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(54) Titre : TRAITEMENT DE MALADIES ASSOCIEES AUX GENES DU METABOLISME ET DU TRANSPORT DES
LIPIDES PAR INHIBITION DE TRANSCRIT ANTISENS NATUREL D'UN GENE DU METABOLISME ET DU
TRANSPORT DES LIPIDES
(54) Title: TREATMENT OF LIPID TRANSPORT AND METABOLISM GENE RELATED DISEASES BY INHIBITION OF
NATURAL ANTISENSE TRANSCRIPT TO A LIPID TRANSPORT AND METABOLISM GENE

(57) **Abrégé/Abstract:**

The present invention relates to antisense oligonucleotides that modulate the expression of and/or function of a Lipid transport and metabolism gene, in particular, by targeting natural antisense polynucleotides of a Lipid transport and metabolism gene. The invention also relates to the identification of these antisense oligonucleotides and their use in treating diseases and disorders associated with the expression of a Lipid transport and metabolism genes.



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(54) **Title**: TREATMENT OF LIPID TRANSPORT AND METABOLISM GENE RELATED DISEASES BY INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO A LIPID TRANSPORT AND METABOLISM GENE

(57) **Abstract**: The present invention relates to antisense oligonucleotides that modulate the expression of and/or function of a Lipid transport and metabolism gene, in particular, by targeting natural antisense polynucleotides of a Lipid transport and metabolism gene. The invention also relates to the identification of these antisense oligonucleotides and their use in treating diseases and disorders associated with the expression of a Lipid transport and metabolism genes.



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**TREATMENT OF LIPID TRANSPORT AND METABOLISM GENE RELATED DISEASES BY
INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO A LIPID TRANSPORT AND METABOLISM
GENE**

FIELD OF THE INVENTION

- 5 [0001] The present application claims the priority of U.S. provisional patent application No. , U.S. provisional patent application 61/175,930 filed May 6, 2009, No. 61/176,267 filed May 7, 2009, U.S. provisional patent application No. 61/180,646 filed May 22, 2009, U.S. provisional patent application No. 61/248,212 filed Oct 2, 2009 and U.S. provisional patent application No. 61/235,227 filed August 19, 2009 which is incorporated herein by reference in its entirety.
- 10 [0002] Embodiments of the invention comprise oligonucleotides modulating expression and/or function of a Lipid transport and metabolism gene and associated molecules.

BACKGROUND

- 15 [0003] DNA-RNA and RNA-RNA hybridization are important to many aspects of nucleic acid function including DNA replication, transcription, and translation. Hybridization is also central to a variety of technologies that either detect a particular nucleic acid or alter its expression. Antisense nucleotides, for example, disrupt gene expression by hybridizing to target RNA, thereby interfering with RNA splicing, transcription, translation, and replication. Antisense DNA has the added feature that DNA-RNA hybrids serve as a substrate for digestion by ribonuclease H, an activity that is present in most cell types. Antisense molecules can be delivered into cells, as is the case for oligodeoxynucleotides (ODNs), or they can be expressed from endogenous genes as RNA molecules. The FDA recently approved an antisense
- 20 drug, VITRAVENE™ (for treatment of cytomegalovirus retinitis), reflecting that antisense has therapeutic utility.

SUMMARY

- [0004] This Summary is provided to present a summary of the invention to briefly indicate the nature and substance of the invention. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.
- 25 [0005] In one embodiment, the invention provides methods for inhibiting the action of a natural antisense transcript by using antisense oligonucleotide(s) targeted to any region of the natural antisense transcript resulting in up-regulation of the corresponding sense gene. It is also contemplated herein that inhibition of the natural antisense transcript can be achieved by siRNA, ribozymes and small molecules, which are considered to be within the scope of the present invention.
- 30 [0006] One embodiment provides a method of modulating function and/or expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues in vivo or in vitro comprising contacting said cells or tissues with an antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1

to 1299 of SEQ ID NO: 8, 1 to 918 of SEQ ID NO: 9, 1 to 1550 of SEQ ID NO: 10, 1 to 329 of SEQ ID NO: 11, 1 to 1826 of SEQ ID NO: 12, 1 to 536 of SEQ ID NO: 13, 1 to 551 of SEQ ID NO: 14, 1 to 672 of SEQ ID NO: 15, 1 to 616 of SEQ ID NO: 16, 1 to 471 of SEQ ID NO: 17, 1 to 707 of SEQ ID NO: 18, 1 to 741 of SEQ ID NO: 19, 1 to 346 of SEQ ID NO: 20, 1 to 867 of SEQ ID NO: 21, 1 to 563 of SEQ ID NO: 22 (Figure 3) thereby modulating function and/or expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

[0007] In another preferred embodiment, an oligonucleotide targets a natural antisense sequence of a Lipid transport and metabolism gene polynucleotide, for example, nucleotides set forth in SEQ ID NO: 8 to 22, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of antisense oligonucleotides are set forth as SEQ ID NOS: 23 to 263 (Figure 4 to 9).

[0008] Another embodiment provides a method of modulating function and/or expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising contacting said cells or tissues with an antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to a reverse complement of the an antisense of the Lipid transport and metabolism gene polynucleotide; thereby modulating function and/or expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

[0009] Another embodiment provides a method of modulating function and/or expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising contacting said cells or tissues with an antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to an antisense oligonucleotide to a Lipid transport and metabolism gene antisense polynucleotide; thereby modulating function and/or expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

[0010] In a preferred embodiment, a composition comprises one or more antisense oligonucleotides which bind to sense and/or antisense Lipid transport and metabolism gene polynucleotides.

[0011] In another preferred embodiment, the oligonucleotides comprise one or more modified or substituted nucleotides.

[0012] In another preferred embodiment, the oligonucleotides comprise one or more modified bonds.

[0013] In yet another embodiment, the modified nucleotides comprise modified bases comprising phosphorothioate, methylphosphonate, peptide nucleic acids, 2'-O-methyl, fluoro- or carbon, methylene or other locked nucleic acid (LNA) molecules. Preferably, the modified nucleotides are locked nucleic acid molecules, including α -L-LNA.

[0014] In another preferred embodiment, the oligonucleotides are administered to a patient subcutaneously, intramuscularly, intravenously or intraperitoneally.

[0015] In another preferred embodiment, the oligonucleotides are administered in a pharmaceutical composition. A treatment regimen comprises administering the antisense compounds at least once to patient; however, this treatment can be modified to include multiple doses over a period of time. The treatment can be combined with one or more other types of therapies.

5 [0016] In another preferred embodiment, the oligonucleotides are encapsulated in a liposome or attached to a carrier molecule (e.g. cholesterol, TAT peptide).

[0017] Other aspects are described *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1:

10 Figure 1A is a graph of real time PCR results showing the fold change + standard deviation in ABCA1 mRNA after treatment of 518A2 cells with siRNA oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of ABCA1 mRNA in 518A2 cells are significantly increased 48 h after treatment with one of the siRNAs designed to ABCA1 antisense AK311445. Bars denoted as CUR-0521, CUR-0519 and CUR-0523 correspond to samples treated with SEQ ID NOS: 23 to 25 respectively.

15 Figure 1B is a graph of real time PCR results showing the fold change + standard deviation in ABCA1 mRNA after treatment of 518A2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of ABCA1 mRNA in 518A2 cells are significantly increased 48 h after treatment with six of the oligos designed to ABCA1 antisense AK311445. Bars denoted as CUR-1214 to CUR-1222 correspond to samples treated with SEQ ID NOS: 26 to 34 respectively.

20 Figure 1C is a graph of real time PCR results showing the fold change + standard deviation in ABCA1 mRNA after treatment of 3T3 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of ABCA1 mRNA in 3T3 cells are significantly increased 48 h after treatment with three of the oligos designed to mouse ABCA1 antisense BF133827. Bars denoted as CUR-1087 to CUR-1090, CUR-1092 and CUR-1091 correspond to samples treated with SEQ ID NOS: 35 to 38, 40 and 39 respectively.

25 Figure 1D is a graph of real time PCR results showing the fold change + standard deviation in LCAT mRNA after treatment of Hek293 cells with siRNA and phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of the LCAT mRNA in Hek293 cells are significantly increased 48 h after treatment with three of the oligos designed to LCAT antisense Hs.668679. Bars denoted as CUR-0476, CUR-0478, CUR-0822, CUR-0820 and CUR-0819 correspond to samples treated with SEQ ID NOS: 41, 42, 58, 56 and 55 respectively.

30 Figure 1E is a graph of real time PCR results showing the fold change + standard deviation in LCAT mRNA after treatment of HepG2 cells with siRNA oligonucleotides introduced using Lipofectamine 2000, as compared to control.

Real time PCR results show that the levels of the LCAT mRNA in HepG2 cells are significantly increased 48 h after treatment with two of the oligos designed to LCAT antisense Hs.668679. Bars denoted as CUR-0476, CUR-0478, CUR-0444, CUR-0446, CUR-0448 and CUR-0450 correspond to samples treated with SEQ ID NOS: 41, 42, and 44 to 47 respectively.

5 Figure 1F is a graph of real time PCR results showing the fold change + standard deviation in LCAT mRNA after treatment of HepG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of the LCAT mRNA in HepG2 cells are significantly increased 48 h after treatment with one of the oligos designed to LCAT antisense Hs.668679. Bars denoted as CUR-0819, CUR-0818, CUR-0817, CUR-0816, CUR-0815, CUR-0820, CUR-0821 and CUR-0822 correspond to samples treated with
10 SEQ ID NOS: 55, 54, 53, 52, 51 and 56 to 58 respectively.

Figure 1G is a graph of real time PCR results showing the fold change + standard deviation in LCAT mRNA after treatment of Vero cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of the LCAT mRNA in Vero cells are significantly increased 48 h after treatment with one of the oligos designed to LCAT antisense Hs.668679. Bars denoted as CUR-0819, CUR-0818,
15 CUR-0817, CUR-0816, CUR-0815, CUR-0820, CUR-0821 and CUR-0822 correspond to samples treated with SEQ ID NOS: 55, 54, 53, 52, 51 and 56 to 58 respectively.

Figure 1H is a graph of real time PCR results showing the fold change + standard deviation in LRP1 mRNA after treatment of HepG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real Time PCR results show that levels of LRP1 mRNA in HepG2 cells are significantly increased 48h after
20 treatment with oligos to LRP1 antisense DC401271. Bars denoted as CUR-0767 to CUR-0769, CUR-0771, CUR-0770, CUR-0775, CUR-0773, CUR-0772 and CUR-0774 correspond to samples treated with SEQ ID NOS: 59 to 61, 63, 62, 67, 65, 64 and 66 respectively.

Figure 1I is a graph of real time PCR results showing the fold change + standard deviation in LRP1 mRNA after treatment of Vero cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to
25 control. Real Time PCR results show that levels of LRP1 mRNA in Vero cells are significantly increased 48h after treatment with oligos to LRP1 antisense DC401271 and Hs.711951. Bars denoted as CUR-0768, CUR-0767, CUR-0774 and CUR-0772 correspond to samples treated with SEQ ID NOS: 60, 59, 66 and 64 respectively.

Figure 1J is a graph of real time PCR results showing the fold change + standard deviation in LRP1 mRNA after treatment of 3T3 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to
30 control. Real Time PCR results show that levels of LRP1 mRNA in 3T3 cells are significantly increased 48h after treatment with oligos to LRP1 antisense DC401271 and AW544265. Bars denoted as CUR-1017 to CUR-1022 correspond to samples treated with SEQ ID NOS: 68 to 73 respectively.

Figure 1K is a graph of real time PCR results showing the fold change + standard deviation in LDLR mRNA after treatment of HepG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real Time PCR results show that levels of LDLR mRNA in HepG2 cells are significantly increased 48h after treatment with antisense oligos to LDLR antisense sherflor.aApr07. Bars denoted as CUR-1054 to CUR-1059 correspond to samples treated with SEQ ID NOS: 74 to 79 respectively.

Figure 1L is a graph of real time PCR results showing the fold change + standard deviation in LDLR mRNA after treatment of HepG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real Time PCR results show that levels of LDLR mRNA in HepG2 cells after treatment with antisense oligos to LDLR antisense bloflor.aApr07. Bars denoted as CUR-1059 to CUR-1063 correspond to samples treated with SEQ ID NOS: 79 to 83 respectively.

Figure 1M is a graph of real time PCR results showing the fold change + standard deviation in ApoE mRNA after treatment of HepG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of APOE mRNA in HepG2 cells are significantly increased 48 h after treatment with three of the antisense oligos designed to APOE antisense Hs.626623. Bars denoted as CUR-0978, CUR-0980, CUR-0981, CUR-0979, CUR-0973, CUR-0975, CUR-0974, CUR-0977 and CUR-0976 correspond to samples treated with SEQ ID NOS: 89, 91, 92, 90, 84, 86, 85, 88 and 87 respectively.

Figure 1N to Figure 1P represent a graphs of real time PCR results showing the fold change + standard deviation in ApoA1 mRNA after treatment of HepG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Real time PCR results show that the levels of ApoA1 mRNA in HepG2 cells are significantly increased 48 h after treatment with some of the antisense oligonucleotides to ApoA1 antisense DA327409ext. Bars RH3-RH597, correspond to samples treated with SEQ ID NOS 171 to 248 respectively.

Figure 1Q is a graph of real time PCR results showing the fold change in ApoA1 mRNA (top panel) and ApoA1 natural antisense DA327409ext RNA (bottom panel) after treatment of HepG2 cells with naked LNA or phosphothioate oligonucleotides over 7 days as compared to control. Bars denoted as #6LNA, #11LNA, #6PS and #11PS represent SEQ ID NOS 249 to 252 respectively.

Figure 1R is a graph of real time PCR results showing the fold change in ApoA1 mRNA (orange bars) and ApoA1 natural antisense DA327409ext RNA (blue bars) after treatment of HepG2 cells with LNA oligonucleotides. Bars denoted as 6-11 correspond to SEQ ID NOS 249, 257 to 260 and 250.

Figure 1S shows dose dependent increase in ApoA1 mRNA (bottom panel) and protein (top panel) after treatment of HepG2 cells with oligonucleotides. Bars denoted CUR-4806 and CUR-4811 correspond to SEQ ID NOS 249 and 250 respectively.

Figure 1T is a graph of the real time PCR results showing upregulation of the ApoA1 mRNA in primary African green monkey hepatocytes after treatment with oligonucleotides against natural ApoA1 antisense DA327409ext. Bars denoted CUR-4816 and CUR-4811 correspond to SEQ ID NOS: 263 and 250 respectively.

Figure 1U is a graph showing that ApoA1 mRNA and protein levels increased in monkey liver biopsies after treatment with CUR-962, an oligonucleotide designed to ApoA1 antisense DA327409ext, compared to the baseline levels, as determined by real time PCR and ELISA respectively (right panels). ApoA1 mRNA or protein levels did not change after the same period of time in the control group dosed with an oligonucleotide that showed no effect on ApoA1 levels *in vitro* (CUR-963) (left panels). Bars denoted CUR-962 and CUR-963 correspond to SEQ ID NOS 260 and 261 respectively.

Figure 1V shows a side-by-side alignment of the human and rhesus ApoA1 natural antisense sequences and the position of some of the oligonucleotides used to target these sequences.

[0019] Figure 2 shows

SEQ ID NO: 1: Homo sapiens ATP-binding cassette, sub-family A (ABC1), member 1(ABCA1), mRNA (NCBI Accession No.: NM_005502).

SEQ ID NO: 2: Homo sapiens lecithin-cholesterol acyltransferase (LCAT), mRNA (NCBI Accession No.: NM_000229.1).

SEQ ID NO: 3: Homo sapiens low density lipoprotein receptor-related protein 1 (LRP1), mRNA (NCBI Accession No.: NM_002332.2).

SEQ ID NO: 4: Mus musculus low density lipoprotein receptor-related protein 1 (Lrp1), mRNA (NCBI Accession No.: NM_008512.2).

SEQ ID NO: 5: Homo sapiens low density lipoprotein receptor (LDLR), mRNA (NCBI Accession No.: NM_000527.3).

SEQ ID NO: 6: Homo sapiens apolipoprotein E (APOE), mRNA (NCBI Accession No.: NM_000041.2).

SEQ ID NO: 7: Homo sapiens apolipoprotein A-I (APOA1), mRNA (NCBI Accession No.: NM_000039).

[0020] Figure 3 shows

SEQ ID NO: 8: Human Natural ABCA1 antisense sequence (AK311445)

SEQ ID NO: 9: Mouse Natural ABCA1 antisense sequence (BF133827)

SEQ ID NO: 10: Human Natural LCAT antisense sequence (Hs.668679)

SEQ ID NO: 11: Human Natural LCAT antisense sequence (Hs.593769)

SEQ ID NO: 12: Human Natural LCAT antisense sequence (Hs.387239)

SEQ ID NO: 13: Human Natural LRP1 antisense sequence (Hs.711951)

SEQ ID NO: 14: Human Natural LRP1 antisense sequence (DC401271)

SEQ ID NO: 15: Human Natural LRP1 antisense sequence (BM933147)

SEQ ID NO: 16: Mouse Natural LRP1 antisense sequence (CK626173)

SEQ ID NO: 17: Mouse Natural LRP1 antisense sequence (AW544265)

SEQ ID NO: 18: Human Natural ABCA1 antisense sequence (bloflor.aApr07)

SEQ ID NO: 19: Human Natural ABCA1 antisense sequence (sherflor.aApr07)

5 SEQ ID NO: 20: Natural APOE antisense sequence (Hs.626623)

SEQ ID NO: 21: Natural APOE antisense sequence (Hs.714236)

SEQ ID NO: 22: Natural APOA1 antisense sequence (DA327409 extended)

[0021] Figure 4 shows the ABCA1 antisense oligonucleotides, SEQ ID NOs: 23 to 40. 'r' indicates RNA and * indicates phosphothioate bond.

10 [0022] Figure 5 shows the LCAT antisense oligonucleotides, SEQ ID NOs: 41 to 58. 'r' indicates RNA and * indicates phosphothioate bond.

[0023] Figure 6 shows the LRP1 antisense oligonucleotides, SEQ ID NOs: 59 to 73. * indicates phosphothioate bond.

[0024] Figure 7 shows the LDLR antisense oligonucleotides, SEQ ID NOs: 74 to 83. * indicates phosphothioate bond.

[0025] Figure 8 shows the ApoE antisense oligonucleotides, SEQ ID NOs: 84 to 92. * indicates phosphothioate bond.

15 [0026] Figure 9 shows the ApoA1 antisense oligonucleotides, SEQ ID NOs: 93 to 263. 'r' indicates RNA and * indicates phosphothioate bond.

[0027] Figure 10 shows the ABCA1 sense oligonucleotides, SEQ ID NOs: 264 to 266. The sense oligonucleotides SEQ ID NO: 264 to 266 are the reverse complements of the antisense oligonucleotides SEQ ID NO: 23 to 24 respectively. 'r' indicates RNA.

20 [0028] Figure 11 shows the LCAT sense oligonucleotides, SEQ ID NOs: 275 to 282. The sense oligonucleotides SEQ ID NO: 267 to 274 are the reverse complements of the antisense oligonucleotides SEQ ID NO: 41 to 48 respectively. 'r' indicates RNA.

[0029] Figure 12 shows:

25 SEQ ID NOs: 275 to 277: correspond to the probe sequence, forward primer sequence and the reverse primer sequence respectively with respect to the custom designed assay for ApoA1 antisense DA327409ext

SEQ ID NO: 278: corresponds to CUR 962, * indicates phosphothioate bond and + indicates LNA.

SEQ ID NO: 279: corresponds to CUR 963, * indicates phosphothioate bond and + indicates LNA.

DETAILED DESCRIPTION

30 [0030] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the ordering of acts or events, as some acts may occur in different orders and/or concurrently with other

acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

[0031] All genes, gene names, and gene products disclosed herein are intended to correspond to homologs from any species for which the compositions and methods disclosed herein are applicable. Thus, the terms include, but are not limited to genes and gene products from humans and mice. It is understood that when a gene or gene product from a particular species is disclosed, this disclosure is intended to be exemplary only, and is not to be interpreted as a limitation unless the context in which it appears clearly indicates. Thus, for example, for the genes disclosed herein, which in some embodiments relate to mammalian nucleic acid and amino acid sequences are intended to encompass homologous and/or orthologous genes and gene products from other animals including, but not limited to other mammals, fish, amphibians, reptiles, and birds. In preferred embodiments, the genes or nucleic acid sequences are human.

Definitions

[0032] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0033] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0034] As used herein, the term "mRNA" means the presently known mRNA transcript(s) of a targeted gene, and any further transcripts which may be elucidated.

[0035] By "antisense oligonucleotides" or "antisense compound" is meant an RNA or DNA molecule that binds to another RNA or DNA (target RNA, DNA). For example, if it is an RNA oligonucleotide it binds to another RNA target by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi *et al.*, (1991) *Ann. Rev. Biochem.* 60, 631-652). An antisense oligonucleotide can upregulate or downregulate expression and/or function of a particular polynucleotide. The definition is meant to include any foreign RNA or DNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such molecules include, for example, antisense RNA or DNA molecules,

interference RNA (RNAi), micro RNA, decoy RNA molecules, siRNA, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

[0036] In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. The term "oligonucleotide", also includes linear or circular oligomers of natural and/or modified monomers or linkages, including deoxyribonucleosides, ribonucleosides, substituted and alpha-anomeric forms thereof, peptide nucleic acids (PNA), locked nucleic acids (LNA), phosphorothioate, methylphosphonate, and the like. Oligonucleotides are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, Hoogsteen or reverse Hoogsteen types of base pairing, or the like.

[0037] The oligonucleotide may be "chimeric", that is, composed of different regions. In the context of this invention "chimeric" compounds are oligonucleotides, which contain two or more chemical regions, for example, DNA region(s), RNA region(s), PNA region(s) etc. Each chemical region is made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotides compound. These oligonucleotides typically comprise at least one region wherein the oligonucleotide is modified in order to exhibit one or more desired properties. The desired properties of the oligonucleotide include, but are not limited, for example, to increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. Different regions of the oligonucleotide may therefore have different properties. The chimeric oligonucleotides of the present invention can be formed as mixed structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide analogs as described above.

[0038] The oligonucleotide can be composed of regions that can be linked in "register" that is, when the monomers are linked consecutively, as in native DNA, or linked via spacers. The spacers are intended to constitute a covalent "bridge" between the regions and have in preferred cases a length not exceeding about 100 carbon atoms. The spacers may carry different functionalities, for example, having positive or negative charge, carry special nucleic acid binding properties (intercalators, groove binders, toxins, fluorophors etc.), being lipophilic, inducing special secondary structures like, for example, alanine containing peptides that induce alpha-helices.

[0039] As used herein "Lipid transport and metabolism genes" are inclusive of all family members, mutants, alleles, fragments, species, coding and noncoding sequences, sense and antisense polynucleotide strands, etc.

[0040] As used herein, the words ATP-binding cassette 1; Lipid transport and metabolism gene; ABC transporter 1; cholesterol efflux regulatory protein (CERP), ABCA1, ABC-1, ABC1, CERP; FLJ14958; HDLDT1; TGD are used interchangeably in the present application.

[0041] As used herein, the words Lecithin-cholesterol acyltransferase, LCAT, Phosphatidylcholine-sterol acyltransferase, Phospholipid-cholesterol acyltransferase are used interchangeably in the present application.

[0042] As used herein, the words A2MR, Alpha-2-macroglobulin receptor, APOER, Apolipoprotein E receptor, APR, CD91, FLJ16451, IGFBP3R, LRP, LRP-1, MGC88725, Prolow-density lipoprotein receptor-related protein 1, TGFR5 are used interchangeably in the present application.

[0043] As used herein, the words LDLR, FH, FHC, LDLCQ2, LDL receptor, Low-density lipoprotein receptor are used interchangeably in the present application.

[0044] As used herein, the words AD2, Apo-E, ApoE, Apolipoprotein E, LDLCQ5, LPG, MGC1571 are used interchangeably in the present application.

[0045] As used herein, the words ApoA1, Apo-A1, Apolipoprotein A1, MGC117399 are used interchangeably in the present application.

[0046] As used herein, the term "oligonucleotide specific for" or "oligonucleotide which targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of a mRNA transcript of the targeted gene. Stability of the complexes and duplexes can be determined by theoretical calculations and/or in vitro assays. Exemplary assays for determining stability of hybridization complexes and duplexes are described in the Examples below.

