELVSTPDMATTFQDGLAAMAARAGDE 788
Human  DEVSEAQSTPDHAIFWLAISFASAHILTSPYRSGCAQDYTGNGVMAKNNPSTGINDPSYLNKCLSLPYLGC 877
Mouse  DEVSEAQSTPDHAIFWLAISFASAHILTSPYRSGCAQDYTGNGVMAKNNPSTGINDPSYLNKCLSLPYLGC 868
Human  DKFFHSESLPREWNNKCALLTPNQVHKEIKOVYTDQQIPIANATISVSGINHGVKTASGGDVKILNPGRYVTANA 957
Mouse  DKFFHSESLPREWNNKCALLTPNQVHKEIKOVYTDQQIPIANATISVSGINHGVKTASGGDVKILNPGRYVTANA 948
Human  BQTTSAKTCNVVDIGATQCNFLAKNNKRIREIMANNRSPRIHIDPSRMTQQRRLQQRRLRLRLAQMRLRL 1037
Mouse  BQTTSAKTCNVVDIGATQCNFLAKNNKRIREILANNRNLGVDSRMTQQRRLQQRRLQYRLRMREQMLRL 1028
Human  LNAITTLGPHVTP...FLPPAPATLLSTTISWGOLIDPTAGWSESSTETITTEVVTGTEVEPEFGTKVPEPEFTQLEPE 1116
Mouse  LNSTA...GATSTPTALNPPSPPTAITLRMEVLTITAGWSESSTETITTEVVTGTEVEPEFTQLEPE 1086
Human  FETQLSEPESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESE 1158
Mouse  YQTLSEVESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESESE 1128
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Fig. 1A