[0047] As used herein, the term "target nucleic acid" encompasses DNA, RNA (comprising premRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA, coding, noncoding sequences, sense or antisense polynucleotides. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds, which specifically hybridize to it, is generally referred to as "antisense". The functions of DNA to be interfered include, for example, replication and transcription. The functions of RNA to be interfered, include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA.

The overall effect of such interference with target nucleic acid function is modulation of the expression of an encoded product or oligonucleotides.

[0048] RNA interference "RNAi" is mediated by double stranded RNA (dsRNA) molecules that have sequence-specific homology to their "target" nucleic acid sequences (Caplen, N. J., *et al.* (2001) *Proc. Natl. Acad. Sci. USA* 98:9742-9747). In certain embodiments of the present invention, the mediators are 5-25 nucleotide "small interfering" RNA duplexes (siRNAs). The siRNAs are derived from the processing of dsRNA by an RNase enzyme known as Dicer (Bernstein, E., *et al.* (2001) *Nature* 409:363-366). siRNA duplex products are recruited into a multi-protein siRNA complex termed RISC (RNA Induced Silencing Complex). Without wishing to be bound by any particular theory, a RISC is then believed to be guided to a target nucleic acid (suitably mRNA), where the siRNA duplex

interacts in a sequence-specific way to mediate cleavage in a catalytic fashion (Bernstein, E., *et al.* (2001) *Nature* 409:363-366; Boutla, A., *et al.* (2001) *Curr. Biol.* 11:1776-1780). Small interfering RNAs that can be used in accordance with the present invention can be synthesized and used according to procedures that are well known in the art and that will be familiar to the ordinarily skilled artisan. Small interfering RNAs for use in the methods of the present invention suitably comprise between about 1 to about 50 nucleotides (nt). In examples of non limiting
5 embodiments, siRNAs can comprise about 5 to about 40 nt, about 5 to about 30 nt, about 10 to about 30 nt, about 15 to about 25 nt, or about 20-25 nucleotides.

[0049] Selection of appropriate oligonucleotides is facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid
10 sequences obtained, for example, by searching databases such as GenBank or by sequencing PCR products. Comparison of nucleic acid sequences from a range of species allows the selection of nucleic acid sequences that display an appropriate degree of identity between species. In the case of genes that have not been sequenced, Southern blots are performed to allow a determination of the degree of identity between genes in target species and other species. By performing Southern blots at varying degrees of stringency, as is well known in the art, it is possible to obtain an
15 approximate measure of identity. These procedures allow the selection of oligonucleotides that exhibit a high degree of complementarity to target nucleic acid sequences in a subject to be controlled and a lower degree of complementarity to corresponding nucleic acid sequences in other species. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

[0050] By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, (1988) *J. American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs
20 through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

[0051] By "decoy RNA" is meant an RNA molecule that mimics the natural binding domain for a ligand. The decoy
25 RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger *et al.* (1990) *Cell*, 63, 601- 608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

[0052] As used herein, the term "monomers" typically indicates monomers linked by phosphodiester bonds or analogs
30 thereof to form oligonucleotides ranging in size from a few monomeric units, e.g., from about 3-4, to about several hundreds of monomeric units. Analogs of phosphodiester linkages include: phosphorothioate, phosphorodithioate, methylphosphonates, phosphoroselenoate, phosphoramidate, and the like, as more fully described below.

[0053] The term "nucleotide" covers naturally occurring nucleotides as well as nonnaturally occurring nucleotides. It should be clear to the person skilled in the art that various nucleotides which previously have been considered "non-naturally occurring" have subsequently been found in nature. Thus, "nucleotides" includes not only the known purine and pyrimidine heterocycles-containing molecules, but also heterocyclic analogues and tautomers thereof. Illustrative examples of other types of nucleotides are molecules containing adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo- N6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N4,N4-ethanocytosin, N6,N6-ethano-2,6- diaminopurine, 5-methylcytosine, 5-(C3-C6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanin, inosine and the "non-naturally occurring" nucleotides described in Benner *et al.*, U.S. Pat No. 5,432,272. The term "nucleotide" is intended to cover every and all of these examples as well as analogues and tautomers thereof. Especially interesting nucleotides are those containing adenine, guanine, thymine, cytosine, and uracil, which are considered as the naturally occurring nucleotides in relation to therapeutic and diagnostic application in humans. Nucleotides include the natural 2'-deoxy and 2'-hydroxyl sugars, e.g., as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992) as well as their analogs.

[0054] "Analog" in reference to nucleotides includes synthetic nucleotides having modified base moieties and/or modified sugar moieties (see e.g., described generally by Scheit, Nucleotide Analogs, John Wiley, New York, 1980; Freier & Altmann, (1997) *Nucl. Acid. Res.*, 25(22), 4429- 4443, Toulmé, J.J., (2001) *Nature Biotechnology* 19:17-18; Manoharan M., (1999) *Biochemica et Biophysica Acta* 1489:117-139; Freier S. M., (1997) *Nucleic Acid Research*, 25:4429-4443, Uhlman, E., (2000) *Drug Discovery & Development*, 3: 203-213, Herdewin P., (2000) *Antisense & Nucleic Acid Drug Dev.*, 10:297-310); 2'-O, 3'-C-linked [3.2.0] bicycloarabinonucleosides (see e.g. N.K Christensen., *et al.*, (1998) *J. Am. Chem. Soc.*, 120: 5458-5463; Prakash TP, Bhat B. (2007) *Curr Top Med Chem.* 7(7):641-9; Cho EJ, *et al.* (2009) *Annual Review of Analytical Chemistry*, 2, 241-264). Such analogs include synthetic nucleotides designed to enhance binding properties, e.g., duplex or triplex stability, specificity, or the like.

[0055] As used herein, "hybridization" means the pairing of substantially complementary strands of oligomeric compounds. One mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoögsteeen or reversed Hoögsteeen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleotides) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleotides which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

[0056] An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a modulation of function and/or activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of

in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

[0057] As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances and in the context of this invention, "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated. In general, stringent hybridization conditions comprise low concentrations ($<0.15\text{M}$) of salts with inorganic cations such as Na^{++} or K^{++} (i.e., low ionic strength), temperature higher than 20°C - 25°C . below the T_m of the oligomeric compound:target sequence complex, and the presence of denaturants such as formamide, dimethylformamide, dimethyl sulfoxide, or the detergent sodium dodecyl sulfate (SDS). For example, the hybridization rate decreases 1.1% for each 1% formamide. An example of a high stringency hybridization condition is 0.1X sodium chloride-sodium citrate buffer (SSC)/0.1% (w/v) SDS at 60°C . for 30 minutes.

[0058] "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides on one or two oligomeric strands. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleotides such that stable and specific binding occurs between the oligomeric compound and a target nucleic acid.

[0059] It is understood in the art that the sequence of an oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure). The oligomeric compounds of the present invention comprise at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an antisense compound in which 18 of 20 nucleotides of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleotides may be clustered or interspersed with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides. As such, an

antisense compound which is 18 nucleotides in length having 4 (four) noncomplementary nucleotides which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using

5 BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, (1990) *J. Mol. Biol.*, 215, 403-410; Zhang and Madden, (1997) *Genome Res.*, 7, 649-656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, (1981) 2, 482-489).

10 [0060] As used herein, the term "Thermal Melting Point (T_m)" refers to the temperature, under defined ionic strength, pH, and nucleic acid concentration, at which 50% of the oligonucleotides complementary to the target sequence hybridize to the target sequence at equilibrium. Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short oligonucleotides (e.g., 10 to 50 nucleotide). Stringent conditions may also be achieved with

15 the addition of destabilizing agents such as formamide.

[0061] As used herein, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene.

[0062] The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic," "splice," "species," or

20 "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type gene products. Variants may result from at least one mutation in the nucleic acid sequence and may result in

25 altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

[0063] The resulting polypeptides generally will have significant amino acid identity relative to each other. A

30 polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs,) or single base mutations in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population with a propensity for a disease state, that is susceptibility versus resistance.

[0064] Derivative polynucleotides include nucleic acids subjected to chemical modification, for example, replacement of hydrogen by an alkyl, acyl, or amino group. Derivatives, e.g., derivative oligonucleotides, may comprise non-naturally-occurring portions, such as altered sugar moieties or inter-sugar linkages. Exemplary among these are phosphorothioate and other sulfur containing species which are known in the art. Derivative nucleic acids may also contain labels, including radionucleotides, enzymes, fluorescent agents, chemiluminescent agents, chromogenic agents, substrates, cofactors, inhibitors, magnetic particles, and the like.

[0065] A "derivative" polypeptide or peptide is one that is modified, for example, by glycosylation, pegylation, phosphorylation, sulfation, reduction/alkylation, acylation, chemical coupling, or mild formalin treatment. A derivative may also be modified to contain a detectable label, either directly or indirectly, including, but not limited to, a radioisotope, fluorescent, and enzyme label.

[0066] As used herein, the term "animal" or "patient" is meant to include, for example, humans, sheep, elks, deer, mule deer, minks, mammals, monkeys, horses, cattle, pigs, goats, dogs, cats, rats, mice, birds, chicken, reptiles, fish, insects and arachnids.

[0067] "Mammal" covers warm blooded mammals that are typically under medical care (e.g., humans and domesticated animals). Examples include feline, canine, equine, bovine, and human, as well as just human.

[0068] "Treating" or "treatment" covers the treatment of a disease-state in a mammal, and includes: (a) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, e.g., arresting its development; and/or (c) relieving the disease-state, e.g., causing regression of the disease state until a desired endpoint is reached. Treating also includes the amelioration of a symptom of a disease (e.g., lessen the pain or discomfort), wherein such amelioration may or may not be directly affecting the disease (e.g., cause, transmission, expression, etc.).

[0069] As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors found in mammals, including, but not limited to: leukemias, lymphomas, melanomas, carcinomas and sarcomas. The cancer manifests itself as a "tumor" or tissue comprising malignant cells of the cancer. Examples of tumors include sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma,

neuroblastoma, and retinoblastoma. Additional cancers which can be treated by the disclosed composition according to the invention include but not limited to, for example, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, and prostate cancer.

[0070] "Neurological disease or disorder" refers to any disease or disorder of the nervous system and/or visual system.

"Neurological disease or disorder" include disease or disorders that involve the central nervous system (brain, brainstem and cerebellum), the peripheral nervous system (including cranial nerves), and the autonomic nervous system (parts of which are located in both central and peripheral nervous system). Examples of neurological disorders include but are not limited to, headache, stupor and coma, dementia, seizure, sleep disorders, trauma, infections, neoplasms, neuroophthalmology, movement disorders, demyelinating diseases, spinal cord disorders, and disorders of peripheral nerves, muscle and neuromuscular junctions. Addiction and mental illness, include, but are not limited to,

bipolar disorder and schizophrenia, are also included in the definition of neurological disorder. The following is a list of several neurological disorders, symptoms, signs and syndromes that can be treated using compositions and methods according to the present invention: acquired epileptiform aphasia; acute disseminated encephalomyelitis; adrenoleukodystrophy; age-related macular degeneration; agenesis of the corpus callosum; agnosia; Aicardi syndrome; Alexander disease; Alpers' disease; alternating hemiplegia; Vascular dementia; amyotrophic lateral sclerosis; anencephaly; Angelman syndrome; angiomas; anoxia; aphasia; apraxia; arachnoid cysts; arachnoiditis; Arnold-Chiari malformation; arteriovenous malformation; Asperger syndrome; ataxia telangiectasia; attention deficit hyperactivity disorder; autism; autonomic dysfunction; back pain; Batten disease; Behcet's disease; Bell's palsy; benign essential blepharospasm; benign focal; amyotrophy; benign intracranial hypertension; Binswanger's disease; blepharospasm; Bloch Sulzberger syndrome; brachial plexus injury; brain abscess; brain injury; brain tumors (including glioblastoma multiforme); spinal tumor; Brown-Sequard syndrome; Canavan disease; carpal tunnel syndrome; causalgia; central pain syndrome; central pontine myelinolysis; cephalic disorder; cerebral aneurysm; cerebral arteriosclerosis; cerebral atrophy; cerebral gigantism; cerebral palsy; Charcot-Marie-Tooth disease; chemotherapy-induced neuropathy and neuropathic pain; Chiari malformation; chorea; chronic inflammatory demyelinating polyneuropathy; chronic pain; chronic regional pain syndrome; Coffin Lowry syndrome; coma, including persistent vegetative state; congenital facial diplegia; corticobasal degeneration; cranial arteritis; craniosynostosis; Creutzfeldt-Jakob disease; cumulative trauma disorders; Cushing's syndrome; cytomegalic inclusion body disease; cytomegalovirus infection; dancing eyes-dancing feet syndrome; DandyWalker syndrome; Dawson disease; De Morsier's syndrome; Dejerine-Klumpke palsy; dementia; dermatomyositis; diabetic neuropathy; diffuse sclerosis;

dysautonomia; dysgraphia; dyslexia; dystonias; early infantile epileptic encephalopathy; empty sella syndrome; encephalitis; encephaloceles; encephalotrigeminal angiomas; epilepsy; Erb's palsy; essential tremor; Fabry's disease; Fahr's syndrome; fainting; familial spastic paralysis; febrile seizures; Fisher syndrome; Friedreich's ataxia; fronto-temporal dementia and other "tauopathies"; Gaucher's disease; Gerstmann's syndrome; giant cell arteritis; giant cell inclusion disease; globoid cell leukodystrophy; Guillain-Barre syndrome; HTLV-1-associated myelopathy; Hallervorden-Spatz disease; head injury; headache; hemifacial spasm; hereditary spastic paraplegia; heredopathia atactica et polyneuritiformis; herpes zoster ophthalmicus; herpes zoster; Hirayama syndrome; HIV-associated dementia and neuropathy (also neurological manifestations of AIDS); holoprosencephaly; Huntington's disease and other polyglutamine repeat diseases; hydranencephaly; hydrocephalus; hypercortisolism; hypoxia; immune-mediated encephalomyelitis; inclusion body myositis; incontinentia pigmenti; infantile phytanic acid storage disease; infantile Refsum disease; infantile spasms; inflammatory myopathy; intracranial cyst; intracranial hypertension; Joubert syndrome; Kearns-Sayre syndrome; Kennedy disease; Kinsbourne syndrome; Klippel Feil syndrome; Krabbe disease; Kugelberg-Welander disease; kuru; Lafora disease; Lambert-Eaton myasthenic syndrome; Landau-Kleffner syndrome; lateral medullary (Wallenberg) syndrome; learning disabilities; Leigh's disease; Lennox-Gustaut syndrome; Lesch-Nyhan syndrome; leukodystrophy; Lewy body dementia; Lissencephaly; locked-in syndrome; Lou Gehrig's disease (i.e., motor neuron disease or amyotrophic lateral sclerosis); lumbar disc disease; Lyme disease--neurological sequelae; Machado-Joseph disease; macrocephaly; megalencephaly; Melkersson-Rosenthal syndrome; Meniere's disease; meningitis; Menkes disease; metachromatic leukodystrophy; microcephaly; migraine; Miller Fisher syndrome; mini-strokes; mitochondrial myopathies; Mobius syndrome; monomelic amyotrophy; motor neuron disease; Moyamoya disease; mucopolysaccharidoses; multi-infarct dementia; multifocal motor neuropathy; multiple sclerosis and other demyelinating disorders; multiple system atrophy with postural hypotension; p muscular dystrophy; myasthenia gravis; myelinoclastic diffuse sclerosis; myoclonic encephalopathy of infants; myoclonus; myopathy; myotonia congenita; narcolepsy; neurofibromatosis; neuroleptic malignant syndrome; neurological manifestations of AIDS; neurological sequelae of lupus; neuromyotonia; neuronal ceroid lipofuscinosis; neuronal migration disorders; Niemann-Pick disease; O'Sullivan-McLeod syndrome; occipital neuralgia; occult spinal dysraphism sequence; Ohtahara syndrome; olivopontocerebellar atrophy; opsoclonus myoclonus; optic neuritis; orthostatic hypotension; overuse syndrome; paresthesia; Neurodegenerative disease or disorder (Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), dementia, multiple sclerosis and other diseases and disorders associated with neuronal cell death); paramyotonia congenita; paraneoplastic diseases; paroxysmal attacks; Parry Romberg syndrome; Pelizaeus-Merzbacher disease; periodic paralysis; peripheral neuropathy; painful neuropathy and neuropathic pain; persistent vegetative state; pervasive developmental disorders; photic sneeze reflex; phytanic acid storage disease; Pick's disease; pinched nerve; pituitary tumors; polymyositis; porencephaly; post-polio syndrome; postherpetic neuralgia; postinfectious encephalomyelitis; postural hypotension; Prader-Willi syndrome; primary lateral sclerosis;

prion diseases; progressive hemifacial atrophy; progressive multifocalleukoencephalopathy; progressive sclerosing poliodystrophy; progressive supranuclear palsy; pseudotumor cerebri; Ramsay-Hunt syndrome (types I and II); Rasmussen's encephalitis; reflex sympathetic dystrophy syndrome; Refsum disease; repetitive motion disorders; repetitive stress injuries; restless legs syndrome; retrovirus-associated myelopathy; Rett syndrome; Reye's syndrome; Saint Vitus dance; Sandhoff disease; Schilder's disease; schizencephaly; septo-optic dysplasia; shaken baby syndrome; shingles; Shy-Drager syndrome; Sjogren's syndrome; sleep apnea; Soto's syndrome; spasticity; spina bifida; spinal cord injury; spinal cord tumors; spinal muscular atrophy; Stiff-Person syndrome; stroke; Sturge-Weber syndrome; subacute sclerosing panencephalitis; subcortical arteriosclerotic encephalopathy; Sydenham chorea; syncope; syringomyelia; tardive dyskinesia; Tay-Sachs disease; temporal arteritis; tethered spinal cord syndrome; Thomsen disease; thoracic outlet syndrome; Tic Douloureux; Todd's paralysis; Tourette syndrome; transient ischemic attack; transmissible spongiform encephalopathies; transverse myelitis; traumatic brain injury; tremor; trigeminal neuralgia; tropical spastic paraparesis; tuberous sclerosis; vascular dementia (multi-infarct dementia); vasculitis including temporal arteritis; Von Hippel-Lindau disease; Wallenberg's syndrome; Werdnig-Hoffman disease; West syndrome; whiplash; Williams syndrome; Wildon's disease; and Zellweger syndrome.

[0071] An "Inflammation" refers to systemic inflammatory conditions and conditions associated locally with migration and attraction of monocytes, leukocytes and/or neutrophils. Examples of inflammation include, but are not limited to, Inflammation resulting from infection with pathogenic organisms (including gram-positive bacteria, gram-negative bacteria, viruses, fungi, and parasites such as protozoa and helminths), transplant rejection (including rejection of solid organs such as kidney, liver, heart, lung or cornea, as well as rejection of bone marrow transplants including graft-versus-host disease (GVHD)), or from localized chronic or acute autoimmune or allergic reactions. Autoimmune diseases include acute glomerulonephritis; rheumatoid or reactive arthritis; chronic glomerulonephritis; inflammatory bowel diseases such as Crohn's disease, ulcerative colitis and necrotizing enterocolitis; granulocyte transfusion associated syndromes; inflammatory dermatoses such as contact dermatitis, atopic dermatitis, psoriasis; systemic lupus erythematosus (SLE), autoimmune thyroiditis, multiple sclerosis, and some forms of diabetes, or any other autoimmune state where attack by the subject's own immune system results in pathologic tissue destruction. Allergic reactions include allergic asthma, chronic bronchitis, acute and delayed hypersensitivity. Systemic inflammatory disease states include inflammation associated with trauma, burns, reperfusion following ischemic events (e.g. thrombotic events in heart, brain, intestines or peripheral vasculature, including myocardial infarction and stroke), sepsis, ARDS or multiple organ dysfunction syndrome. Inflammatory cell recruitment also occurs in atherosclerotic plaques. Inflammation includes, but is not limited to, Non-Hodgkin's lymphoma, Wegener's granulomatosis, Hashimoto's thyroiditis, hepatocellular carcinoma, thymus atrophy, chronic pancreatitis, rheumatoid arthritis, reactive lymphoid hyperplasia, osteoarthritis, ulcerative colitis, papillary carcinoma, Crohn's disease, ulcerative colitis, acute cholecystitis, chronic cholecystitis, cirrhosis, chronic sialadenitis, peritonitis, acute pancreatitis, chronic pancreatitis,

chronic Gastritis, adenomyosis, endometriosis, acute cervicitis, chronic cervicitis, lymphoid hyperplasia, multiple sclerosis, hypertrophy secondary to idiopathic thrombocytopenic purpura, primary IgA nephropathy, systemic lupus erythematosus, psoriasis, pulmonary emphysema, chronic pyelonephritis, and chronic cystitis.

[0072] A cardiovascular disease or disorder includes those disorders that can either cause ischemia or are caused by reperfusion of the heart. Examples include, but are not limited to, atherosclerosis, coronary artery disease, granulomatous myocarditis, chronic myocarditis (non-granulomatous), primary hypertrophic cardiomyopathy, peripheral artery disease (PAD), stroke, angina pectoris, myocardial infarction, cardiovascular tissue damage caused by cardiac arrest, cardiovascular tissue damage caused by cardiac bypass, cardiogenic shock, and related conditions that would be known by those of ordinary skill in the art or which involve dysfunction of or tissue damage to the heart or vasculature, especially, but not limited to, tissue damage related to a Lipid transport and metabolism gene activation. CVS diseases include, but are not limited to, atherosclerosis, granulomatous myocarditis, myocardial infarction, myocardial fibrosis secondary to valvular heart disease, myocardial fibrosis without infarction, primary hypertrophic cardiomyopathy, and chronic myocarditis (non-granulomatous).

[0073] A "Metabolic disease or disorder" refers to a wide range of diseases and disorders of the endocrine system including, for example, insulin resistance, diabetes, obesity, impaired glucose tolerance, high blood cholesterol, hyperglycemia, hyperinsulinemia, dyslipidemia and hyperlipidemia.

Polynucleotide and Oligonucleotide Compositions and Molecules

Targets

[0074] In one embodiment, the targets comprise nucleic acid sequences of a Lipid transport and metabolism genes, including without limitation sense and/or antisense noncoding and/or coding sequences associated with a Lipid transport and metabolism gene.

[0075] In one embodiment, the targets comprise nucleic acid sequences of ABCA1, including without limitation sense and/or antisense noncoding and/or coding sequences associated with ABCA1 gene.

[0076] In one embodiment, the targets comprise nucleic acid sequences of LCAT, including without limitation sense and/or antisense noncoding and/or coding sequences associated with LCAT gene.

[0077] In one embodiment, the targets comprise nucleic acid sequences of LRP1, including without limitation sense and/or antisense noncoding and/or coding sequences associated with LRP1 gene.

[0078] In one embodiment, the targets comprise nucleic acid sequences of low density lipoprotein receptor (LDLR), including without limitation sense and/or antisense noncoding and/or coding sequences associated with LDLR.

[0079] In one embodiment, the targets comprise nucleic acid sequences of apolipoprotein (ApoA1), including without limitation sense and/or antisense noncoding and/or coding sequences associated with ApoA. Human apolipoprotein A-I (ApoA-I) is the major protein constituent of high-density lipoproteins (HDL and lymph chylomicrons. In human plasma four major circulating lipoproteins have been named: chylomicrons (CM), very low-density lipoproteins

(VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). HDL is involved in the removal of cholesterol from peripheral tissues by transporting it to the liver or to other lipoproteins.

[0080] ATP-binding cassette, sub family-A (ABCAI) member I ABCAI functions as a cholesterol efflux pump in the cellular lipid removal pathway.

5 [0081] ATP-binding cassette transporters (ABC-transporter) are members of a protein superfamily that is one of the largest and most ancient families with representatives in all extant phyla from prokaryotes to humans. ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair. They transport a wide variety of substrates across extra- and
10 intracellular membranes, including metabolic products, lipids and sterols, and drugs. Proteins are classified as ABC transporters based on the sequence and organization of their ATP-binding cassette (ABC) domain(s). ABC transporters are involved in tumor resistance, cystic fibrosis, bacterial multidrug resistance, and a range of other inherited human diseases.

[0082] The membrane-associated protein encoded by ABC1 gene is a member of the superfamily of ATP-binding
15 cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intracellular membranes. ABC genes are divided into seven distinct subfamilies (ABCA, MDRITAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the ABCA subfamily. Members of the ABCA subfamily comprise the only major ABC subfamily found exclusively in multicellular eukaryotes. With cholesterol as its substrate, this protein functions as a cholesterol efflux pump in the cellular lipid removal pathway.

20 [0083] In preferred embodiments, antisense oligonucleotides are used to prevent or treat diseases or disorders associated with ATP-Binding cassette molecules. ATP-binding cassette transporter ABC1 (member 1 of human transporter sub-family ABCA), also known as the cholesterol efflux regulatory protein (CERP) is a protein which in humans is encoded by the ABC1 gene. This transporter is a major regulator of cellular cholesterol and phospholipid homeostasis.

25 [0084] ABC1 is present in high-density lipoproteins (HDL) which permits the removal of excessive cholesterol and phospholipids from human cell membranes. Since this protein is needed throughout the body it is synthesized ubiquitously as a 220-kDa protein. It is present in higher quantities in tissues that shuttle or are involved in the turnover of lipids such as the liver, the small intestine and adipose tissue.

[0085] Factors that act upon the ABC1 transporter's expression or its posttranslational modification are also molecules
30 that are involved in its subsequent function like fatty acids, cholesterol and also cytokines and cyclic adenosine monophosphate.

[0086] Low-density lipoprotein receptor-related protein 1 (LPR1) is about 4544 amino acids; 504575 Da. It is a heterodimer of an 85-kDa membrane-bound carboxyl subunit and a noncovalently attached 515-kDa amino-terminal

subunit. Intracellular domain interacts with MAFB. LRP1 is found in a complex with PID1PCLI1, LRP1 and CUBN1. Interacts with SNX17, PID1PCLI1, PDGF, LRPAP1 and CUBN. The intracellular domain interacts with SHC1, GULP1 and DAB1.

[0087] LRP1 is an endocytic receptor involved in endocytosis and in phagocytosis of apoptotic cells; early embryonic development; cellular lipid homeostasis; plasma clearance of chylomicron remnants and activated LRPAP1 (alpha 2-macroglobulin); local metabolism of complexes between plasminogen activators and their endogenous inhibitors. Without wishing to be bound by theory, it may modulate cellular events, such as APP metabolism, kinase-dependent intracellular signaling, neuronal calcium signaling as well as neurotransmission.

[0088] High density lipoprotein (HDL) picks up extra cholesterol in the blood and returns it to the liver. Low density lipoprotein (or LDL) is the main transporter of cholesterol in the body. But too much LDL over many years can result in atherosclerosis (the narrowing and hardening of arteries) and lead to heart disease or a heart attack. The ratio is determined by dividing the LDL cholesterol into the HDL cholesterol. For example, if a person has an HDL cholesterol of 50 mg/dL and an LDL cholesterol of 150 mg/dL, the HDL/LDL ratio would be 0.33. The goal is to keep the HDL/LDL ratio above 0.3, with the ideal HDL/LDL ratio being above 0.4.

[0089] HDL are synthesized de novo in both the liver and small intestine as protein-rich disc-shaped particles. The primary apoproteins of HDL are apoA-I, apoA-II, apoC-I, apoC-II, and apoE. Newly formed HDL contain very little cholesterol and cholesteryl esters. HDL are converted from their initial discoidal shape into spherical lipoprotein particles through the accumulation of cholesteryl esters in the neutral core of the lipoprotein particle. Cholesterol is accumulated by HDL from chylomicron remnants VLDL remnants (also called intermediate density Lipoproteins or IDL) and directly from cell surface membranes. The cholesterol is esterified through the action of an HDL-associated enzyme lecithin:cholesterol acyltransferase ("LCAT"). For LCAT to transfer a fatty acid from lecithin (phosphatidylcholine) to the C-3-OH group of cholesterol, interaction with ApoA-I found on the HDL surface is required. This accumulation of core cholesteryl esters converts nascent HDL to HDL2 and HDL3. See R. I. Levy et al., "The structure, function and metabolism of high-density lipoproteins: A status report," *Circulation*, vol. 62, pp. IV4-8 (1980); and D. I. Silverman et al., "High-density lipoprotein subfractions," *Am. J. Med.*, vol. 94, pp. 636-45 (1993).

[0090] HDL are usually isolated from the plasma by ultracentrifugation. The normal HDL density range is from 1.063 g/mL to 1.21 g/mL, which divides roughly into two ranges HDL2 (1.063 g/mL to 1.125 g/mL) and HDL3 (1.125 g/mL to 1.21 g/mL). More recently, two major populations of particles in HDL have been identified by two dimensional electrophoresis followed by immunoblotting and enzyme-linked differential antibody immunosorbent assay. One of these populations contains particles with apoA-I alone, and the other contains particles with both apoA-I and apoA-II. The relative proportion of apoA-I particles is highest in the HDL2 fraction, while HDL3 is more a combination of apoA-I and apoA-II. See J. C. Fruchart et al., "Apolipoprotein A-containing lipoprotein particles: physiological role, quantification, and clinical significance," *Clin. Chem.*, vol. 38, pp. 793-7 (1992); and B. F. Asztalos

et al., "Normolipidemic subjects with low HDL cholesterol levels have altered HDL subpopulations," *Arterioscler. Thromb. Vasc. Biol.*, vol. 17, pp. 1885-1893 (1997).

[0091] Human apolipoprotein A-I (ApoA-I) is the major protein constituent of HDL and lymph chylomicrons. ApoA-I is primarily synthesized in the liver and small intestine as a precursor protein (preproapo A-I). Preproapo A-I is cleaved intracellularly to form proapo A-I, the form secreted into the plasma and lymph. In the plasma, six amino acids are cleaved from proapo A-I to form mature ApoA-I.

[0092] Mature ApoA-I is a single unglycosylated polypeptide composed of 243 amino acids of known sequence. ApoA-I serves as a cofactor of a plasma enzyme (lecithin-cholesterol acyltransferase (LCAT)), responsible for the formation of most cholesterol esters in plasma. Decreased levels of ApoA-I may result in disorders of the plasma lipid transport system and in the development of coronary heart disease. Low levels of both ApoA-I and HDL has been shown to be a strong risk factor for heart attacks and other atherosclerotic vascular diseases. See U.S. Pat. Nos. 5,059,528 and 6,258,596.

[0093] Apolipoprotein E (ApoE) is an apoprotein found in the chylomicron and intermediate-density lipoproteins (IDLs) that binds to a specific receptor on liver cells and peripheral cells. Intermediate-density lipoproteins belong to the lipoprotein particle family and are formed from the degradation of very low-density lipoproteins. IDL is one of the five major groups of lipoproteins (chylomicrons, VLDL, IDL, LDL, HDL) that enable fats and cholesterol to move within the water-based solution of the bloodstream. Apolipoprotein E (ApoE) is important for the normal catabolism of triglyceride-rich lipoprotein constituents.

[0094] The APOE gene, ApoE, is mapped to chromosome 19 in a cluster with Apolipoprotein C1 and Apolipoprotein C2. ApoE consists of four exons and three introns, totaling 3597 base pairs. In melanocytic cells APOE gene expression may be regulated by microphthalmia-associated transcription factor (MITF). The gene is polymorphic with three major alleles, ApoE2, ApoE3, ApoE4, which translate into three isoforms of the protein: normal - ApoE-E3; dysfunctional- ApoE-E2 and ApoE-E4. These isoforms differ from each other only by single amino acid substitutions at positions 112 and 158.

[0095] Lecithin-cholesterol acyltransferase (LCAT), is a plasma enzyme produced by the liver and catalyzes the conversion of cholesterol to cholesteryl esters on lipoproteins by the transacylation of fatty acid from the sn-2 position of phosphatidylcholine to the 3-hydroxyl group on the A-ring of cholesterol. Most LCAT activity is found on high-density lipoprotein (HDL) but approximately 30% is also on apolipoprotein (Apo) B-containing lipoproteins.

[0096] The apolipoprotein E gene is polymorphic with three major alleles, ApoE2, ApoE3, ApoE4. E2 is associated with the genetic disorder type III hyperlipoproteinemia and with both increased and decreased risk for atherosclerosis. E3 is found in approximately 64 percent of the population. It is considered the "neutral" Apo E genotype. E4 may contribute to atherosclerosis and Alzheimer's disease, impaired cognitive function, and reduced neurite outgrowth.

[0097] LCAT promotes the reverse cholesterol transport pathway, the pathway by which excess cellular cholesterol is returned to the liver for excretion. Without wishing to be bound by theory, mechanisms include, for example: LCAT increases the level of HDL, which in itself may increase the flux of cholesterol from cells by increasing the amount of extracellular acceptors of cholesterol. Also, esterification of cholesterol by LCAT on HDL could limit the spontaneous back exchange of cholesterol from HDL to cells and promotes the net delivery of cholesterol on HDL and on to the liver.

[0098] In preferred embodiments, antisense oligonucleotides are used to prevent or treat diseases or disorders associated with Lipid transport and metabolism gene family members. Exemplary Lipid transport and metabolism gene mediated diseases and disorders which can be treated with cell/tissues regenerated from stem cells obtained using the antisense compounds comprise: a cardiovascular disease or disorder, a metabolic disease or disorder (e.g., diabetes, obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, hypercholesterolemia etc.), a disease or disorder associated with impaired lipid metabolism, a coronary artery disease, atherosclerosis, an HDL metabolism disease or disorder (e.g., familial HDL deficiency (FHD), Sea-blue histiocytosis, Tangier's Disease, Fish-eye disease, LCAT deficiency, low-HDL cholesterolemia etc.), a disease or disorder associated with cellular cholesterol and/or phospholipid homeostasis, Familial amyloid nephropathy, a disease or disorder associated with impaired cholesterol regulation, a disease or disorder associated with a deficiency of the Lipid transport and metabolism gene transporter, Apolipoprotein A-I deficiency, a disease or disorder associated with abnormally fast or abnormally slow rate of cholesterol efflux in a cell, a disease or disorder associated with pancreatic beta cell function, diabetes, a metabolic disease or disorder, arthritis, inflammation, an autoimmune disease or disorder, acquired immune deficiency syndrome (AIDS), inflammation, a neurological disease or disorder, a neurodegenerative disease or disorder, cancer, dyslipidemia, metabolic syndrome, a senile plaque, cerebral amyloid angiopathy, Amyloidosis, glioblastoma, a disease or disorder associated with amyloid deposition, neurofibrillary tangles, choriocarcinoma, astrocytoma, amyloidosis, hyperlipidemia, neoplastic transformation, atherosclerotic plaque, obstruction, metastasis, pulmonary fibrosis, necrosis, shock, melanoma, genetic susceptibility, psoriasis, glioma, neuropathology, a vascular disease, cell damage, Nonsmall cell lung carcinomas (NSCLCs), liposarcoma, an immunodeficiency disease or disorder, an organ transplant rejection, an allergy, glomerulonephritis, venous thrombosis, pathological processes or leukemia, a skeletal disease or disorder, a muscular disease or disorder, a disease or disorder associated with infectious organisms, an immune related disease or disorder, nerve repair and paralysis, neuroendocrine differentiation, systemic non-neuropathic amyloidosis, an amyloid disease, tumor growth dependent on angiogenesis, non-cancerous diseases with symptoms include an increase in angiogenesis, e.g., psoriasis, retinopathy of prematurity, a Choroid disease, neovascular glaucoma, diabetic retinopathy, substance abuse, impaired cognitive function, and reduced neurite outgrowth, ApoE abnormal expression, function, activity as compared to a normal control, psoriasis, a disease or disorder caused by foreign organisms such as viral, bacterial, parasitic, fungal, and the like.

[0099] In a preferred embodiment the Lipid transport and metabolism gene antisense oligonucleotides are therapeutically used in organ transplantation (e.g., liver transplant, kidney transplant, bone marrow transplant, heart transplant etc.).

[00100] In a preferred embodiment, the oligonucleotides are specific for polynucleotides of a Lipid transport and metabolism gene, which includes, without limitation noncoding regions. The Lipid transport and metabolism gene targets comprise variants of a Lipid transport and metabolism gene; mutants of a Lipid transport and metabolism gene, including SNPs; noncoding sequences of a Lipid transport and metabolism gene; alleles, fragments and the like. Preferably the oligonucleotide is an antisense RNA molecule.

[00101] In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to a Lipid transport and metabolism gene polynucleotides alone but extends to any of the isoforms, receptors, homologs, non-coding regions and the like of a Lipid transport and metabolism gene.

[00102] In another preferred embodiment, an oligonucleotide targets a natural antisense sequence (natural antisense to the coding and non-coding regions) of a Lipid transport and metabolism gene targets, including, without limitation, variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense RNA or DNA molecule.

[00103] In another preferred embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenine, variants may be produced which contain thymidine, guanosine, cytidine or other natural or unnatural nucleotides at this position. This may be done at any of the positions of the antisense compound. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

[00104] In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

[00105] An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired. Such conditions include, i.e., physiological conditions in the case of in vivo assays or therapeutic treatment, and conditions in which assays are performed in the case of in vitro assays.

[00106] An antisense compound, whether DNA, RNA, chimeric, substituted etc, is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

[00107] In another preferred embodiment, targeting of a Lipid transport and metabolism gene including without limitation, antisense sequences which are identified and expanded, using for example, PCR, hybridization etc., one or more of the sequences set forth as SEQ ID NO: 8 to 22, and the like, modulate the expression or function of a Lipid transport and metabolism gene. In one embodiment, expression or function is up-regulated as compared to a control. In another preferred embodiment, expression or function is down-regulated as compared to a control.

[00108] In another preferred embodiment, oligonucleotides comprise nucleic acid sequences set forth as SEQ ID NOS: 23 to 263 including antisense sequences which are identified and expanded, using for example, PCR, hybridization etc. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like. Examples of modified bonds or internucleotide linkages comprise phosphorothioate, phosphorodithioate or the like. In another preferred embodiment, the nucleotides comprise a phosphorus derivative. The phosphorus derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphorothioate and the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides, *per se*, is also known and need not be described here.

[00109] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

[00110] In embodiments of the present invention oligomeric antisense compounds, particularly oligonucleotides, bind to target nucleic acid molecules and modulate the expression and/or function of molecules encoded by a target gene. The functions of DNA to be interfered comprise, for example, replication and transcription. The functions of RNA to be interfered comprise all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The functions may be up-regulated or inhibited depending on the functions desired.

[00111] The antisense compounds, include, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

5 [00112] Targeting an antisense compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target nucleic acid encodes a Lipid transport and metabolism gene.

10 [00113] The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in
15 the present invention, are defined as positions within a target nucleic acid.

[00114] In a preferred embodiment, the antisense oligonucleotides bind to the natural antisense sequences of a Lipid transport and metabolism gene and modulate the expression and/or function of a Lipid transport and metabolism gene (SEQ ID NO: 1). Examples of antisense sequences include SEQ ID NOS: 8 to 263.

[00115] In another preferred embodiment, the antisense oligonucleotides bind to one or more segments of a Lipid
20 transport and metabolism gene polynucleotide and modulate the expression and/or function of a Lipid transport and metabolism gene. The segments comprise at least five consecutive nucleotides of a Lipid transport and metabolism gene sense or antisense polynucleotides.

[00116] In another preferred embodiment, the antisense oligonucleotides are specific for natural antisense sequences of a Lipid transport and metabolism gene wherein binding of the oligonucleotides to the natural antisense sequences of
25 a Lipid transport and metabolism gene modulate expression and/or function of a Lipid transport and metabolism gene.

[00117] In another preferred embodiment, oligonucleotide compounds comprise sequences set forth as SEQ ID NOS: 23 to 263, antisense sequences which are identified and expanded, using for example, PCR, hybridization etc These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like. Examples of modified bonds or internucleotide linkages comprise phosphorothioate, phosphorodithioate or the
30 like. In another preferred embodiment, the nucleotides comprise a phosphorus derivative. The phosphorus derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphorothioate and the like. The preparation of the above-noted phosphate analogs, and their

incorporation into nucleotides, modified nucleotides and oligonucleotides, per se, is also known and need not be described here.

[00118] Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes has a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG; and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). Eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA transcribed from a gene encoding a Lipid transport and metabolism gene, regardless of the sequence(s) of such codons. A translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

[00119] The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions that may be targeted effectively with the antisense compounds of the present invention.

[00120] The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Within the context of the present invention, a targeted region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

[00121] Another target region includes the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene). Still another target region includes the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an

mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. Another target region for this invention is the 5' cap region.

[00122] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. In one embodiment, targeting splice sites, i.e., intron-exon junctions or exon-intron junctions, is particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. An aberrant fusion junction due to rearrangement or deletion is another embodiment of a target site. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts". Introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA.

[00123] In another preferred embodiment, the antisense oligonucleotides bind to coding and/or non-coding regions of a target polynucleotide and modulate the expression and/or function of the target molecule.

[00124] In another preferred embodiment, the antisense oligonucleotides bind to natural antisense polynucleotides and modulate the expression and/or function of the target molecule.

[00125] In another preferred embodiment, the antisense oligonucleotides bind to sense polynucleotides and modulate the expression and/or function of the target molecule.

[00126] Alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants". More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

[00127] Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

[00128] Variants can be produced through the use of alternative signals to start or stop transcription. Pre-mRNAs and mRNAs can possess more than one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate

at unique polyA sites. Within the context of the invention, the types of variants described herein are also embodiments of target nucleic acids.

[00129] The locations on the target nucleic acid to which the antisense compounds hybridize are defined as at least a 5-nucleotide long portion of a target region to which an active antisense compound is targeted.

5 [00130] While the specific sequences of certain exemplary target segments are set forth herein, one of skill in the art will recognize that these serve to illustrate and describe particular embodiments within the scope of the present invention. Additional target segments are readily identifiable by one having ordinary skill in the art in view of this disclosure.

10 [00131] Target segments 5-100 nucleotides in length comprising a stretch of at least five (5) consecutive nucleotides selected from within the illustrative preferred target segments are considered to be suitable for targeting as well.

[00132] Target segments can include DNA or RNA sequences that comprise at least the 5 consecutive nucleotides from the 5'-terminus of one of the illustrative preferred target segments (the remaining nucleotides being a consecutive stretch of the same DNA or RNA beginning immediately upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleotides). Similarly preferred target segments are
15 represented by DNA or RNA sequences that comprise at least the 5 consecutive nucleotides from the 3'-terminus of one of the illustrative preferred target segments (the remaining nucleotides being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleotides). One having skill in the art armed with the target segments illustrated herein will be able, without undue experimentation, to identify further preferred target segments.

20 [00133] Once one or more target regions, segments or sites have been identified, antisense compounds are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

[00134] In embodiments of the invention the oligonucleotides bind to an antisense strand of a particular target. The oligonucleotides are at least 5 nucleotides in length and can be synthesized so each oligonucleotide targets overlapping
25 sequences such that oligonucleotides are synthesized to cover the entire length of the target polynucleotide. The targets also include coding as well as non coding regions.

[00135] In one embodiment, it is preferred to target specific nucleic acids by antisense oligonucleotides. Targeting an antisense compound to a particular nucleic acid, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene
30 (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a non coding polynucleotide such as for example, non coding RNA (ncRNA).

[00136] RNAs can be classified into (1) messenger RNAs (mRNAs), which are translated into proteins, and (2) non-protein-coding RNAs (ncRNAs). ncRNAs comprise microRNAs, antisense transcripts and other Transcriptional Units

(TU) containing a high density of stop codons and lacking any extensive "Open Reading Frame". Many ncRNAs appear to start from initiation sites in 3' untranslated regions (3'UTRs) of protein-coding loci. ncRNAs are often rare and at least half of the ncRNAs that have been sequenced by the FANTOM consortium seem not to be polyadenylated. Most researchers have for obvious reasons focused on polyadenylated mRNAs that are processed and exported to the cytoplasm. Recently, it was shown that the set of non-polyadenylated nuclear RNAs may be very large, and that many such transcripts arise from so-called intergenic regions (Cheng, J. *et al.* (2005) *Science* 308 (5725), 1149-1154; Kapranov, P. *et al.* (2005). *Genome Res* 15 (7), 987-997). The mechanism by which ncRNAs may regulate gene expression is by base pairing with target transcripts. The RNAs that function by base pairing can be grouped into (1) cis encoded RNAs that are encoded at the same genetic location, but on the opposite strand to the RNAs they act upon and therefore display perfect complementarity to their target, and (2) trans-encoded RNAs that are encoded at a chromosomal location distinct from the RNAs they act upon and generally do not exhibit perfect base-pairing potential with their targets.

[00137] Without wishing to be bound by theory, perturbation of an antisense polynucleotide by the antisense oligonucleotides described herein can alter the expression of the corresponding sense messenger RNAs. However, this regulation can either be discordant (antisense knockdown results in messenger RNA elevation) or concordant (antisense knockdown results in concomitant messenger RNA reduction). In these cases, antisense oligonucleotides can be targeted to overlapping or non-overlapping parts of the antisense transcript resulting in its knockdown or sequestration. Coding as well as non-coding antisense can be targeted in an identical manner and that either category is capable of regulating the corresponding sense transcripts – either in a concordant or discordant manner. The strategies that are employed in identifying new oligonucleotides for use against a target can be based on the knockdown of antisense RNA transcripts by antisense oligonucleotides or any other means of modulating the desired target.

[00138] *Strategy 1:* In the case of discordant regulation, knocking down the antisense transcript elevates the expression of the conventional (sense) gene. Should that latter gene encode for a known or putative drug target, then knockdown of its antisense counterpart could conceivably mimic the action of a receptor agonist or an enzyme stimulant.

[00139] *Strategy 2:* In the case of concordant regulation, one could concomitantly knock down both antisense and sense transcripts and thereby achieve synergistic reduction of the conventional (sense) gene expression. If, for example, an antisense oligonucleotide is used to achieve knockdown, then this strategy can be used to apply one antisense oligonucleotide targeted to the sense transcript and another antisense oligonucleotide to the corresponding antisense transcript, or a single energetically symmetric antisense oligonucleotide that simultaneously targets overlapping sense and antisense transcripts.

[00140] According to the present invention, antisense compounds include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference

(RNAi) compounds such as siRNA compounds, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, doublestranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleotide positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines, however, in some embodiments, the gene expression or function is up regulated. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

[00141] Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (i.e., generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (i.e., generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-formlike structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

[00142] In another preferred embodiment, the desired oligonucleotides or antisense compounds, comprise at least one of: antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

[00143] dsRNA can also activate gene expression, a mechanism that has been termed "small RNA-induced gene activation" or RNAa. dsRNAs targeting gene promoters induce potent transcriptional activation of associated genes. RNAa was demonstrated in human cells using synthetic dsRNAs, termed "small activating RNAs" (saRNAs). It is currently not known whether RNAa is conserved in other organisms.

5 [00144] Small double-stranded RNA (dsRNA), such as small interfering RNA (siRNA) and microRNA (miRNA), have been found to be the trigger of an evolutionary conserved mechanism known as RNA interference (RNAi). RNAi invariably leads to gene silencing via remodeling chromatin to thereby suppress transcription, degrading complementary mRNA, or blocking protein translation. However, in instances described in detail in the examples section which follows, oligonucleotides are shown to increase the expression and/or function of the Lipid transport and
10 metabolism gene polynucleotides and encoded products thereof. dsRNAs may also act as small activating RNAs (saRNA). Without wishing to be bound by theory, by targeting sequences in gene promoters, saRNAs would induce target gene expression in a phenomenon referred to as dsRNA-induced transcriptional activation (RNAa).

[00145] In a further embodiment, the "preferred target segments" identified herein may be employed in a screen for additional compounds that modulate the expression of a Lipid transport and metabolism gene polynucleotide.
15 "Modulators" are those compounds that decrease or increase the expression of a nucleic acid molecule encoding a Lipid transport and metabolism gene and which comprise at least a 5-nucleotide portion that is complementary to a preferred target segment. The screening method comprises the steps of contacting a preferred target segment of a nucleic acid molecule encoding sense or natural antisense polynucleotides of a Lipid transport and metabolism gene with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the
20 expression of a nucleic acid molecule encoding a Lipid transport and metabolism gene polynucleotide, e.g. SEQ ID NOS: 23 to 263. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the expression of a nucleic acid molecule encoding a Lipid transport and metabolism gene polynucleotide, the modulator may then be employed in further investigative studies of the function of a Lipid transport and metabolism gene polynucleotide, or for use as a research, diagnostic, or therapeutic agent in accordance with the
25 present invention.

[00146] Targeting the natural antisense sequence preferably modulates the function of the target gene. For example, the Lipid transport and metabolism gene (e.g. accession numbers NM_005502, NM_000229, NM_002332, NM_008512, NM_000527.3, NM_000041, NM_000039, Fig. 2). In a preferred embodiment, the target is an antisense polynucleotide of the Lipid transport and metabolism gene. In a preferred embodiment, an antisense oligonucleotide
30 targets sense and/or natural antisense sequences of a Lipid transport and metabolism gene polynucleotide (e.g. accession numbers NM_005502, NM_000229, NM_002332, NM_008512, NM_000527, NM_000041, NM_000039, Fig. 2), variants, alleles, isoforms, homologs, mutants, derivatives, fragments and complementary sequences thereto.

Preferably the oligonucleotide is an antisense molecule and the targets include coding and noncoding regions of antisense and/or sense Lipid transport and metabolism gene polynucleotides.

[00147] The preferred target segments of the present invention may be also be combined with their respective complementary antisense compounds of the present invention to form stabilized double-stranded (duplexed) oligonucleotides.

[00148] Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications (Fire *et al.*, (1998) *Nature*, 391, 806-811; Timmons and Fire, (1998) *Nature*, 395, 854; Timmons *et al.*, (2001) *Gene*, 263, 103-112; Tabara *et al.*, (1998) *Science*, 282, 430-431; Montgomery *et al.*, (1998) *Proc. Natl. Acad. Sci. USA*, 95, 15502-15507; Tuschl *et al.*, (1999) *Genes Dev.*, 13, 3191-3197; Elbashir *et al.*, (2001) *Nature*, 411, 494-498; Elbashir *et al.*, (2001) *Genes Dev.* 15, 188-200). For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target (Tijsterman *et al.*, (2002) *Science*, 295, 694-697).

[00149] In a preferred embodiment, an antisense oligonucleotide targets Lipid transport and metabolism gene polynucleotides (e.g. accession numbers NM_005502, NM_000229, NM_002332, NM_008512, NM_000527, NM_000041, NM_000039), variants, alleles, isoforms, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense molecule.

[00150] In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to Lipid transport and metabolism gene alone but extends to any of the isoforms, receptors, homologs and the like of a Lipid transport and metabolism gene molecule.

[00151] In another preferred embodiment, an oligonucleotide targets a natural antisense sequence of a Lipid transport and metabolism gene polynucleotide, for example, polynucleotides set forth as SEQ ID NO: 8 to 22, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of antisense oligonucleotides are set forth as SEQ ID NOS: 23 to 263.

[00152] In one embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of a Lipid transport and metabolism gene antisense, including without limitation noncoding sense and/or antisense sequences associated with a Lipid transport and metabolism gene polynucleotide and modulate expression and/or function of a Lipid transport and metabolism gene molecule.

[00153] In another preferred embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of a Lipid transport and metabolism gene natural antisense, set forth as SEQ ID NO: 8 to 22 and modulate expression and/or function of a Lipid transport and metabolism gene molecule.

[00154] In a preferred embodiment, oligonucleotides comprise sequences of at least 5 consecutive nucleotides of SEQ ID NOS: 23 to 263 and modulate expression and/or function of a Lipid transport and metabolism gene molecule.

[00155] The polynucleotide targets comprise Lipid transport and metabolism gene, including family members thereof, variants of a Lipid transport and metabolism gene; mutants of a Lipid transport and metabolism gene, including SNPs; noncoding sequences of a Lipid transport and metabolism gene; alleles of a Lipid transport and metabolism gene; species variants, fragments and the like. Preferably the oligonucleotide is an antisense molecule.

5 [00156] In another preferred embodiment, the oligonucleotide targeting Lipid transport and metabolism gene polynucleotides, comprise: antisense RNA, interference RNA (RNAi), short interfering RNA (siRNA); micro interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); or, small activating RNA (saRNA).

10 [00157] In another preferred embodiment, targeting of a Lipid transport and metabolism gene polynucleotide, e.g. SEQ ID NO: 8 to 22 modulate the expression or function of these targets. In one embodiment, expression or function is up-regulated as compared to a control. In another preferred embodiment, expression or function is down-regulated as compared to a control.

15 [00158] In another preferred embodiment, antisense compounds comprise sequences set forth as SEQ ID NOS: 23 to 263. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like.

[00159] In another preferred embodiment, SEQ ID NOS: 23 to 263 comprise one or more LNA nucleotides.

20 [00160] The modulation of a desired target nucleic acid can be carried out in several ways known in the art. For example, antisense oligonucleotides, siRNA etc. Enzymatic nucleic acid molecules (e.g., ribozymes) are nucleic acid molecules capable of catalyzing one or more of a variety of reactions, including the ability to repeatedly cleave other separate nucleic acid molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid molecules can be used, for example, to target virtually any RNA transcript (Zaug *et al.*, 324, *Nature* 429 1986; Cech, 260 *JAMA* 3030, 1988; and Jefferies *et al.*, 17 *Nucleic Acids Research* 1371, 1989).

25 [00161] Because of their sequence-specificity, trans-cleaving enzymatic nucleic acid molecules show promise as therapeutic agents for human disease (Usman & McSwiggen, (1995) *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, (1995) *J. Med. Chem.* 38, 2023-2037). Enzymatic nucleic acid molecules can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the mRNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

30 [00162] In general, enzymatic nucleic acids with RNA cleaving activity act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis

of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

[00163] Several approaches such as in vitro selection (evolution) strategies (Orgel, (1979) *Proc. R. Soc. London*, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing a variety of reactions, such as cleavage and ligation of phosphodiester linkages and amide linkages, (Joyce, (1989) *Gene*, 82, 83-87; Beaudry *et al.*, (1992) *Science* 257, 635-641; Joyce, (1992) *Scientific American* 267, 90-97; Breaker *et al.*, (1994) *TIBTECH* 12, 268; Bartel *et al.*, (1993) *Science* 261:1411- 1418; Szostak, (1993) *TIBS* 17, 89-93; Kumar *et al.*, (1995) *FASEB J.*, 9, 1183; Breaker, (1996) *Curr. Op. Biotech.*, 7, 442).

[00164] The development of ribozymes that are optimal for catalytic activity would contribute significantly to any strategy that employs RNA-cleaving ribozymes for the purpose of regulating gene expression. The hammerhead ribozyme, for example, functions with a catalytic rate (kcat) of about 1 min⁻¹ in the presence of saturating (10 mM) concentrations of Mg²⁺ cofactor. An artificial "RNA ligase" ribozyme has been shown to catalyze the corresponding self-modification reaction with a rate of about 100 min⁻¹. In addition, it is known that certain modified hammerhead ribozymes that have substrate binding arms made of DNA catalyze RNA cleavage with multiple turn-over rates that approach 100 min⁻¹. Finally, replacement of a specific residue within the catalytic core of the hammerhead with certain nucleotide analogues gives modified ribozymes that show as much as a 10-fold improvement in catalytic rate. These findings demonstrate that ribozymes can promote chemical transformations with catalytic rates that are significantly greater than those displayed in vitro by most natural self-cleaving ribozymes. It is then possible that the structures of certain selfcleaving ribozymes may be optimized to give maximal catalytic activity, or that entirely new RNA motifs can be made that display significantly faster rates for RNA phosphodiester cleavage.

[00165] Intermolecular cleavage of an RNA substrate by an RNA catalyst that fits the "hammerhead" model was first shown in 1987 (Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600). The RNA catalyst was recovered and reacted with multiple RNA molecules, demonstrating that it was truly catalytic.

[00166] Catalytic RNAs designed based on the "hammerhead" motif have been used to cleave specific target sequences by making appropriate base changes in the catalytic RNA to maintain necessary base pairing with the target sequences (Haseloff and Gerlach, (1988) *Nature*, 334, 585; Walbot and Bruening, (1988) *Nature*, 334, 196; Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600; Koizumi, M., *et al.* (1988) *FEBS Lett.*, 228: 228-230). This has allowed use of the catalytic RNA to cleave specific target sequences and indicates that catalytic RNAs designed according to the "hammerhead" model may possibly cleave specific substrate RNAs in vivo. (see Haseloff and Gerlach, (1988) *Nature*, 334, 585; Walbot and Bruening, (1988) *Nature*, 334, 196; Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600).

[00167] RNA interference (RNAi) has become a powerful tool for modulating gene expression in mammals and mammalian cells. This approach requires the delivery of small interfering RNA (siRNA) either as RNA itself or as DNA, using an expression plasmid or virus and the coding sequence for small hairpin RNAs that are processed to

siRNAs. This system enables efficient transport of the pre-siRNAs to the cytoplasm where they are active and permit the use of regulated and tissue specific promoters for gene expression.

[00168] In a preferred embodiment, an oligonucleotide or antisense compound comprises an oligomer or polymer of ribonucleic acid (RNA) and/or deoxyribonucleic acid (DNA), or a mimetic, chimera, analog or homolog thereof. This term includes oligonucleotides composed of naturally occurring nucleotides, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often desired over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

[00169] According to the present invention, the oligonucleotides or "antisense compounds" include antisense oligonucleotides (e.g. RNA, DNA, mimetic, chimera, analog or homolog thereof), ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, saRNA, aRNA, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleotide positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines (Hammond *et al.*, (1991) *Nat. Rev. Genet.*, 2, 110-119; Matzke *et al.*, (2001) *Curr. Opin. Genet. Dev.*, 11, 221-227; Sharp, (2001) *Genes Dev.*, 15, 485-490). When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

[00170] Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (i.e., generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (i.e., generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally, U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-formlike structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

[00171] The antisense compounds in accordance with this invention can comprise an antisense portion from about 5 to about 80 nucleotides (i.e. from about 5 to about 80 linked nucleosides) in length. This refers to the length of the antisense strand or portion of the antisense compound. In other words, a single-stranded antisense compound of the invention comprises from 5 to about 80 nucleotides, and a double-stranded antisense compound of the invention (such as a dsRNA, for example) comprises a sense and an antisense strand or portion of 5 to about 80 nucleotides in length. One of ordinary skill in the art will appreciate that this comprehends antisense portions of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleotides in length, or any range therewithin.

[00172] In one embodiment, the antisense compounds of the invention have antisense portions of 10 to 50 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies oligonucleotides having antisense portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length, or any range therewithin. In some embodiments, the oligonucleotides are 15 nucleotides in length.

[00173] In one embodiment, the antisense or oligonucleotide compounds of the invention have antisense portions of 12 or 13 to 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies antisense compounds having antisense portions of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length, or any range therewithin.

[00174] In another preferred embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the antisense or dsRNA compounds. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

[00175] In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is from about 40% to about 60%. In some embodiments, homology, sequence identity or complementarity, is

from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

5 [00176] In another preferred embodiment, the antisense oligonucleotides, such as for example, nucleic acid molecules set forth in SEQ ID NOS: 8 to 263 comprise one or more substitutions or modifications. In one embodiment, the nucleotides are substituted with locked nucleic acids (LNA).

[00177] In another preferred embodiment, the oligonucleotides target one or more regions of the nucleic acid molecules sense and/or antisense of coding and/or non-coding sequences associated with Lipid transport and metabolism gene and the sequences set forth as SEQ ID NOS: 1 to 7 and 8 to 22. The oligonucleotides are also targeted to overlapping regions of SEQ ID NOS: 1 to 7 and 8 to 22.

[00178] Certain preferred oligonucleotides of this invention are chimeric oligonucleotides. "Chimeric oligonucleotides" or "chimeras," in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of antisense modulation of gene expression.

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20 Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. In one preferred embodiment, a chimeric oligonucleotide comprises at least one region modified to increase target binding affinity, and, usually, a region that acts as a substrate for RNase H. Affinity of an oligonucleotide for its target (in this case, a nucleic acid encoding ras) is routinely determined by measuring the T_m of an oligonucleotide/target pair, which is the temperature at which the oligonucleotide and target dissociate; dissociation is detected spectrophotometrically. The higher the T_m , the greater is the affinity of the oligonucleotide for the target.

[00179] Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotides mimetics as described above.

30 Such; compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, US patent nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

[00180] In another preferred embodiment, the region of the oligonucleotide which is modified comprises at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other preferred embodiments, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher T_m (i.e., higher target binding affinity) than; 2'-deoxyoligonucleotides against a given target. The effect of such increased affinity is to greatly enhance RNAi oligonucleotide inhibition of gene expression. RNase H is a cellular endonuclease that cleaves the RNA strand of RNA:DNA duplexes; activation of this enzyme therefore results in cleavage of the RNA target, and thus can greatly enhance the efficiency of RNAi inhibition. Cleavage of the RNA target can be routinely demonstrated by gel electrophoresis. In another preferred embodiment, the chimeric oligonucleotide is also modified to enhance nuclease resistance. Cells contain a variety of exo- and endo-nucleases which can degrade nucleic acids. A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide. Nuclease resistance is routinely measured by incubating oligonucleotides with cellular extracts or isolated nuclease solutions and measuring the extent of intact oligonucleotide remaining over time, usually by gel electrophoresis. Oligonucleotides which have been modified to enhance their nuclease resistance survive intact for a longer time than unmodified oligonucleotides. A variety of oligonucleotide modifications have been demonstrated to enhance or confer nuclease resistance. Oligonucleotides which contain at least one phosphorothioate modification are presently more preferred. In some cases, oligonucleotide modifications which enhance target binding affinity are also, independently, able to enhance nuclease resistance. Some desirable modifications can be found in De Mesmaeker *et al.* (1995) *Acc. Chem. Res.*, 28:366-374.

[00181] Specific examples of some preferred oligonucleotides envisioned for this invention include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly $CH_2-NH-O-CH_2$, $CH_2-N(CH_3)-O-CH_2$ [known as a methylene(methylimino) or MMI backbone], $CH_2-O-N(CH_3)-CH_2$, $CH_2-N(CH_3)-N(CH_3)-CH_2$ and $O-N(CH_3)-CH_2-CH_2$ backbones, wherein the native phosphodiester backbone is represented as $O-P-O-CH_2$. The amide backbones disclosed by De Mesmaeker *et al.* (1995) *Acc. Chem. Res.* 28:366-374 are also preferred. Also preferred are oligonucleotides having morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other preferred embodiments, such as the peptide nucleic acid (PNA) backbone, the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (Nielsen *et al.* (1991) *Science* 254, 1497). Oligonucleotides may also comprise one or more substituted sugar moieties. Preferred

oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH₃, F, OCN, OCH₃, OCH₃, OCH₃, O(CH₂)_n CH₃, O(CH₂)_n NH₂ or O(CH₂)_n CH₃ where n is from 1 to about 10; C₁ to C₁₀ lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF₃; OCF₃; O--, S--, or N-alkyl; O--, S--, or N-alkenyl; SOCH₃; SO₂ CH₃; ONO₂; NO₂; N₃; NH₂; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy [2'-O-CH₂ CH₂ OCH₃, also known as 2'-O-(2-methoxyethyl)] (Martin *et al.*, (1995) *Helv. Chim. Acta*, 78, 486). Other preferred modifications include 2'-methoxy (2'-O--CH₃), 2'-propoxy (2'-OCH₂ CH₂CH₃) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

[00182] Oligonucleotides may also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleotides include nucleotides found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic nucleotides, e.g., 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalkylamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N₆ (6-aminohexyl)adenine and 2,6-diaminopurine. (Kornberg, A., DNA Replication, W. H. Freeman & Co., San Francisco, 1980, pp75-77; Gebeyehu, G., (1987) *et al. Nucl. Acids Res.* 15:4513). A "universal" base known in the art, e.g., inosine, may be included. 5-Me-C substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanghvi, Y. S., in Crooke, S. T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions.

[00183] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, a cholesteryl moiety (Letsinger *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86, 6553), cholic acid (Manoharan *et al.* (1994) *Bioorg. Med. Chem. Lett.* 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660, 306; Manoharan *et al.* (1993) *Bioorg. Med. Chem. Lett.* 3, 2765), a thiocholesterol (Oberhauser *et al.*, (1992) *Nucl. Acids Res.* 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras *et al.* EMBO J. 1991, 10, 111; Kabanov *et al.* (1990) *FEBS Lett.* 259, 327; Svinarchuk *et al.* (1993) *Biochimie* 75, 49), a phospholipid, e.g., di-

hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero- 3-H-phosphonate (Manoharan *et al.* (1995) *Tetrahedron Lett.* 36, 3651; Shea *et al.* (1990) *Nucl. Acids Res.* 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.* (1995) *Nucleosides & Nucleotides*, 14, 969), or adamantane acetic acid (Manoharan *et al.* (1995) *Tetrahedron Lett.* 36, 3651). Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides are known in the art, for example, U.S. Pat. Nos. 5,138,045, 5,218,105 and 5,459,255.

[00184] It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide. The present invention also includes oligonucleotides which are chimeric oligonucleotides as hereinbefore defined.

[00185] In another embodiment, the nucleic acid molecule of the present invention is conjugated with another moiety including but not limited to abasic nucleotides, polyether, polyamine, polyamides, peptides, carbohydrates, lipid, or polyhydrocarbon compounds. Those skilled in the art will recognize that these molecules can be linked to one or more of any nucleotides comprising the nucleic acid molecule at several positions on the sugar, base or phosphate group.

[00186] The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of one of ordinary skill in the art. It is also well known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives. It is also well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products such as biotin, fluorescein, acridine or psoralen-modified amidites and/or CPG (available from Glen Research, Sterling VA) to synthesize fluorescently labeled, biotinylated or other modified oligonucleotides such as cholesterol-modified oligonucleotides.

[00187] In accordance with the invention, use of modifications such as the use of LNA monomers to enhance the potency, specificity and duration of action and broaden the routes of administration of oligonucleotides comprised of current chemistries such as MOE, ANA, FANA, PS etc (Uhlman, *et al.* (2000) *Current Opinions in Drug Discovery & Development* Vol. 3 No 2). This can be achieved by substituting some of the monomers in the current oligonucleotides by LNA monomers. The LNA modified oligonucleotide may have a size similar to the parent compound or may be larger or preferably smaller. It is preferred that such LNA-modified oligonucleotides contain less than about 70%, more preferably less than about 60%, most preferably less than about 50% LNA monomers and that their sizes are between about 5 and 25 nucleotides, more preferably between about 12 and 20 nucleotides.

[00188] Preferred modified oligonucleotide backbones comprise, but not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates

comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

5 [00189] Representative United States patents that teach the preparation of the above phosphorus containing linkages comprise, but are not limited to, US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

10 [00190] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl
15 and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

[00191] Representative United States patents that teach the preparation of the above oligonucleosides comprise, but are not limited to, US patent nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,
20 264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

[00192] In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an
25 appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds
30 comprise, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen, *et al.* (1991) *Science* 254, 1497-1500.

[00193] In another preferred embodiment of the invention the oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular- CH₂-NH-O-CH₂-, -CH₂-N (CH₃)-O-CH₂-known as a methylene (methylimino) or MMI backbone, - CH₂-O-N (CH₃)-CH₂-, -CH₂N(CH₃)-N(CH₃) CH₂-and-O-N(CH₃)-CH₂-CH₂- wherein the native phosphodiester backbone is represented as-O-P-O-CH₂- of the above referenced US patent no. 5,489,677, and the amide backbones of the above referenced US patent no. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced US patent no. 5,034,506.

[00194] Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C to CO alkyl or C₂ to CO alkenyl and alkynyl. Particularly preferred are O (CH₂)_n OmCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON(CH₂)_nCH₃)₂ where n and m can be from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C to CO, (lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification comprises 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2- methoxyethyl) or 2'-MOE) (Martin *et al.*, (1995) *Helv. Chim. Acta*, 78, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification comprises 2'-dimethylaminoethoxyethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'- DMAEOE), i.e., 2'-O-CH₂-O-CH₂-N (CH₂)₂.

[00195] Other preferred modifications comprise 2'-methoxy (2'-O CH₃), 2'-aminopropoxy (2'-O CH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures comprise, but are not limited to, US patent nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference.

[00196] Oligonucleotides may also comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides comprise the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleotides comprise other

synthetic and natural nucleotides such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudo-uracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylquanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[00197] Further, nucleotides comprise those disclosed in United States Patent No. 3,687,808, those disclosed in 'The Concise Encyclopedia of Polymer Science And Engineering', pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch *et al.*, 'Angewandte Chemie, International Edition', 1991, 30, page 613, and those disclosed by Sanghvi, Y.S., Chapter 15, 'Antisense Research and Applications', pages 289-302, Crooke, S.T. and Lebleu, B. ea., CRC Press, 1993. Certain of these nucleotides are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These comprise 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, comprising 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds, 'Antisense Research and Applications', CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[00198] Representative United States patents that teach the preparation of the above noted modified nucleotides as well as other modified nucleotides comprise, but are not limited to, US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

[00199] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates, which enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide.

[00200] Such moieties comprise but are not limited to, lipid moieties such as a cholesterol moiety (Letsinger *et al.*, (1989) *Proc. Natl. Acad. Sci. USA*, 86, 6553-6556), cholic acid (Manoharan *et al.*, (1994) *Bioorg. Med. Chem. Lett.*, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan *et al.*, (1992) *Ann. N. Y. Acad. Sci.*, 660, 306-309; Manoharan *et al.*, (1993) *Bioorg. Med. Chem. Lett.*, 3, 2765-2770), a thiocholesterol (Oberhauser *et al.*, (1992) *Nucl. Acids Res.*, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Kabanov *et al.*, (1990) *FEBS Lett.*, 259, 327-330; Svinarchuk *et al.*, (1993) *Biochimie* 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36,

3651-3654; Shea *et al.*, (1990) *Nucl. Acids Res.*, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Mancharan *et al.*, (1995) *Nucleosides & Nucleotides*, 14, 969-973), or adamantane acetic acid (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36, 3651-3654), a palmityl moiety (Mishra *et al.*, (1995) *Biochim. Biophys. Acta*, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-t oxysterol moiety (Crooke *et al.*, (1996) *J. Pharmacol. Exp. Ther.*, 277, 923-937).

[00201] Representative United States patents that teach the preparation of such oligonucleotides conjugates comprise, but are not limited to, US patent nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552, 538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486, 603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762, 779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082, 830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5, 245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391, 723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5, 565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599, 928 and 5,688,941, each of which is herein incorporated by reference.

[00202] *Drug discovery*: The compounds of the present invention can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the compounds and preferred target segments identified herein in drug discovery efforts to elucidate relationships that exist between a Lipid transport and metabolism gene polynucleotide and a disease state, phenotype, or condition. These methods include detecting or modulating a Lipid transport and metabolism gene polynucleotide comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of a Lipid transport and metabolism gene polynucleotide and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

Assessing Up-regulation or Inhibition of Gene Expression:

[00203] Transfer of an exogenous nucleic acid into a host cell or organism can be assessed by directly detecting the presence of the nucleic acid in the cell or organism. Such detection can be achieved by several methods well known in the art. For example, the presence of the exogenous nucleic acid can be detected by Southern blot or by a polymerase chain reaction (PCR) technique using primers that specifically amplify nucleotide sequences associated with the nucleic acid. Expression of the exogenous nucleic acids can also be measured using conventional methods including gene expression analysis. For instance, mRNA produced from an exogenous nucleic acid can be detected and quantified using a Northern blot and reverse transcription PCR (RT-PCR).

[00204] Expression of RNA from the exogenous nucleic acid can also be detected by measuring an enzymatic activity or a reporter protein activity. For example, antisense modulatory activity can be measured indirectly as a decrease or increase in target nucleic acid expression as an indication that the exogenous nucleic acid is producing the effector RNA. Based on sequence conservation, primers can be designed and used to amplify coding regions of the target genes. Initially, the most highly expressed coding region from each gene can be used to build a model control gene, although any coding or non coding region can be used. Each control gene is assembled by inserting each coding region between a reporter coding region and its poly(A) signal. These plasmids would produce an mRNA with a reporter gene in the upstream portion of the gene and a potential RNAi target in the 3' non-coding region. The effectiveness of individual antisense oligonucleotides would be assayed by modulation of the reporter gene. Reporter genes useful in the methods of the present invention include acetohydroxyacid synthase (AHAS), alkaline phosphatase (AP), beta galactosidase (LacZ), beta glucuronidase (GUS), chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), horseradish peroxidase (HRP), luciferase (Luc), nopaline synthase (NOS), octopine synthase (OCS), and derivatives thereof. Multiple selectable markers are available that confer resistance to ampicillin, bleomycin, chloramphenicol, gentamycin, hygromycin, kanamycin, lincomycin, methotrexate, phosphinothricin, puromycin, and tetracycline. Methods to determine modulation of a reporter gene are well known in the art, and include, but are not limited to, fluorometric methods (e.g. fluorescence spectroscopy, Fluorescence Activated Cell Sorting (FACS), fluorescence microscopy), antibiotic resistance determination.

[00205] Lipid transport and metabolism gene protein and mRNA expression can be assayed using methods known to those of skill in the art and described elsewhere herein. For example, immunoassays such as the ELISA can be used to measure protein levels. Lipid transport and metabolism gene antibodies for ELISAs are available commercially, e.g., from R&D Systems (Minneapolis, MN), Abcam, Cambridge, MA.

[00206] In embodiments, Lipid transport and metabolism gene expression (e.g., mRNA or protein) in a sample (e.g., cells or tissues in vivo or in vitro) treated using an antisense oligonucleotide of the invention is evaluated by comparison with Lipid transport and metabolism gene expression in a control sample. For example, expression of the protein or nucleic acid can be compared using methods known to those of skill in the art with that in a mock-treated or untreated sample. Alternatively, comparison with a sample treated with a control antisense oligonucleotide (e.g., one having an altered or different sequence) can be made depending on the information desired. In another embodiment, a difference in the expression of the Lipid transport and metabolism gene protein or nucleic acid in a treated vs. an untreated sample can be compared with the difference in expression of a different nucleic acid (including any standard deemed appropriate by the researcher, e.g., a housekeeping gene) in a treated sample vs. an untreated sample.

[00207] Observed differences can be expressed as desired, e.g., in the form of a ratio or fraction, for use in a comparison with control. In embodiments, the level of a Lipid transport and metabolism gene mRNA or protein, in a

sample treated with an antisense oligonucleotide of the present invention, is increased or decreased by about 1.25-fold to about 10-fold or more relative to an untreated sample or a sample treated with a control nucleic acid. In embodiments, the level of a Lipid transport and metabolism gene mRNA or protein is increased or decreased by at least about 1.25-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, or at least about 10-fold or more.

Kits, Research Reagents, Diagnostics, and Therapeutics

[00208] The compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis, and as research reagents and components of kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

[00209] For use in kits and diagnostics and in various biological systems, the compounds of the present invention, either alone or in combination with other compounds or therapeutics, are useful as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

[00210] As used herein the term "biological system" or "system" is defined as any organism, cell, cell culture or tissue that expresses, or is made competent to express products of the Lipid transport and metabolism genes. These include, but are not limited to, humans, transgenic animals, cells, cell cultures, tissues, xenografts, transplants and combinations thereof.

[00211] As one non limiting example, expression patterns within cells or tissues treated with one or more antisense compounds are compared to control cells or tissues not treated with antisense compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds that affect expression patterns.

[00212] Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, (2000) *FEBS Lett.*, 480, 17-24; Celis, *et al.*, (2000) *FEBS Lett.*, 480, 2-16), SAGE (serial analysis of gene expression) (Madden, *et al.*, (2000) *Drug Discov. Today*, 5, 415- 425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, (1999) *Methods Enzymol.*, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, *et al.*, (2000) *Proc. Natl. Acad. Sci. U.S.A.*, 97, 1976-81), protein arrays and proteomics (Celis, *et al.*, (2000) *FEBS Lett.*, 480, 2-16; Jungblut, *et al.*, *Electrophoresis*, 1999, 20, 2100-10), expressed sequence tag (EST)

sequencing (Celis, et al., FEBS Lett., 2000, 480, 2-16; Larsson, et al., J. Biotechnol., 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, *et al.*, (2000) Anal. Biochem. 286, 91-98; Larson, *et al.*, (2000) *Cytometry* 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, (2000) *Curr. Opin. Microbiol.* 3, 316-21), comparative genomic hybridization (Carulli, *et al.*, (1998) *J. Cell Biochem. Suppl.*, 31, 286-96), FISH (fluorescent in situ hybridization) techniques (Going and Gusterson, (1999) *Eur. J. Cancer*, 35, 1895-904) and mass spectrometry methods (To, Comb. (2000) *Chem. High Throughput Screen*, 3, 235-41).

[00213] The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding a Lipid transport and metabolism gene. For example, oligonucleotides that hybridize with such efficiency and under such conditions as disclosed herein as to be effective Lipid transport and metabolism gene modulators are effective primers or probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding a Lipid transport and metabolism gene and in the amplification of said nucleic acid molecules for detection or for use in further studies of a Lipid transport and metabolism gene. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding a Lipid transport and metabolism gene can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabeling of the oligonucleotide, or any other suitable detection means. Kits using such detection means for detecting the level of a Lipid transport and metabolism gene in a sample may also be prepared.

[00214] The specificity and sensitivity of antisense are also harnessed by those of skill in the art for therapeutic uses. Antisense compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that antisense compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

[00215] For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of a Lipid transport and metabolism gene polynucleotide is treated by administering antisense compounds in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of a Lipid transport and metabolism gene modulator. The Lipid transport and metabolism gene modulators of the present invention effectively modulate the activity of a Lipid transport and metabolism gene or modulate the expression of a Lipid transport and metabolism gene protein. In one embodiment, the activity or expression of a Lipid transport and metabolism gene in an animal is inhibited by about 10% as compared to a control. Preferably, the activity or expression of a Lipid transport and metabolism gene in an animal is inhibited by about 30%. More preferably, the activity or expression of a Lipid transport and metabolism gene in an animal is inhibited by 50% or more. Thus, the oligomeric

compounds modulate expression of a Lipid transport and metabolism gene mRNA by at least 10%, by at least 50%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100% as compared to a control.

5 [00216] In one embodiment, the activity or expression of a Lipid transport and metabolism gene and/or in an animal is increased by about 10% as compared to a control. Preferably, the activity or expression of a Lipid transport and metabolism gene in an animal is increased by about 30%. More preferably, the activity or expression of a Lipid transport and metabolism gene in an animal is increased by 50% or more. Thus, the oligomeric compounds modulate expression of a Lipid transport and metabolism gene mRNA by at least 10%, by at least 50%, by at least 25%, by at
10 least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100% as compared to a control.

[00217] For example, the reduction of the expression of a Lipid transport and metabolism gene may be measured in serum, blood, adipose tissue, liver or any other body fluid, tissue or organ of the animal. Preferably, the cells contained within said fluids, tissues or organs being analyzed contain a nucleic acid molecule encoding Lipid transport and
15 metabolism gene peptides and/or the Lipid transport and metabolism gene protein itself.

[00218] The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the compounds and methods of the invention may also be useful prophylactically.

Conjugates

20 [00219] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic
25 properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context
30 of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application No. PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860, which are incorporated herein by reference. Conjugate moieties include, but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether,

e.g., hexyl-5- tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. Oligonucleotides of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indomethacin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

[00220] Representative United States patents that teach the preparation of such oligonucleotides conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

Formulations

[00221] The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as forexample, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption.

Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,165; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

[00222] Although, the antisense oligonucleotides do not need to be administered in the context of a vector in order to modulate a target expression and/or function, embodiments of the invention relates to expression vector constructs for the expression of antisense oligonucleotides, comprising promoters, hybrid promoter gene sequences and possess a strong constitutive promoter activity, or a promoter activity which can be induced in the desired case.

[00223] In an embodiment, invention practice involves administering at least one of the foregoing antisense oligonucleotides with a suitable nucleic acid delivery system. In one embodiment, that system includes a non-viral vector operably linked to the polynucleotide. Examples of such nonviral vectors include the oligonucleotide alone (e.g. any one or more of SEQ ID NOS: 23 to 263) or in combination with a suitable protein, polysaccharide or lipid formulation.

[00224] Additionally suitable nucleic acid delivery systems include viral vector, typically sequence from at least one of an adenovirus, adenovirus-associated virus (AAV), helper-dependent adenovirus, retrovirus, or hemagglutinin virus of Japan-liposome (HVJ) complex. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide e.g., a cytomegalovirus (CMV) promoter.

5 [00225] Additionally preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. One preferred HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the env gene is from another virus. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector [Geller, A.I. *et al.*, (1995) *J. Neurochem*, 64: 487; 10 Lim, F., *et al.*, in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A.I. *et al.*, (1993) *Proc Natl. Acad. Sci.*: U.S.A.:90 7603; Geller, A.I., *et al.*, (1990) *Proc Natl. Acad. Sci USA*: 87:1149], Adenovirus Vectors (LeGal LaSalle *et al.*, *Science*, 259:988 (1993); Davidson, *et al.*, (1993) *Nat. Genet.* 3: 219; Yang, *et al.*, (1995) *J. Virol.* 69: 2004) and Adeno-associated Virus Vectors (Kaplit, M.G., *et al.*, (1994) *Nat. Genet.* 8:148).

15 [00226] The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

[00227] The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do 20 not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

[00228] The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a 25 number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, 30 administration.

[00229] For treating tissues in the central nervous system, administration can be made by, e.g., injection or infusion into the cerebrospinal fluid. Administration of antisense RNA into cerebrospinal fluid is described, e.g., in U.S. Pat.

App. Pub. No. 2007/0117772, "Methods for slowing familial ALS disease progression," incorporated herein by reference in its entirety.

[00230] When it is intended that the antisense oligonucleotide of the present invention be administered to cells in the central nervous system, administration can be with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier. Injection can be made, e.g., in the entorhinal cortex or hippocampus. Delivery of neurotrophic factors by administration of an adenovirus vector to motor neurons in muscle tissue is described in, e.g., U.S. Pat. No. 6,632,427, "Adenoviral-vector-mediated gene transfer into medullary motor neurons," incorporated herein by reference. Delivery of vectors directly to the brain, e.g., the striatum, the thalamus, the hippocampus, or the substantia nigra, is known in the art and described, e.g., in U.S. Pat. No. 6,756,523, "Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain," incorporated herein by reference. Administration can be rapid as by injection or made over a period of time as by slow infusion or administration of slow release formulations.

[00231] The subject antisense oligonucleotides can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, the antisense oligonucleotide can be coupled to any substance, known in the art to promote penetration or transport across the blood-brain barrier, such as an antibody to the transferrin receptor, and administered by intravenous injection. The antisense compound can be linked with a viral vector, for example, that makes the antisense compound more effective and/or increases the transport of the antisense compound across the blood-brain barrier. Osmotic blood brain barrier disruption can also be accomplished by, e.g., infusion of sugars including, but not limited to, meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids including, but not limited to, glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, e.g., in U. S. Patent No. 4,866,042, "Method for the delivery of genetic material across the blood brain barrier," 6,294,520, "Material for passage through the blood-brain barrier," and 6,936,589, "Parenteral delivery systems," all incorporated herein by reference in their entirety.

[00232] The subject antisense compounds may be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. For example, cationic lipids may be included in the formulation to facilitate oligonucleotide uptake. One such composition shown to facilitate uptake is LIPOFECTIN (available from GIBCO-BRL, Bethesda, MD).

[00233] Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

[00234] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[00235] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[00236] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

[00237] Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug that may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860.

[00238] Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes that are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

[00239] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids. When incorporated into liposomes, these specialized lipids result in liposomes with enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of

sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860.

[00240] The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

[00241] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating nonsurfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

[00242] One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

[00243] Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoyl-phosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoyl-phosphatidyl ethanolamine DOTMA).

[00244] For topical or other administration, oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters, pharmaceutically acceptable salts thereof, and their uses are further described in U.S. Pat. No. 6,287,860.

[00245] Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitables. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference. Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether,

polyoxyethylene-20-cetyl ether. Oligonucleotides of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Oligonucleotide complexing agents and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

[00246] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[00247] Certain embodiments of the invention provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents that function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bischloroethyl- nitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5- azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclo-phosphoramidate, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[00248] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. For example, the first target may be a particular antisense sequence of a Lipid transport and metabolism gene, and the second target may be a region from another nucleotide sequence. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same Lipid transport and metabolism gene nucleic acid target. Numerous examples of antisense compounds are illustrated herein and others may be selected from among suitable compounds known in the art. Two or more combined compounds may be used together or sequentially.

Dosing:

[00249] The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC50s found to be effective in in vitro and in vivo animal models. In general, dosage is from 0.01 µg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 µg to 100 g per kg of body weight, once or more daily, to once every 20 years.

[00250] In embodiments, a patient is treated with a dosage of drug that is at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, or at least about 100 mg/kg body weight. Certain injected dosages of antisense oligonucleotides are described, e.g., in U.S. Pat. No. 7,563,884, "Antisense modulation of PTP1B expression," incorporated herein by reference in its entirety.

[00251] While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. Numerous changes to the disclosed embodiments can be made in accordance with the disclosure herein without departing from the spirit or scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above described embodiments.

[00252] All documents mentioned herein are incorporated herein by reference. All publications and patent documents cited in this application are incorporated by reference for all purposes to the same extent as if each individual publication or patent document were so individually denoted. By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention. Embodiments of inventive compositions and methods are illustrated in the following examples.

EXAMPLES

[00253] The following non-limiting Examples serve to illustrate selected embodiments of the invention. It will be appreciated that variations in proportions and alternatives in elements of the components shown will be apparent to those skilled in the art and are within the scope of embodiments of the present invention.

Example 1: Design of antisense oligonucleotides specific for a nucleic acid molecule antisense to a Lipid transport and metabolism gene and/or a sense strand of a Lipid transport and metabolism gene polynucleotide

[00254] As indicated above the term "oligonucleotide specific for" or "oligonucleotide targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of an mRNA transcript of the targeted gene.

[00255] Selection of appropriate oligonucleotides is facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid sequences obtained, for example, by searching databases such as GenBank or by sequencing PCR products. Comparison of nucleic acid sequences from a range of species allows the selection of nucleic acid sequences that display an appropriate degree of identity between species. In the case of genes that have not been sequenced, Southern blots are performed to allow a determination of the degree of identity between genes in target species and other species. By performing Southern blots at varying degrees of stringency, as is well known in the art, it is possible to obtain an approximate measure of identity. These procedures allow the selection of oligonucleotides that exhibit a high degree of complementarity to target nucleic acid sequences in a subject to be controlled and a lower degree of complementarity to corresponding nucleic acid sequences in other species. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

[00256] An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a modulation of function and/or activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays

[00257] The hybridization properties of the oligonucleotides described herein can be determined by one or more in vitro assays as known in the art. For example, the properties of the oligonucleotides described herein can be obtained by determination of binding strength between the target natural antisense and a potential drug molecules using melting curve assay.

[00258] The binding strength between the target natural antisense and a potential drug molecule (Molecule) can be estimated using any of the established methods of measuring the strength of intermolecular interactions, for example, a melting curve assay.

[00259] Melting curve assay determines the temperature at which a rapid transition from double-stranded to single-stranded conformation occurs for the natural antisense/Molecule complex. This temperature is widely accepted as a reliable measure of the interaction strength between the two molecules.

[00260] A melting curve assay can be performed using a cDNA copy of the actual natural antisense RNA molecule or a synthetic DNA or RNA nucleotide corresponding to the binding site of the Molecule. Multiple kits containing all necessary reagents to perform this assay are available (e.g. Applied Biosystems Inc. MeltDoctor kit). These kits include a suitable buffer solution containing one of the double strand DNA (dsDNA) binding dyes (such as ABI HRM dyes, SYBR Green, SYTO, etc.). The properties of the dsDNA dyes are such that they emit almost no fluorescence in free form, but are highly fluorescent when bound to dsDNA.

[00261] To perform the assay the cDNA or a corresponding oligonucleotide are mixed with Molecule in concentrations defined by the particular manufacturer's protocols. The mixture is heated to 95 °C to dissociate all pre-formed dsDNA complexes, then slowly cooled to room temperature or other lower temperature defined by the manufacturer to allow the DNA molecules to anneal. The newly formed complexes are then slowly heated to 95 °C with simultaneous continuous collection of data on the amount of fluorescence that is produced by the reaction. The fluorescence intensity is inversely proportional to the amounts of dsDNA present in the reaction. The data can be collected using a real time PCR instrument compatible with the kit (e.g. ABI's StepOne Plus Real Time PCR System or LightTyper instrument, Roche Diagnostics, Lewes, UK).

[00262] Melting peaks are constructed by plotting the negative derivative of fluorescence with respect to temperature ($-d(\text{Fluorescence})/dT$) on the y-axis) against temperature (x-axis) using appropriate software (for example LightTyper (Roche) or SDS Dissociation Curve, ABI). The data is analyzed to identify the temperature of the rapid transition from dsDNA complex to single strand molecules. This temperature is called T_m and is directly proportional to the strength of interaction between the two molecules. Typically, T_m will exceed 40 °C.

Example 2: Modulation of a Lipid transport and metabolism gene polynucleotide

Treatment of 518A2 cells with antisense oligonucleotides

[00263] 518A2 cells obtained from Albert Einstein-Montefiore Cancer Center, NY were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35- 011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^5 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was changed to fresh growth media. All antisense oligonucleotides were diluted to the concentration of 20 µM. Two µl of this solution was incubated with 400 µl of Opti-MEM media (Gibco cat#31985-070) and 4 µl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with 518A2 cells. A Similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was

added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) or High Capacity cDNA Reverse Transcription Kit (cat# 4368813 as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Taqman Gene Expression Assay by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using StepOne Plus Real Time PCR Machine (Applied Biosystems).

Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

10 *Results*

[00264] Real time PCR results show that the levels of ABCA1 mRNA in 518A2 cells are significantly increased 48 h after treatment with one of the siRNAs designed to ABCA1 antisense AK311445 (Fig 1A).

[00265] Real time PCR results show that the levels of ABCA1 mRNA in 518A2 cells are significantly increased 48 h after treatment with six of the oligos designed to ABCA1 antisense AK311445 (Fig 1B).

15 *Treatment of 3T3 cells with antisense oligonucleotides*

[00266] 3T3 cells from ATCC (cat# CRL-1658) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35- 011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^5 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was changed to fresh growth media. All antisense oligonucleotides were diluted to the concentration of 20 µM. Two µl of this solution was incubated with 400 µl of Opti-MEM media (Gibco cat#31985-070) and 4 µl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with 3T3 cells. A Similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) or High Capacity cDNA Reverse Transcription Kit (cat# 4368813) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Taqman Gene Expression Assay by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using StepOne Plus Real Time PCR Machine (Applied Biosystems).

[00267] Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Results:

[00268] Real time PCR results show that the levels of ABCA1 mRNA in 3T3 cells are significantly increased 48 h after treatment with three of the oligos designed to mouse ABCA1 antisense BF133827 (Fig 1C).

[00269] Real Time PCR results show that levels of LRP1 mRNA in 3T3 cells are significantly increased 48h after treatment with oligos to LRP1 antisense DC401271 and AW544265 (Fig 1J).

Treatment of HepG2 cells with antisense oligonucleotides

Method 1: Treatment of HepG2 cells with naked antisense oligonucleotides:

[00270] HepG2 cells from ATCC (cat# HB-8065) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35-011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 0.5×10^4 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was replaced with 1.5 ml/well of fresh growth media. All antisense oligonucleotides were diluted in water to the concentration of 20 µM. 2 µl of this solution was mixed with 400 µl of fresh growth media and applied to each well of the 6 well plates with HepG2 cells. A similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-treated controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 72 h after addition of antisense oligonucleotides the cells were redosed as described in above. 48-72 h after second dosing the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using Mx4000 thermal cycler (Stratagene). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Method Two: Treatment of HepG2 cells with antisense oligonucleotides:

[00271] HepG2 cells from ATCC (cat# HB-8065) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35-011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^5 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media

in the 6 well plates was changed to fresh growth media. All antisense oligonucleotides were diluted to the concentration of 20 μ M. 2 μ l of this solution was incubated with 400 μ l of Opti-MEM media (Gibco cat#31985-070) and 4 μ l of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with HepG2 cells. A similar mixture including 2 μ l of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using Mx4000 thermal cycler (Stratagene). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Results:

Real time PCR results show that the levels of the LCAT mRNA in HepG2 cells are significantly increased 48 h after treatment with two of the oligos designed to LCAT antisense Hs.668679 (Fig 1E).

Real time PCR results show that the levels of the LCAT mRNA in HepG2 cells are significantly increased 48 h after treatment with one of the oligos designed to LCAT antisense Hs.668679 (Fig 1F).

Real Time PCR results show that levels of LRP1 mRNA in HepG2 cells are significantly increased 48h after treatment with oligos to LRP1 antisense DC401271 (Fig 1H).

Real Time PCR results show that levels of LDLr mRNA in HepG2 cells are significantly increased 48h after treatment with antisense oligos to LDLR antisense sherflor.aApr07.Oligos designed to LDLr antisense bloflor.aApr07 (CUR-1059-CUR-1063) did not elevate LDLr levels (Fig 1K and 1L).

Real time PCR results show that the levels of APOE mRNA in HepG2 cells are significantly increased 48 h after treatment with three of the antisense oligos designed to APOE antisense Hs.626623. Oligos designed to APOE4 antisense Hs.714236 did not significantly elevate APOE mRNA (Fig M).

Real time PCR results show that the levels of ApoA1 mRNA in HepG2 cells are significantly increased 48 h after treatment with some of the antisense oligonucleotides to ApoA1 antisense DA327409ext (Fig 1N to Fig 1P).

Real time PCR results showing the fold change in ApoA1 mRNA (top panel) and ApoA1 natural antisense DA327409ext RNA (bottom panel) after treatment of HepG2 cells with naked LNA or phosphothioate oligonucleotides over 7 days as compared to control (Fig Q).

Real time PCR results showing the fold change in ApoA1 mRNA (orange bars) and ApoA1 natural antisense DA327409ext RNA (blue bars) after treatment of HepG2 cells with LNA oligonucleotides (Fig R).

Treatment of Hek293 cells with antisense oligonucleotides

[00272] Hek293 cells from ATCC (cat# CRL-1573) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35- 011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^5 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was changed to fresh growth media. All antisense oligonucleotides were diluted to the concentration of 20 µM. Two µl of this solution was incubated with 400 µl of Opti-MEM media (Gibco cat#31985-070) and 4 µl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with Hek293 cells. A Similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) or High Capacity cDNA Reverse Transcription Kit (cat# 4368813) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Taqman Gene Expression Assay by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using using Mx4000 thermal cycler (Stratagene) or StepOne Plus Real Time PCR Machine (Applied Biosystems).

[00273] Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Results:

Real time PCR results show that the levels of the LCAT mRNA in Hek293 cells are significantly increased 48 h after treatment with three of the oligos designed to LCAT antisense Hs.668679 (Fig 1D).

Treatment of Vero 76 cells with antisense oligonucleotides

[00274] Vero 76 cells from ATCC (cat# CRL-1587) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35- 011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^5 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was changed to fresh growth media. All antisense oligonucleotides were diluted to the concentration

of 20 μ M. Two μ l of this solution was incubated with 400 μ l of Opti-MEM media (Gibco cat#31985-070) and 4 μ l of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with Vero 76 cells. A Similar mixture including 2 μ l of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) or High Capacity cDNA Reverse Transcription Kit (cat# 4368813) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Taqman Gene Expression Assay by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using using Mx4000 thermal cycler (Stratagene) or StepOne Plus Real Time PCR Machine (Applied Biosystems).

[00275] Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Results:

[00276] Real time PCR results show that the levels of the LCAT mRNA in Vero cells are significantly increased 48 h after treatment with one of the oligos designed to LCAT antisense Hs.668679 (Fig. 1G).

[00277] Real Time PCR results show that levels of LRP1 mRNA in Vero cells are significantly increased 48h after treatment with oligos to LRP1 antisense DC401271 and Hs.711951 (Fig 1I).

Detection probes used in Applied Biosystems Gene Expression Assays:

ABCA1: Hs00194045_m1 (human), Mm01350760_m1 (mouse)

LCAT: Hs00173415_m1

LRP1: Hs00233856_m1 (human), Mm00464608_m1 (mouse)

LDLR: Hs00181192_m1

ApoE: Hs00171168_m1

ApoA1: Hs00163641_m1, 18S cat# 4319413E

Custom designed assay for ApoA1 antisense DA327409ext:

FAM labeled: TTTGGATCTGGACGACTTC (SEQ ID NO: 275)

Example 3: Modulation of a Lipid transport and metabolism gene Expression

Materials and Methods

[00278] Cells were treated with either of the following methods:

Method 1: Treatment of HepG2 cells with naked antisense oligonucleotides:

[00279] HepG2 cell were grown in MEM/EBSS (Hyclone cat #SH30024) +10% FBS+ penicillin+streptomycin at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^4 /ml into 6 well plates and left at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was changed to fresh MEM/EBSS +10% FBS. All antisense oligonucleotides manufactured by IDT were diluted to the concentration of 20 μ M. 2 μ l of this solution was incubated with 400 μ l of Opti-MEM media (Gibco cat#31985-070) and applied to each well of the 6 well plates with HepG2 cells. Similar mixture including 2 μ l of water instead of the oligonucleotide solution was used for the mock-transfected controls. 72 h after addition of antisense oligonucleotides the media was removed and the dosing procedure was repeated as described in above.

48-72 h after repeated dosing RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using Mx4000 thermal cycler (Stratagene).

Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Primers and probe for the custom designed Taqman assay for the ApoA1 natural antisense DA327409ext. Capital letters indicate unmodified deoxyribonucleotides

Probe sequence (FAM labeled) TTTGGATCTGGACGACTTC (SEQ ID NO: 275)

Forward Primer Seq. CTCCTCCTGCCACTTCTTCTG (SEQ ID NO: 276)

Reverse Primer Seq. CTGGTGGATGAAGAAGGTTTGC (SEQ ID NO: 277)

Method Two: Treatment of HepG2 cells with antisense oligonucleotides:

[00280] HepG2 cells from ATCC (cat# HB-8065) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35-011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO₂. One day before the experiment the cells were replated at the density of 1.5×10^5 /ml into 6 well plates and incubated at 37°C and 5% CO₂. On the day of the experiment the media in the 6 well plates was changed to fresh growth media. All antisense oligonucleotides were diluted to the concentration of 20 μ M. 2 μ l of this solution was incubated with 400 μ l of Opti-MEM media (Gibco cat#31985-070) and 4 μ l of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of

the 6 well plates with HepG2 cells. Similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions.

600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using Mx4000 thermal cycler (Stratagene). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

Primers and probe for the custom designed Taqman assay for the ApoA1 natural antisense DA327409ext. Capital letters indicate unmodified deoxyribonucleotides

Probe sequence (FAM labeled) TTTGGATCTGGACGACTTC (SEQ ID NO: 275)

Forward Primer Seq. CTCCTCCTGCCACTTCTTCTG (SEQ ID NO: 276)

Reverse Primer Seq. CTGGTGGATGAAGAAGGTTTGC (SEQ ID NO: 277)

Treatment of primary monkey hepatocytes

Primary monkey hepatocytes were introduced into culture by RxGen Inc. and plated in 6 well plates. They were treated with oligonucleotides as follows. The media in the 6 well plates was changed to fresh growth media consisting of William's Medium E (Sigma cat#W4128) supplemented with 5% FBS, 50 U/ml penicillin and 50 ug/ml streptomycin, 4 ug/ml insulin, 1 uM dexamethasone, 10 ug/ml Fungin (Invivogen, San Diego CA). All antisense oligonucleotides were diluted to the concentration of 20 µM. 2 µl of this solution was incubated with 400 µl of Opti-MEM media (Gibco cat#31985-070) and 4 µl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with cells. Similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO₂ the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions.

600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Inc., Foster City CA). The following PCR

cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using Mx4000 thermal cycler (Stratagene). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

ELISA was conducted using MabTech Inc. ApoA1 ELISA kit cat# 3710-11-6 according to manufacturer's instructions.

5 [00281] The results are shown in Fig. 1Q to Fig. 1T. Figure Q shows that both oligonucleotides with the phosphothioate backbone, i.e. internucleotide linkages and LNA oligonucleotides were effective in modulating the target gene expression as measured by ApoA1 mRNA (top panel) and ApoA1 antisense DA327409ext RNA (bottom panel) amounts detected. Figure R shows the levels of ApoA1 mRNA (orange bars) and ApoA1 antisense DA327409ext RNA (blue bars) in HepG2 cells treated with oligonucleotides designed against DA327409ext. Figure S
10 shows dose dependent upregulation of ApoA1 mRNA (bottom panel) and protein (top panel) in HepG2 cultures treated with oligonucleotides designed against DA327409ext. Figure T shows upregulation of ApoA1 mRNA in primary African green monkey hepatocytes after treatment with oligonucleotides designed against DA327409ext.

Example 4: Efficacy and Duration of Action Study of CUR-962 in the African Green Monkey

[00282] The objective of this study was to assess and compare the effect of antisense knockdown of the discordant
15 noncoding antisense sequences that regulate a Lipid transport and metabolism gene following intravenous administration in a nonhuman primate model. The antisense oligonucleotide test articles designed to inhibit the APOA1 regulatory sequences were designated as CUR-962.

CUR-962: +G*+C*T* A*G*T* C*T*G* +T*+T*+G (SEQ ID NO: 278)

CUR-963 (control): +G*+T*C* T*G*A* T*G*G* +A*+G*+A (SEQ ID NO: 279)

20 REGULATORY TEST GUIDELINES

[00283] This study was designed in accordance with accepted toxicological principles and to comply with International Conference of Harmonization (ICH) Harmonized Tripartite Guidelines (Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals ICH M3(m), 2000 November 9), and generally accepted procedures for the testing of therapeutic agents.

25 TEST AND CONTROL ARTICLES

Test Article Identity and Preparation

[00284] The test article, CUR-962, is a chemically stabilized antisense oligonucleotide. The vehicle for intravenous delivery is phosphate-buffered saline (PBS).

Vehicle characterization

30 [00285] For the PBS vehicle, the composition, batch number, expiry date and storage conditions (temperature and light/dark) was obtained from the supplier.

Test Article Storage and Handling

[00286] The test substance and vehicle were stored according to the received storage conditions supplied by the Sponsor and manufacturer, accordingly.

Analysis of the Test Article Formulations

[00287] Samples of the test article formulation will be cryopreserved for analysis of the concentration, stability and homogeneity of the test substance formulations.

TEST SYSTEM RATIONALE

[00288] The primate is a suitable non rodent species, acceptable to regulatory authorities as an indicator of potential hazards, and for which extensive background data are available. The African green monkey specifically is a highly clinically relevant model of multiple human physiologic and disease states.

[00289] The intravenous route of administration corresponds to a possible human therapeutic route. The dose of the test articles was based on the results of the dose finding studies of analogous compounds previously performed in the African green monkey.

[00290] African green monkey were chosen as the primate of choice as the test substances' target sequence are conserved across species with 100 % homology in primates. Additionally, the test substance is a synthetic oligonucleotide. Consequently, dosing in primates allows for a superior assessment of the efficacy of these compounds that would be more reflective of the uptake likely to be seen in humans than in any other species.

ANIMALS

[00291] *Species:* *Chlorocebus sabaeus*, non-human primate

[00292] *Breed:* African green monkey indigenous to St. Kitts.

[00293] *Source:* RxGen, Lower Bourryeau, St. Kitts, West Indies.

[00294] *Expected Age:* The test animals were adults.

[00295] *Expected Body Weight:* The monkeys weigh approximately 3-4 kg. The actual range may vary but will be documented in the data.

[00296] *Sex:* The test animals were adult females.

[00297] *Number of Animals:* Ten animals were screened to ensure identification of 8 animals appropriate for enrollment in the study.

[00298] *Number on Study:* Females: 8

Justification for Number on Study:

[00299] This study was designed to use the fewest number of animals possible, consistent with the primary objective of evaluating the therapeutic efficacy of the test article in the African green monkey and prior studies of the systemic administration of this type of oligonucleotide in this species.

Animal Specification:

[00300] Ten adult African Green monkeys in the weight range of 3 to 4 kg, were employed in the study. The monkeys were drug-naïve adult animals humanely trapped from the feral population that inhabits the island. Trapped monkeys were treated with antihelminthics to eliminate any possible intestinal parasite burden and were observed in quarantine for a minimum of 4 weeks prior to screening for study enrollment. The age of trapped monkeys were estimated by size and dentation, with the exclusion of older animals from the study. Prior to study enrollment, a clinical exam was performed on each monkey, including evaluation of locomotion and dexterity. Blood samples were taken and sent to Antech Diagnostics (Memphis, TN) for comprehensive clinical chemistries and a complete blood count and lipid profiles (see sections 9.2 and 319567928 for specifications). Monkeys with abnormal lab values, as determined by comparison to the established normal range for monkeys in the St. Kitts colony, were excluded from the study. In order to identify 8 monkeys that satisfy this criterion, 10 monkeys were screened, with the screening of additional animals as needed. Before study initiation, the selected monkeys will be transferred to individual cages to acclimate to individual housing for a one-week period. Only animals deemed suitable for experimentation will be enrolled in the study. The actual (or estimated) age and weight ranges at the start of the study will be detailed in the raw data and final report.

Animal Health and Welfare

[00301] The highest standards of animal welfare were followed and adhered to guidelines stipulated by the St. Kitts Department of Agriculture and the U.S. Department of Health and Human Services. All studies will be conducted in accordance with these requirements and all applicable codes of practice for the care and housing of laboratory animals. All applicable standards for veterinary care, operation, and review as contained in the NIH Guide for the Care and Use of Animals. The St. Kitts facility maintains an animal research committee that reviews the protocols and inspects the facilities as required by the Guide. The Foundation has an approved assurance filed with the Office of Laboratory Animal Welfare, as required by the Guide, #A4384-01 (Axion Research Foundation/St. Kitts Biomedical Foundation). There are no special nonhuman primate veterinary care issues and biohazard issues raised by the research specified in this study.

Housing and Environment

[00302] To allow detection of any treatment-related clinical signs, the animals were housed individually prior to surgery and postoperatively until sacrifice. The primate building in which the individual cages were situated were illuminated entirely by ambient light, which at 17 degrees north latitude approximates a 12hr:12hr light-dark cycle as recommended in the U.S. D.H.H.S guidelines. The RxGen primate building was completely ventilated to the outside. Additional air movement was assured by ceiling fans to maintain a constant target temperature of 23-35°C, as is typical of St. Kitts throughout the year. Twenty-four hour extremes of temperature and relative humidity (which also will not be controlled) were measured daily. During the study, the cages were cleaned at regular intervals.

Diet and Water

[00303] Each animal was offered approximately 90 grams per day of a standard monkey chow diet (TekLad, Madison, WI). The specific nutritional composition of the diet was recorded. The water was periodically analyzed for microbiological purity. The criteria for acceptable levels of contaminants in stock diet and water supply were within the analytical specifications established by the diet manufacturer and the periodic facility water evaluations, respectively. The water met all criteria necessary for certification as acceptable for human consumption.

EXPERIMENTAL DESIGN

Animal Identification and Randomization

[00304] Allocation was done by means of a stratified randomization procedure based on bodyweight and plasma cholesterol profiles. Prior to and after allocation to a group, each animal was identified by a tattoo on the abdomen. Tattoos are placed on all colony animals as a means of identification in the course of routine health inspections. A cage plan was drawn up to identify the individuals housed within, and individual monkeys were further identified by a labeled tag attached to their respective cage.

Group sizes, doses and identification numbers

[00305] The animals were assigned to 2 treatment groups, comprised of 4 monkeys in each group. Specific animal identification numbers were provided to each monkey according to the facility numbering system. This system uniquely identifies each monkey by a letter followed by a three-digit number, e.g. Y032.

Route and Frequency of Administration

[00306] Animals were dosed once daily on Days 1, 3, and 5 delivered intravenously by manual infusion over ~10 min. The infusion rate will be 24 mL/kg/h. The animals were sedated with ketamine and xylazine prior to and during the dosing procedure. A venous catheter (Terumo mini vein infusion set, 20 gauge needle, or similar appropriate infusion set) was inserted into the saphenous vein. Dosing took place in each monkey between 8:00 and 10:00 a.m. shortly after the animals wake and prior to feeding. A blood sample to assess plasma cholesterol and other lipid levels as described in Blood Chemistry section below, was collected just prior to each infusion. Blood collection preceded feeding at both sampling intervals to minimize dietary effects on cholesterol measurements.

Clinical Observations

[00307] All visible signs of reaction to treatment were recorded on each day of dosing. In addition, the animals were examined at least once each week for physical attributes such as appearance and general condition.

Body Weights

[00308] Body weights were recorded at weekly intervals during the treatment and post-treatment periods.

Food Consumption

[00309] Individual food consumption was not be quantified. Feeding patterns, however, were monitored and a note made of any major changes.

Mortality and Morbidity

[00310] Mortality and morbidity will be recorded. Any decision regarding premature sacrifice will be made after consultation with the Study Director and with the Sponsor's Monitoring Scientist, if possible. Animals that are found dead or killed prematurely will be subjected to necropsy with collection of liver, kidney, heart and spleen lung tissues for histopathology. In the event of premature sacrifice a blood sample will also be taken (if possible) and the parameters determined. Animals that are found dead after regular working hours will be refrigerated overnight and necropsies performed at the start of the next working day. If the condition of an animal requires premature sacrifice, it will be euthanized by intravenous overdose of sodium pentobarbital. All research is governed by the Principles for Use of Animals. RxGen is required by law to comply with the U.S. Department of Health and Human Services standards for primate facility, which dictates the levels of severity that the procedures within this study, specified as mild, must abide.

CLINICAL LABORATORY STUDIES

Blood Samples

[00311] Three blood samples were obtained from all animals prior to treatment, to establish a plasma cholesterol baseline. Blood samples were collected post treatment and were taken via superficial venipuncture. The volume collected at any one sampling time point was not to exceed 8 ml, which represents approximately 4% total blood volume of an adult monkey.

[00312] Animals had blood drawn at two baseline time points and on study days 1, 3, 5, 7, 9, 11, 13 and 15, with continued ~weekly collection thereafter until total plasma cholesterol normalizes in group 1 (APOA1), if a perturbation is appreciated. Eight milliliters of blood were collected on days 1, 6 and 11 to allow for assessment of clinical chemistries, lipid profiles and coagulation profiles. On all other days only 5 mls of blood were collected, sufficient for clinical chemistries and lipid profiles.

[00313] Blood samples were split into three parts on days on which both chemistry and hematology measures will be made. One sample was collected into plasma collection tubes containing 25µl of heparin and labeled with the study number, dose level, day number, date, unique animal identification number. Following separation 1ml of plasma was removed to a sterile cryotube carrying the above details and stored appropriately until shipment, for blood chemistry analysis. One aliquot of the plasma (0.5 ml) was removed to a sterile cryotube labeled with the details described above and stored appropriately until shipment for plasma cholesterol distribution and Lipid profile analysis. An additional 1ml and 0.5ml aliquot of plasma was flash frozen and stored in liquid nitrogen to serve as back-up samples for potential additional analyses.

[00314] Two additional whole blood sample aliquots (2.5ml each) were treated with acid citrate dextrose (ACD) anticoagulant and labeled, and stored at 4°C until shipped for coagulation and CBC measures detailed below.

[00315] The samples were shipped to arrive within 24 h of sampling, or stored under stable conditions for shipment at a time determined appropriate.

[00316] Repeat samples were taken only if the method of sampling or the method of assay was thought to be outside normal quality limits. Samples were taken into labeled tubes.

Hematology

[00317] A complete blood count (CBC), Prothrombin Time, PTT, Fibrinogen and D-Dimer were measured on all samples collected on days 1, 6 and 11 (and on additional days if perturbations are detected at any of these time points). Blood counts were assessed on 1 ml of whole blood collected in vacutainers containing EDTA. Coagulation profile determinations were performed on approximately 2.0 mL blood collected in vacutainers containing acid citrate dextrose (ACD) anticoagulant.

Blood Chemistry

[00318] Glucose, Blood Urea Nitrogen, Creatinine, Total protein, Albumin, Total bilirubin, Alkaline Phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Cholesterol, Calcium, Phosphorus, Sodium, Potassium, Chloride, A/G ratio, BUN/Creatine (calculated) Globulins (calculated), Lipase, Amylase, Triglycerides, CPK, Lactate dehydrogenase, Gamma glutamyl transferase (GGT), Magnesium, Total Cholesterol LDL, VLDL, HDL, ApoA1, ApoA2, ApoB, ApoE, ApoLp(a). Superchemistries and LDL and HDL measures were made on every plasma sample. ApoA1 measures were made on select samples after assessment of the LDL and HDL data.

[00319] Determinations were performed on approximately 1.0 mL plasma for the superchemistry and 0.5ml plasma for the cholesterol distribution and Lipid transport and metabolism gene measures. An additional aliquot of plasma was collected and stored for possible future analyses.

Liver Biopsies

[00320] A percutaneous liver biopsy was performed on all monkeys at baseline and on days 7 and 17. A 14 gauge biopsy needle (INRAD) will be employed to obtain 2 core biopsies (~1.0 cm in length) from both the right and left lobe of the liver. Successful biopsy was confirmed by visual inspection of the biopsy sample on the biopsy needle prior to subdividing as indicated below.

[00321] The samples were pooled and then split in the following manner. Half of one biopsy (~0.5 cm) from the left lobe was immersed in paraformaldehyde for sectioning for histopathology and *in situ* analysis. The remaining half of each of the divided biopsies, as well the other two intact biopsies were immediately immersed in a labeled cryotube containing 2 mls of RNAlater (Qiagen) and incubated at 4°C overnight, following which the RNAlater was aspirated and the sample tube flash frozen in liquid nitrogen. Following transportation in liquid nitrogen total RNA was isolated employing the Trizol or TriReagent method, with an expected yield of ~40 µg per 1.0 cm 14 g core biopsy (~80-100 µg total for the pooled RNA derived from all 4 pooled core biopsies from a single monkey, absent the component saved for histopathology and *in situ*). 5µg of the RNA fraction were used for target-specific real-time qPCR (TaqMan miRNA assay, ABI). The remaining RNA fraction was reserved for possible genome wide expression analysis.

[00322] The fixed tissue was processed for paraffin embedding. Sections were stained for H&E and histopathological findings reported under Gross Histological findings. All slides generated in this work carried a label with the study number, dose level, day number, date, unique animal identification number.

STATISTICAL ANALYSIS

5 *Statistics*

[00323] Descriptive statistics on hematology, clinical chemistries and lipid profiles were performed. Appropriate bioinformatic analyses was conducted on expression data.

Sample Size

10 [00324] Sample size determinations were made on the basis of prior experiments administering modified anti-sense oligonucleotides to African green monkeys and resulting clinical chemistry and lipid profile changes and associated variability. The total number of subjects for efficacy evaluation were twenty enrolled animals, with four animals per treatment group, and four additional screened animals.

Results:

15 [00325] The results are shown in the following figures. Figure 1U: ApoA1 mRNA (top panels) and protein (bottom panels) levels increased in monkey liver biopsies after treatment with CUR-962, an oligonucleotide designed to ApoA1 antisense DA327409ext, compared to the baseline levels, as determined by real time PCR and ELISA respectively (two left panels). ApoA1 mRNA and protein levels did not change after the same period of time in the control group dosed with an oligonucleotide that showed no effect on ApoA1 levels in vitro (CUR-963, two right panels).

20 [00326] Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations and modifications will occur to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the other implementations as may be desired and advantageous for any given or particular application.

25 [00327] The Abstract of the disclosure will allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the following claims.

CLAIMS

What is claimed is:

1. A method of modulating a function of and/or the expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 1299 of SEQ ID NO: 8, 1 to 918 of SEQ ID NO: 9, 1 to 1550 of SEQ ID NO: 10, 1 to 329 of SEQ ID NO: 11, 1 to 1826 of SEQ ID NO: 12, 1 to 536 of SEQ ID NO: 13, 1 to 551 of SEQ ID NO: 14, 1 to 672 of SEQ ID NO: 15, 1 to 616 of SEQ ID NO: 16, 1 to 471 of SEQ ID NO: 17, 1 to 707 of SEQ ID NO: 18, 1 to 741 of SEQ ID NO: 19, 1 to 346 of SEQ ID NO: 20, 1 to 867 of SEQ ID NO: 21, 1 to 563 of SEQ ID NO: 22 (Figure 3); thereby modulating a function of and/or the expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.
2. A method of modulating a function of and/or the expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a natural antisense of a Lipid transport and metabolism gene polynucleotide; thereby modulating a function of and/or the expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.
3. A method of modulating a function of and/or the expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to an antisense oligonucleotide to the Lipid transport and metabolism gene polynucleotide; thereby modulating a function of and/or the expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.
4. A method of modulating a function of and/or the expression of a Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one antisense oligonucleotide that targets a region of a natural antisense oligonucleotide of the Lipid transport and metabolism gene polynucleotide; thereby modulating a function of and/or the expression of the Lipid transport and metabolism gene polynucleotide in patient cells or tissues *in vivo* or *in vitro*.
5. The method of claim 4, wherein a function of and/or the expression of the Lipid transport and metabolism gene is increased *in vivo* or *in vitro* with respect to a control.
6. The method of claim 4, wherein the at least one antisense oligonucleotide targets a natural antisense sequence of a Lipid transport and metabolism gene polynucleotide.

7. The method of claim 4, wherein the at least one antisense oligonucleotide targets a nucleic acid sequence comprising coding and/or non-coding nucleic acid sequences of a Lipid transport and metabolism gene polynucleotide.
8. The method of claim 4, wherein the at least one antisense oligonucleotide targets overlapping and/or non-overlapping sequences of a Lipid transport and metabolism gene polynucleotide.
9. The method of claim 4, wherein the at least one antisense oligonucleotide comprises one or more modifications selected from: at least one modified sugar moiety, at least one modified internucleoside linkage, at least one modified nucleotide, and combinations thereof.
10. The method of claim 9, wherein the one or more modifications comprise at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and combinations thereof.
11. The method of claim 9, wherein the one or more modifications comprise at least one modified internucleoside linkage selected from: a phosphorothioate, 2'-O-methoxyethyl (MOE), 2'-fluoro, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.
12. The method of claim 9, wherein the one or more modifications comprise at least one modified nucleotide selected from: a peptide nucleic acid (PNA), a locked nucleic acid (LNA), an arabino-nucleic acid (FANA), an analogue, a derivative, and combinations thereof.
13. The method of claim 1, wherein the at least one oligonucleotide comprises at least one oligonucleotide sequences set forth as SEQ ID NOS: 23 to 263.
14. A method of modulating a function of and/or the expression of a Lipid transport and metabolism gene in mammalian cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one short interfering RNA (siRNA) oligonucleotide 5 to 30 nucleotides in length, said at least one siRNA oligonucleotide being specific for an antisense polynucleotide of a Lipid transport and metabolism gene polynucleotide, wherein said at least one siRNA oligonucleotide has at least 50% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense nucleic acid molecule of the Lipid transport and metabolism gene polynucleotide; and, modulating a function of and/or the expression of a Lipid transport and metabolism gene in mammalian cells or tissues *in vivo* or *in vitro*.
15. The method of claim 14, wherein said oligonucleotide has at least 80% sequence identity to a sequence of at least about five consecutive nucleic acids that is complementary to the antisense and/or sense nucleic acid molecule of the Lipid transport and metabolism gene polynucleotide.
16. A method of modulating a function of and/or the expression of a Lipid transport and metabolism gene in mammalian cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one antisense

oligonucleotide of about 5 to 30 nucleotides in length specific for noncoding and/or coding sequences of a sense and/or natural antisense strand of a Lipid transport and metabolism gene polynucleotide wherein said at least one antisense oligonucleotide has at least 50% sequence identity to at least one nucleic acid sequence set forth as SEQ ID NOS: 1 to 7 and 8 to 22; and, modulating the function and/or expression of the Lipid transport and metabolism gene in mammalian cells or tissues *in vivo* or *in vitro*.

17. A synthetic, modified oligonucleotide comprising at least one modification wherein the at least one modification is selected from: at least one modified sugar moiety; at least one modified internucleotide linkage; at least one modified nucleotide, and combinations thereof; wherein said oligonucleotide is an antisense compound which hybridizes to and modulates the function and/or expression of a Lipid transport and metabolism gene *in vivo* or *in vitro* as compared to a normal control.

18. The oligonucleotide of claim 17, wherein the at least one modification comprises an internucleotide linkage selected from the group consisting of: phosphorothioate, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.

19. The oligonucleotide of claim 17, wherein said oligonucleotide comprises at least one phosphorothioate internucleotide linkage.

20. The oligonucleotide of claim 17, wherein said oligonucleotide comprises a backbone of phosphorothioate internucleotide linkages.

21. The oligonucleotide of claim 17, wherein the oligonucleotide comprises at least one modified nucleotide, said modified nucleotide selected from: a peptide nucleic acid, a locked nucleic acid (LNA), analogue, derivative, and a combination thereof.

22. The oligonucleotide of claim 17, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified nucleotides selected from: phosphorothioate, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and a combination thereof.

23. The oligonucleotide of claim 17, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified nucleotides selected from: peptide nucleic acids, locked nucleic acids (LNA), analogues, derivatives, and a combination thereof.

24. The oligonucleotide of claim 17, wherein the oligonucleotide comprises at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and a combination thereof.

25. The oligonucleotide of claim 17, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified sugar moieties selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-

methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and a combination thereof.

26. The oligonucleotide of claim 17, wherein the oligonucleotide is of at least about 5 to 30 nucleotides in length and hybridizes to an antisense and/or sense strand of a Lipid transport and metabolism gene polynucleotide wherein said oligonucleotide has at least about 20% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense coding and/or noncoding nucleic acid sequences of the Lipid transport and metabolism gene polynucleotide.
27. The oligonucleotide of claim 17, wherein the oligonucleotide has at least about 80% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense coding and/or noncoding nucleic acid sequence of the Lipid transport and metabolism gene polynucleotide.
28. The oligonucleotide of claim 17, wherein said oligonucleotide hybridizes to and modulates expression and/or function of at least one Lipid transport and metabolism gene polynucleotide *in vivo* or *in vitro*, as compared to a normal control.
29. The oligonucleotide of claim 17, wherein the oligonucleotide comprises the sequences set forth as SEQ ID NOS: 23 to 263.
30. A composition comprising one or more oligonucleotides specific for one or more Lipid transport and metabolism gene polynucleotides, said polynucleotides comprising antisense sequences, complementary sequences, alleles, homologs, isoforms, variants, derivatives, mutants, fragments, or combinations thereof.
31. The composition of claim 30, wherein the oligonucleotides have at least about 40% sequence identity as compared to any one of the nucleotide sequences set forth as SEQ ID NOS: 23 to 263.
32. The composition of claim 30, wherein the oligonucleotides comprise nucleotide sequences set forth as SEQ ID NOS: 23 to 263.
33. The composition of claim 32, wherein the oligonucleotides set forth as SEQ ID NOS: 23 to 263 comprise one or more modifications or substitutions.
34. The composition of claim 33, wherein the one or more modifications are selected from: phosphorothioate, methylphosphonate, peptide nucleic acid, locked nucleic acid (LNA) molecules, and combinations thereof.
35. A method of preventing or treating a disease associated with at least one Lipid transport and metabolism gene polynucleotide and/or at least one encoded product thereof, comprising: administering to a patient a therapeutically effective dose of at least one antisense oligonucleotide that binds to a natural antisense sequence of said at least one Lipid transport and metabolism gene polynucleotide and modulates expression of said at least one Lipid transport and metabolism gene polynucleotide; thereby preventing or treating the disease associated with the at least one Lipid transport and metabolism gene polynucleotide and/or at least one encoded product thereof.

36. The method of claim 35, wherein a disease associated with the at least one Lipid transport and metabolism gene polynucleotide is selected from: a cardiovascular disease or disorder, a metabolic disease or disorder (e.g., diabetes, obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, hypercholesterolemia etc.), a disease or disorder associated with impaired lipid metabolism, a coronary artery disease, atherosclerosis, an HDL metabolism disease or disorder (e.g., familial HDL deficiency (FHD), Sea-blue histiocytosis, Tangier's Disease, Fish-eye disease, LCAT deficiency, low-HDL cholesterolemia etc.), a disease or disorder associated with cellular cholesterol and/or phospholipid homeostasis, Familial amyloid nephropathy, a disease or disorder associated with impaired cholesterol regulation, a disease or disorder associated with a deficiency of the Lipid transport and metabolism gene transporter, Apolipoprotein A-I deficiency, a disease or disorder associated with abnormally fast or abnormally slow rate of cholesterol efflux in a cell, a disease or disorder associated with pancreatic beta cell function, diabetes, a metabolic disease or disorder, arthritis, inflammation, an autoimmune disease or disorder, acquired immune deficiency syndrome (AIDS), inflammation, a neurological disease or disorder, a neurodegenerative disease or disorder, cancer, dyslipidemia, metabolic syndrome, a senile plaque, cerebral amyloid angiopathy, Amyloidosis, glioblastoma, a disease or disorder associated with amyloid deposition, neurofibrillary tangles, choriocarcinoma, astrocytoma, amyloidosis, hyperlipidemia, neoplastic transformation, atherosclerotic plaque, obstruction, metastasis, pulmonary fibrosis, necrosis, shock, melanoma, genetic susceptibility, psoriasis, glioma, neuropathology, a vascular disease, cell damage, Nonsmall cell lung carcinomas (NSCLCs), liposarcoma, an immunodeficiency disease or disorder, an allergy, an organ transplant rejection, glomerulonephritis, venous thrombosis, pathological processes or leukemia, a skeletal disease or disorder, a muscular disease or disorder, a disease or disorder associated with infectious organisms, an immune related disease or disorder, nerve repair and paralysis, neuroendocrine differentiation, systemic non-neuropathic amyloidosis, an amyloid disease, tumor growth dependent on angiogenesis, non-cancerous diseases with symptoms include an increase in angiogenesis, e.g., psoriasis, retinopathy of prematurity, a Choroid disease, neovascular glaucoma, diabetic retinopathy, substance abuse, impaired cognitive function, and reduced neurite outgrowth, ApoE abnormal expression, function, activity as compared to a normal control, psoriasis, a disease or disorder caused by foreign organisms such as viral, bacterial, parasitic, fungal, and the like.
37. A method of identifying and selecting at least one oligonucleotide for in vivo administration comprising: selecting a target polynucleotide associated with a disease state; identifying at least one oligonucleotide comprising at least five consecutive nucleotides which are complementary to the selected target polynucleotide or to a polynucleotide that is antisense to the selected target polynucleotide; measuring the thermal melting point of a hybrid of an antisense oligonucleotide and the target polynucleotide or the polynucleotide that is antisense to the selected target polynucleotide under stringent hybridization conditions; and selecting at least one oligonucleotide for in vivo administration based on the information obtained.

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FIGURE 1A

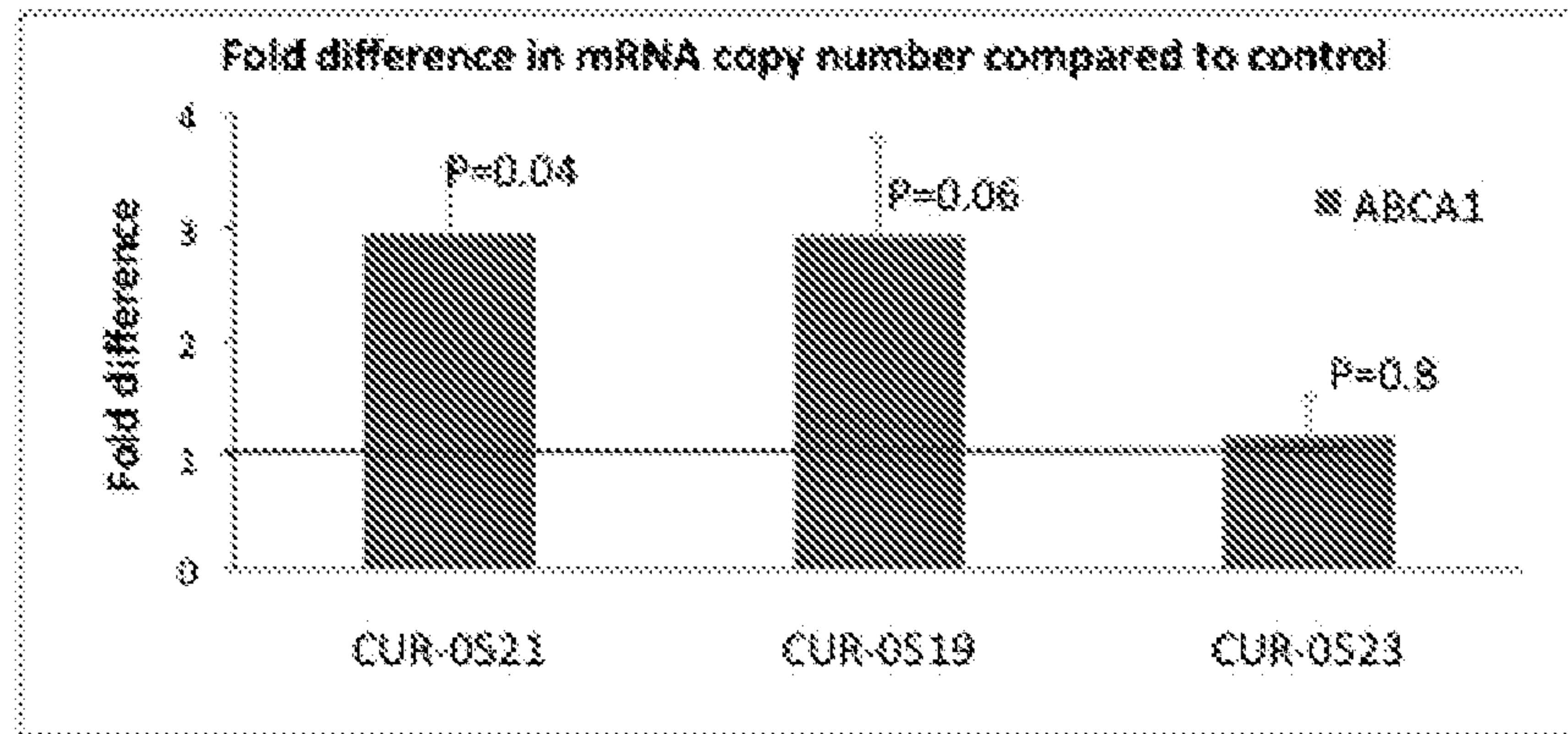
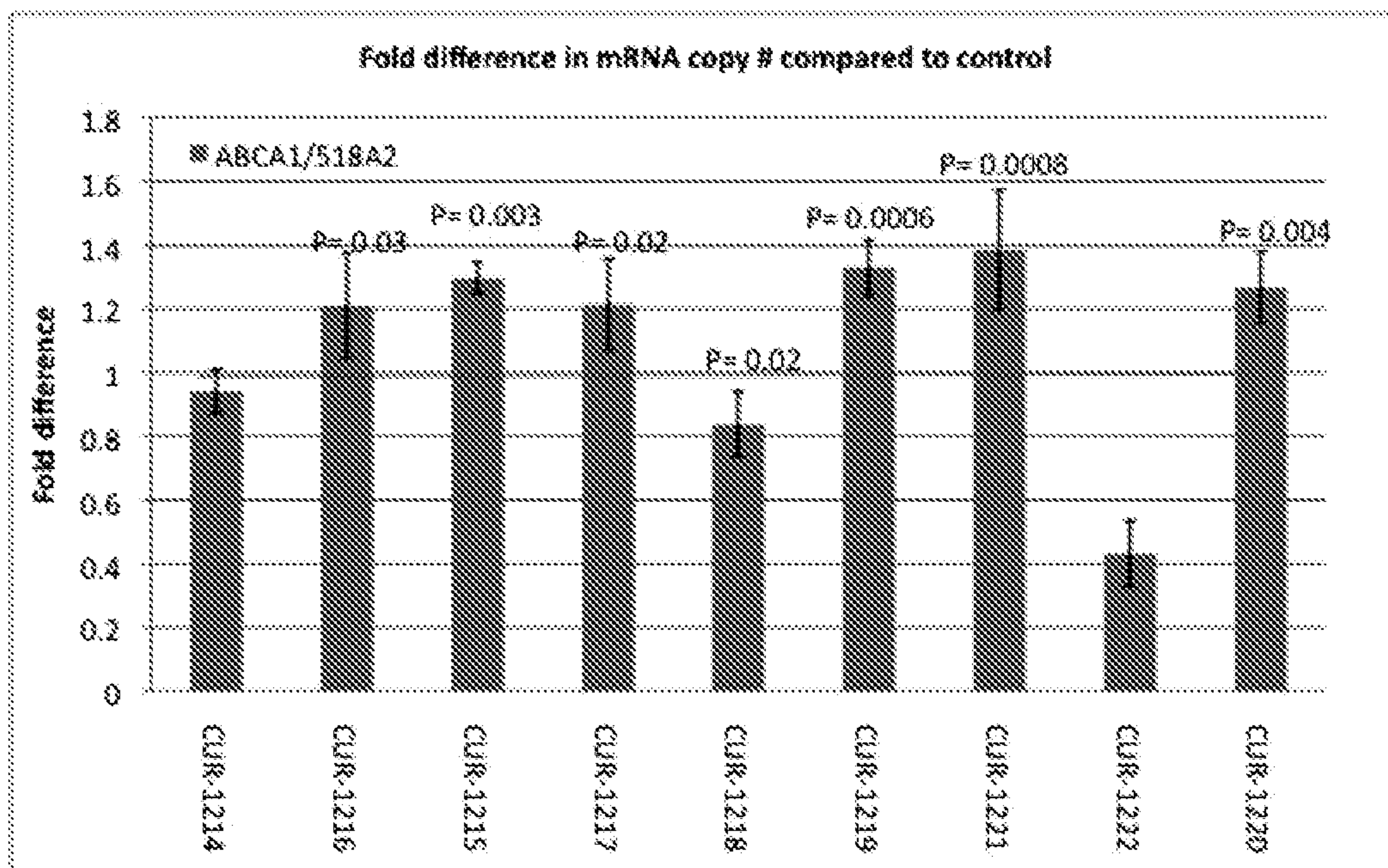


FIGURE 1B



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FIGURE 1C

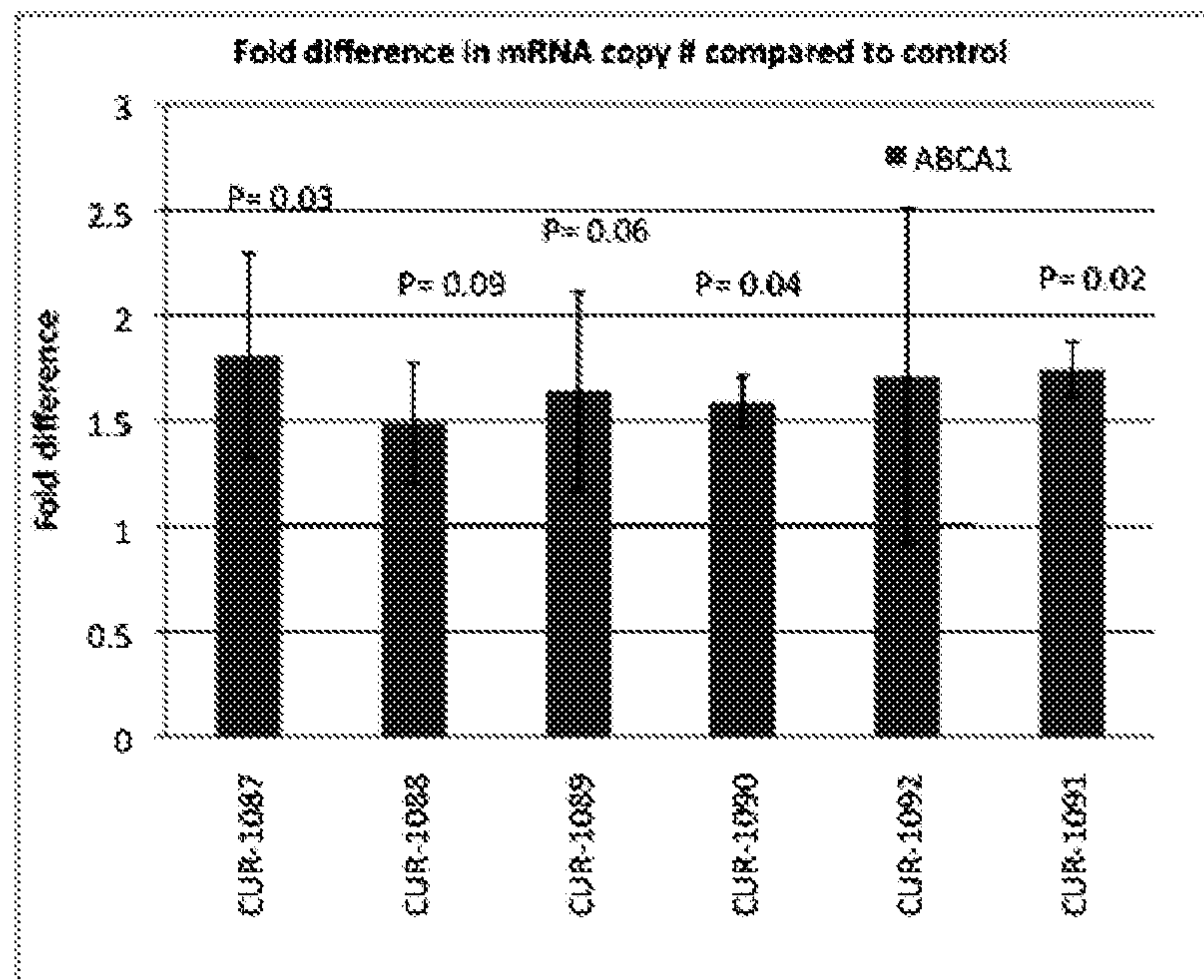
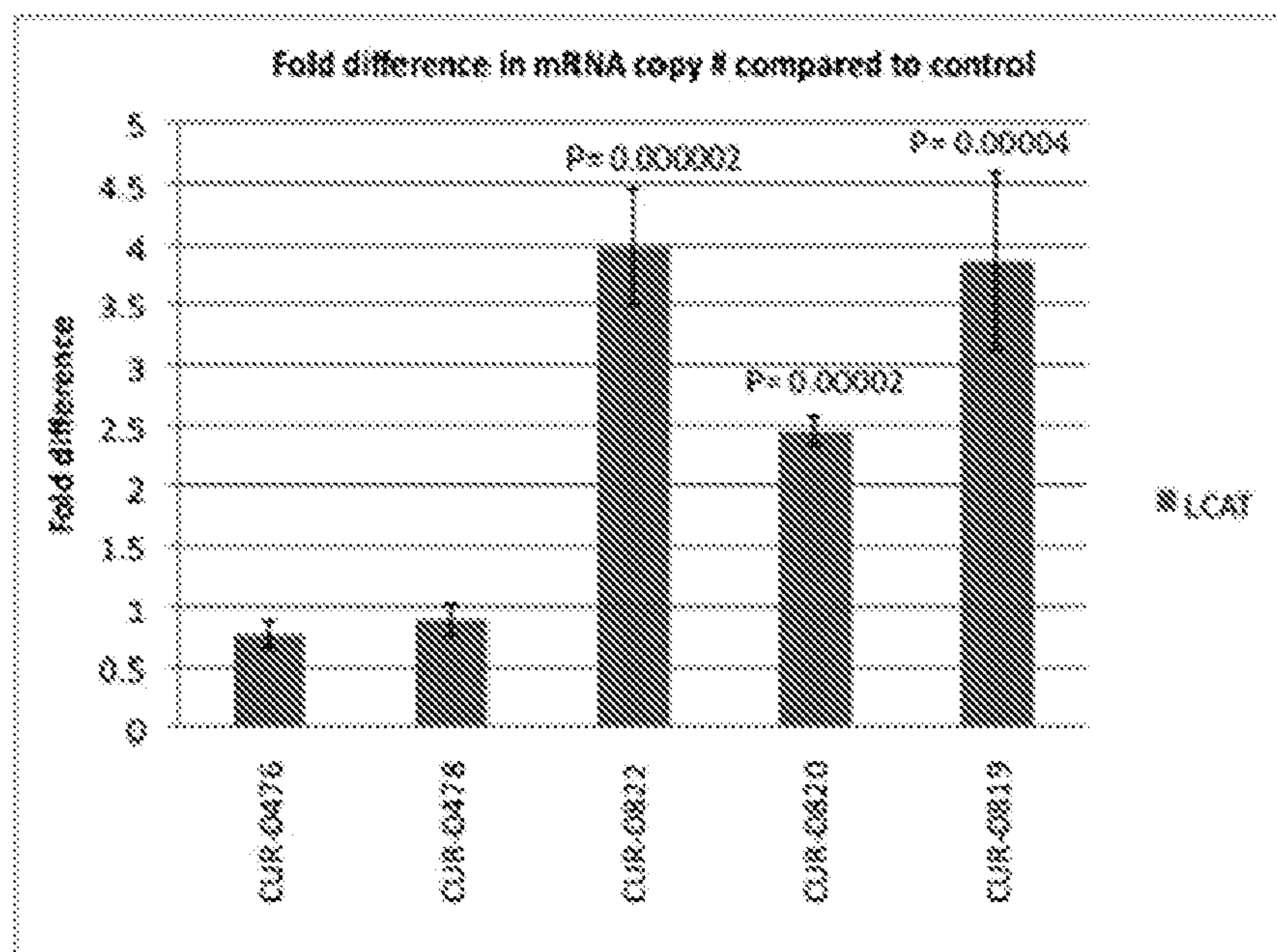


FIGURE 1D



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FIGURE 1E

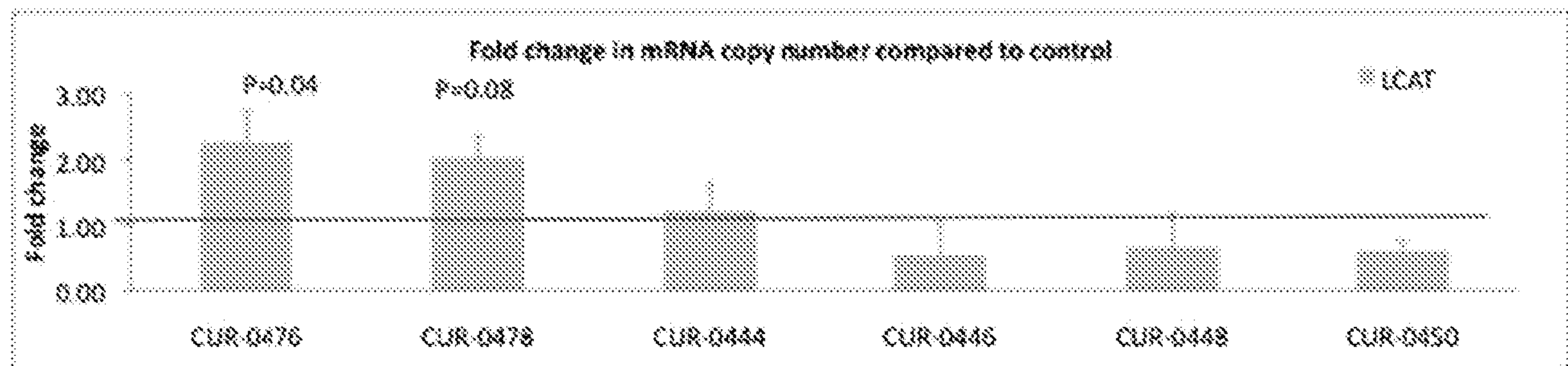


FIGURE 1F

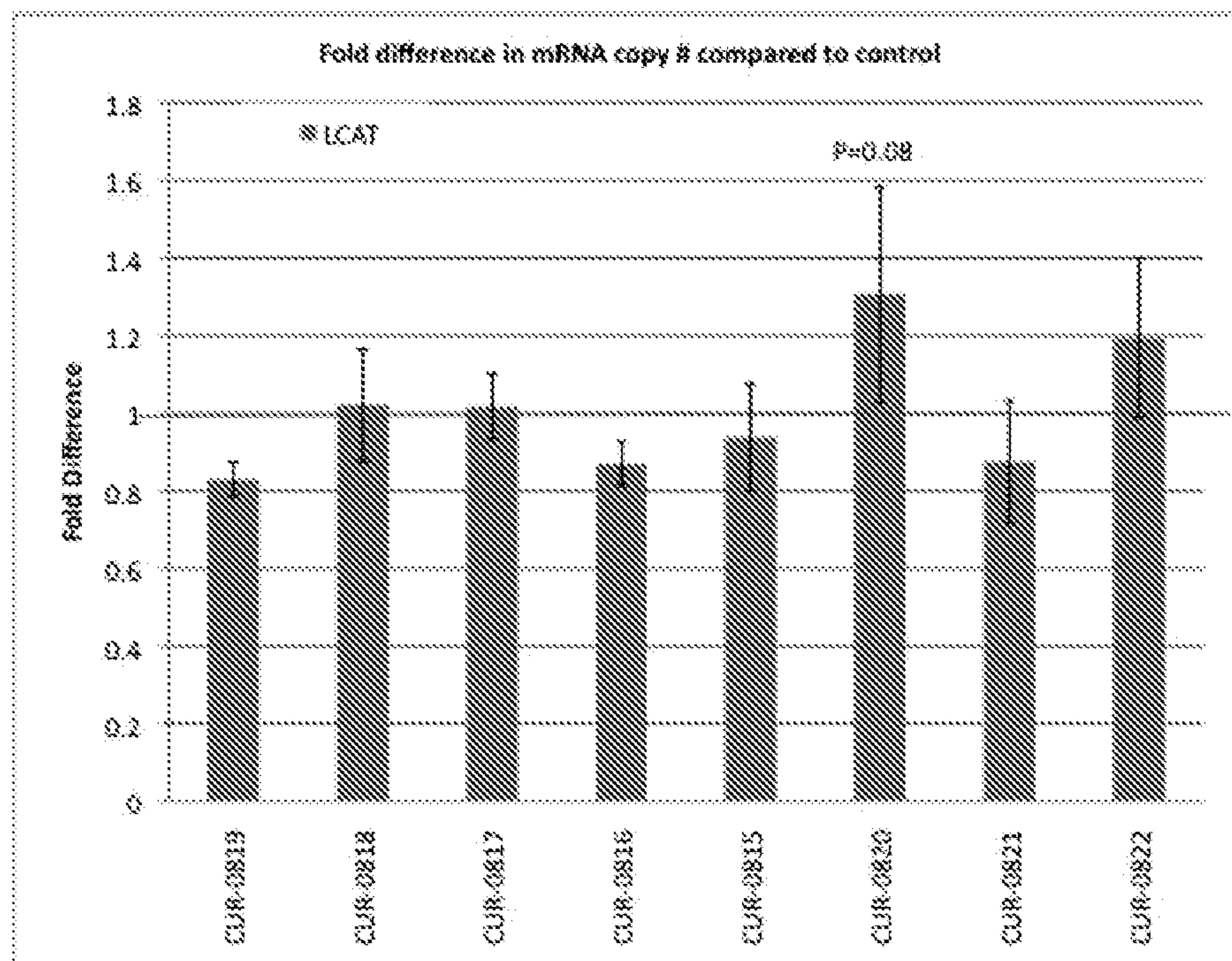


FIGURE 1G

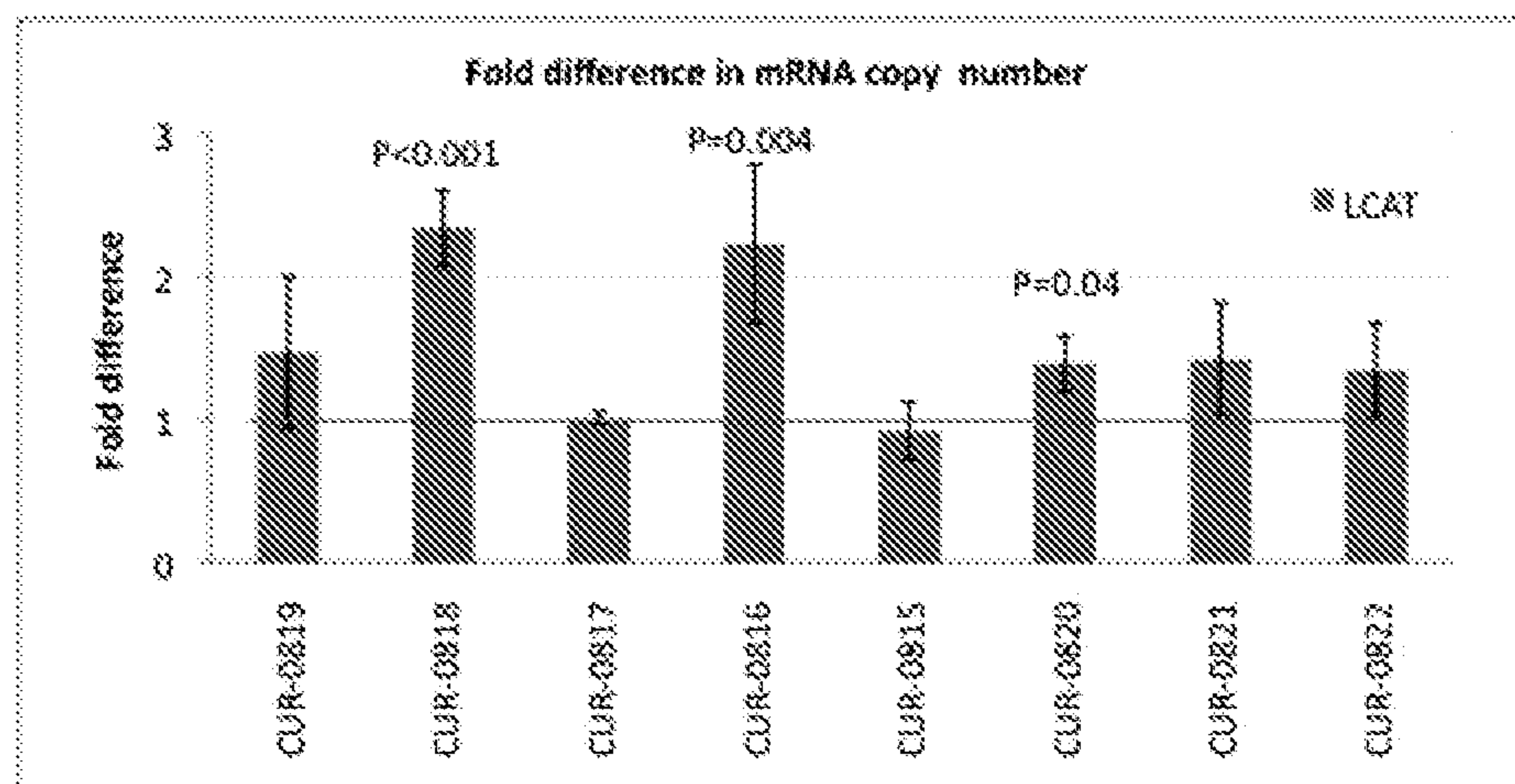


FIGURE 1H

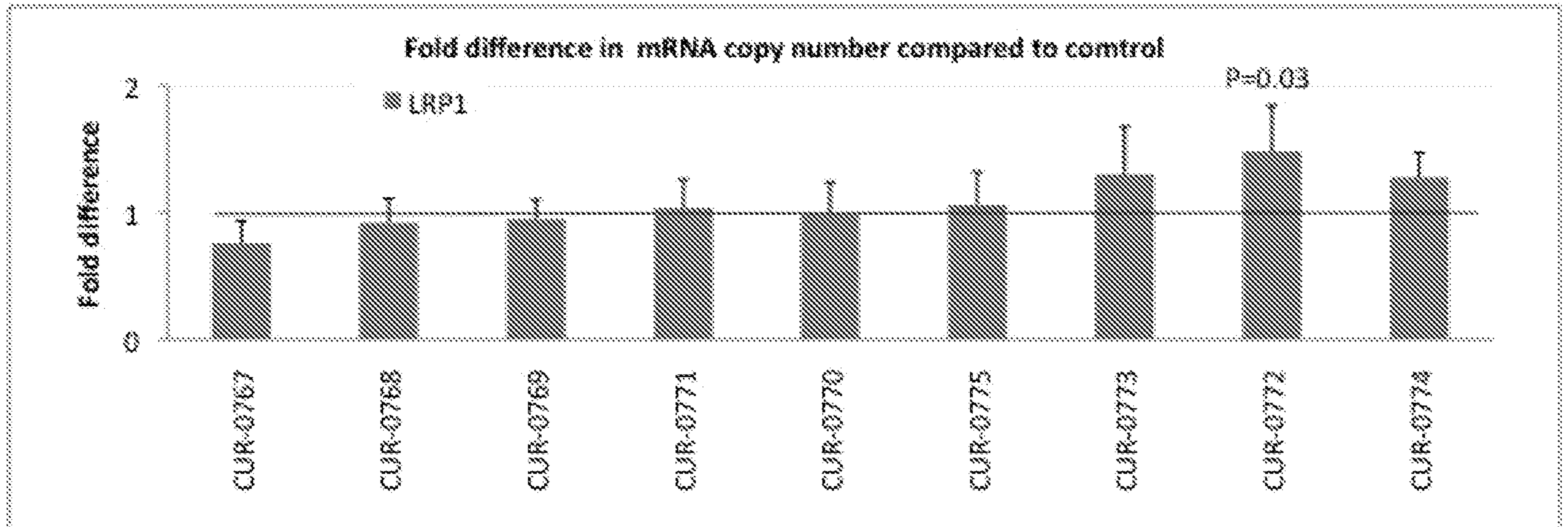
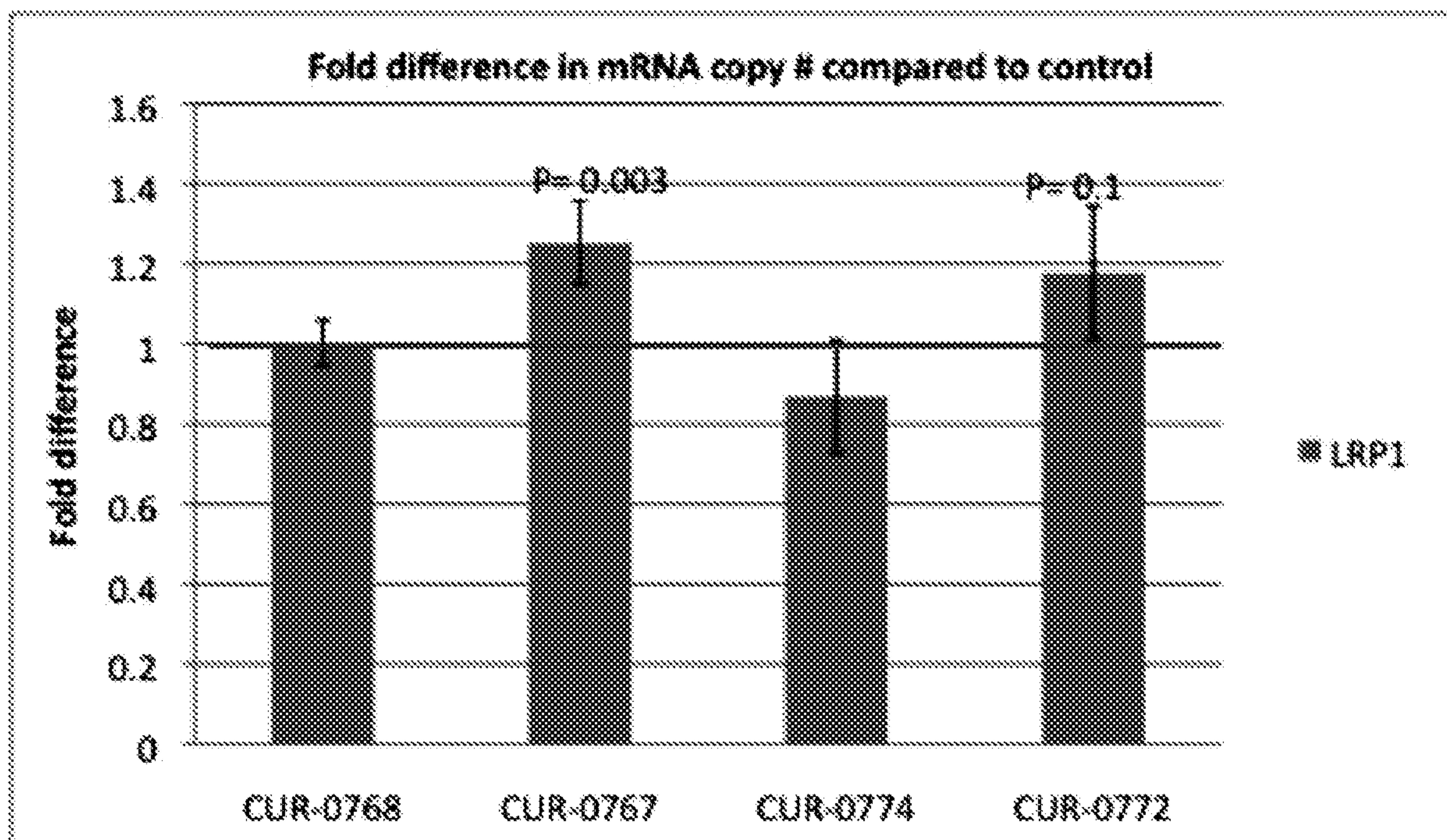


FIGURE 1I



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FIGURE 1J

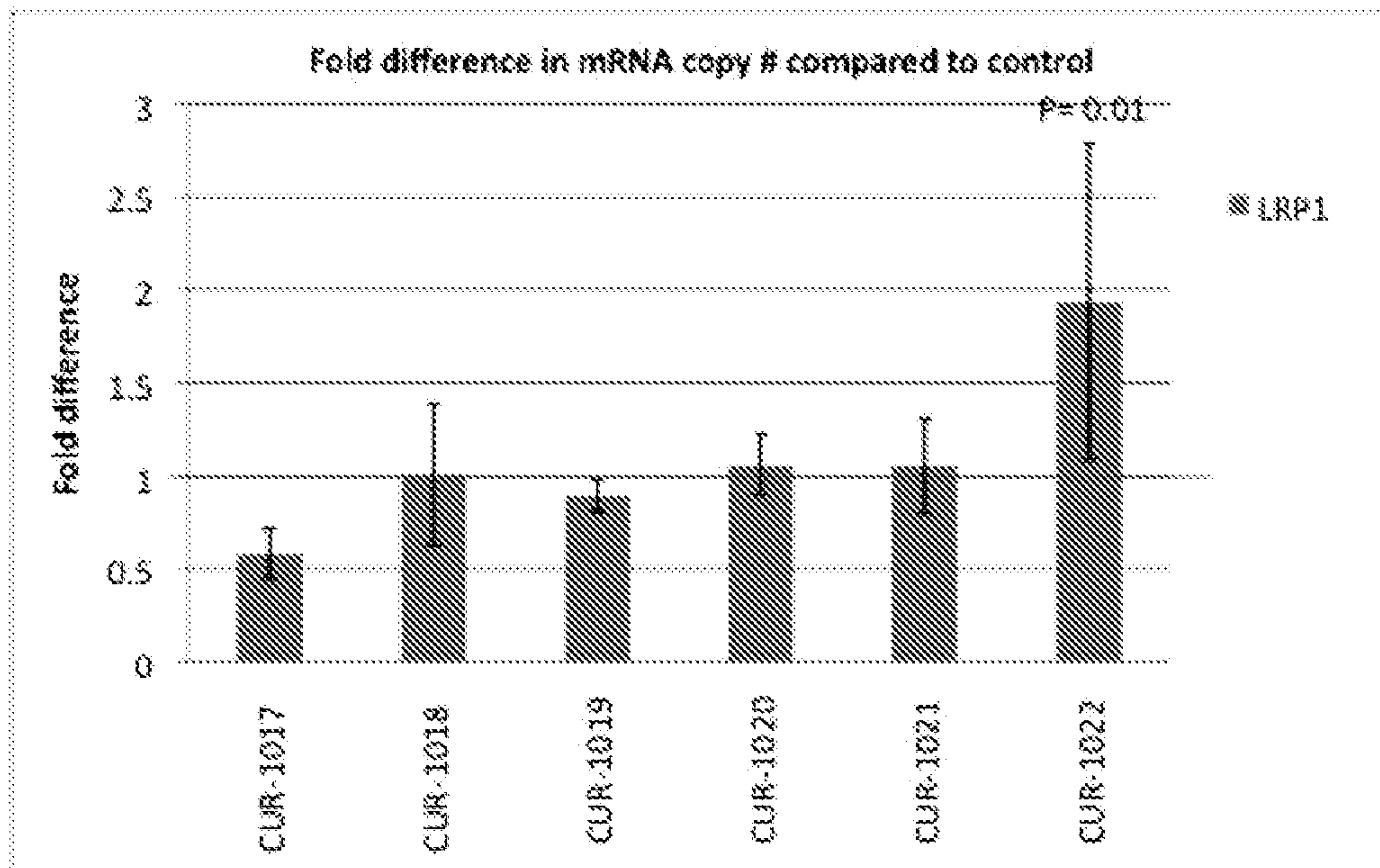
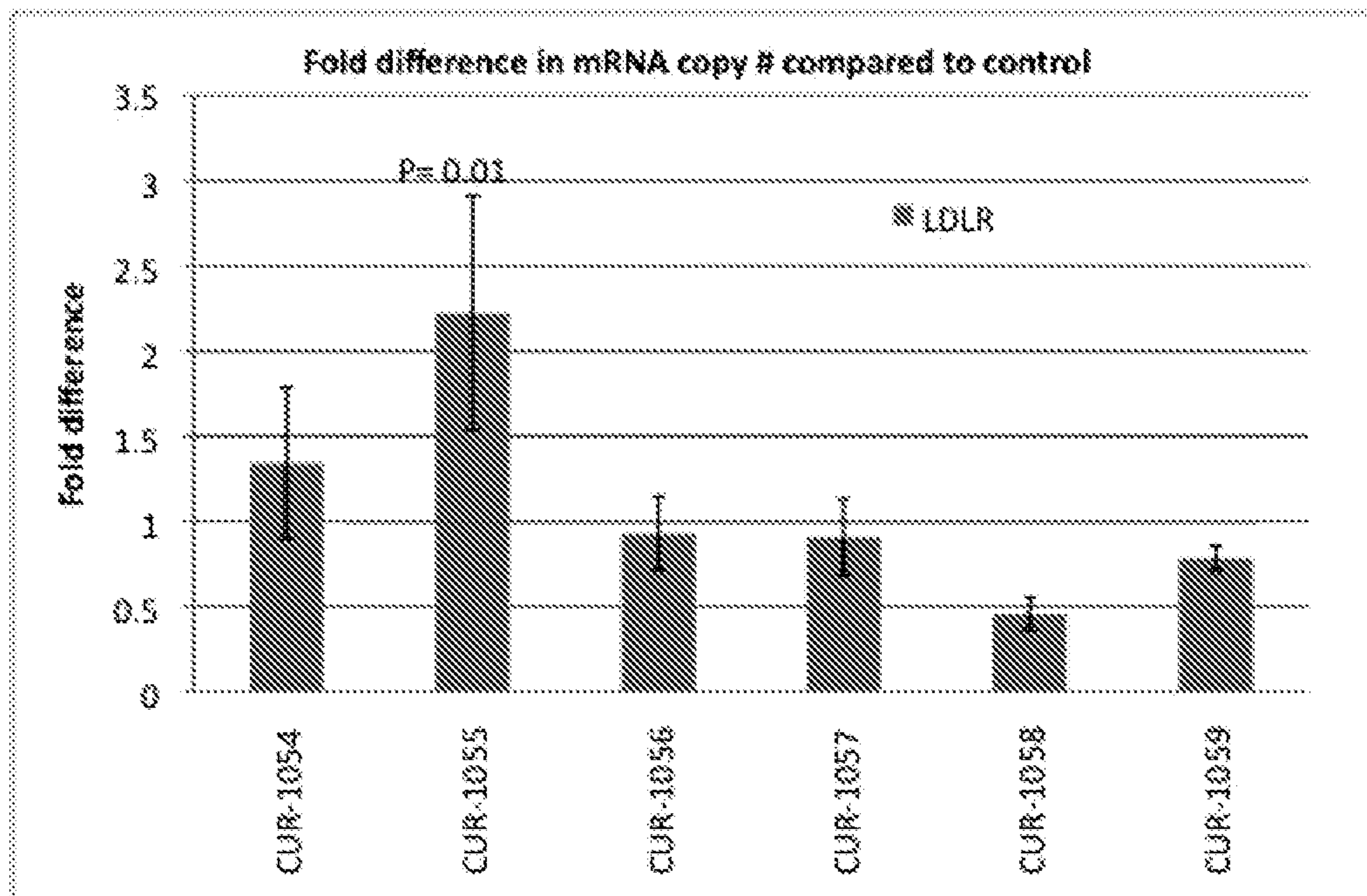


FIGURE 1K



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FIGURE 1L

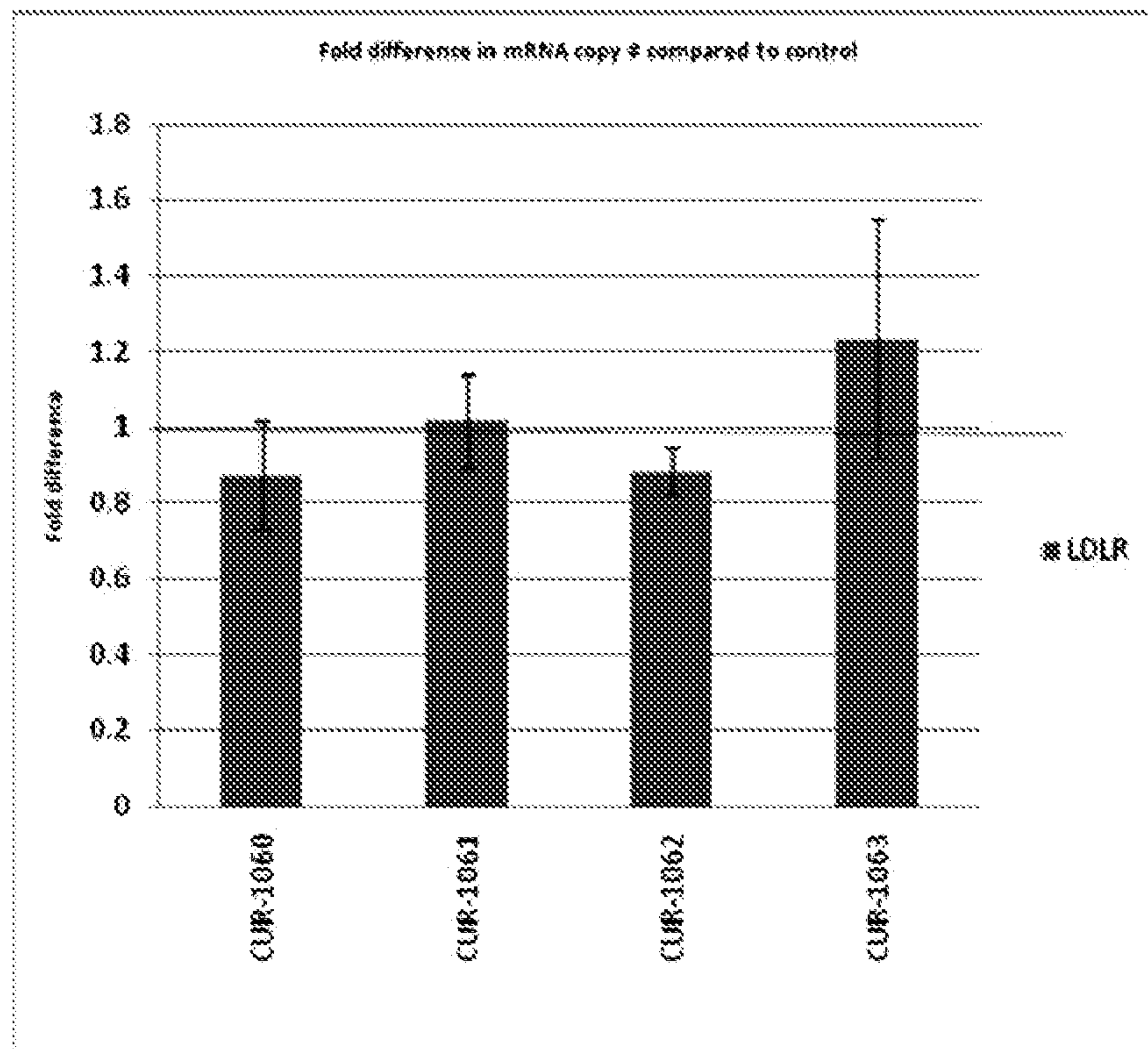
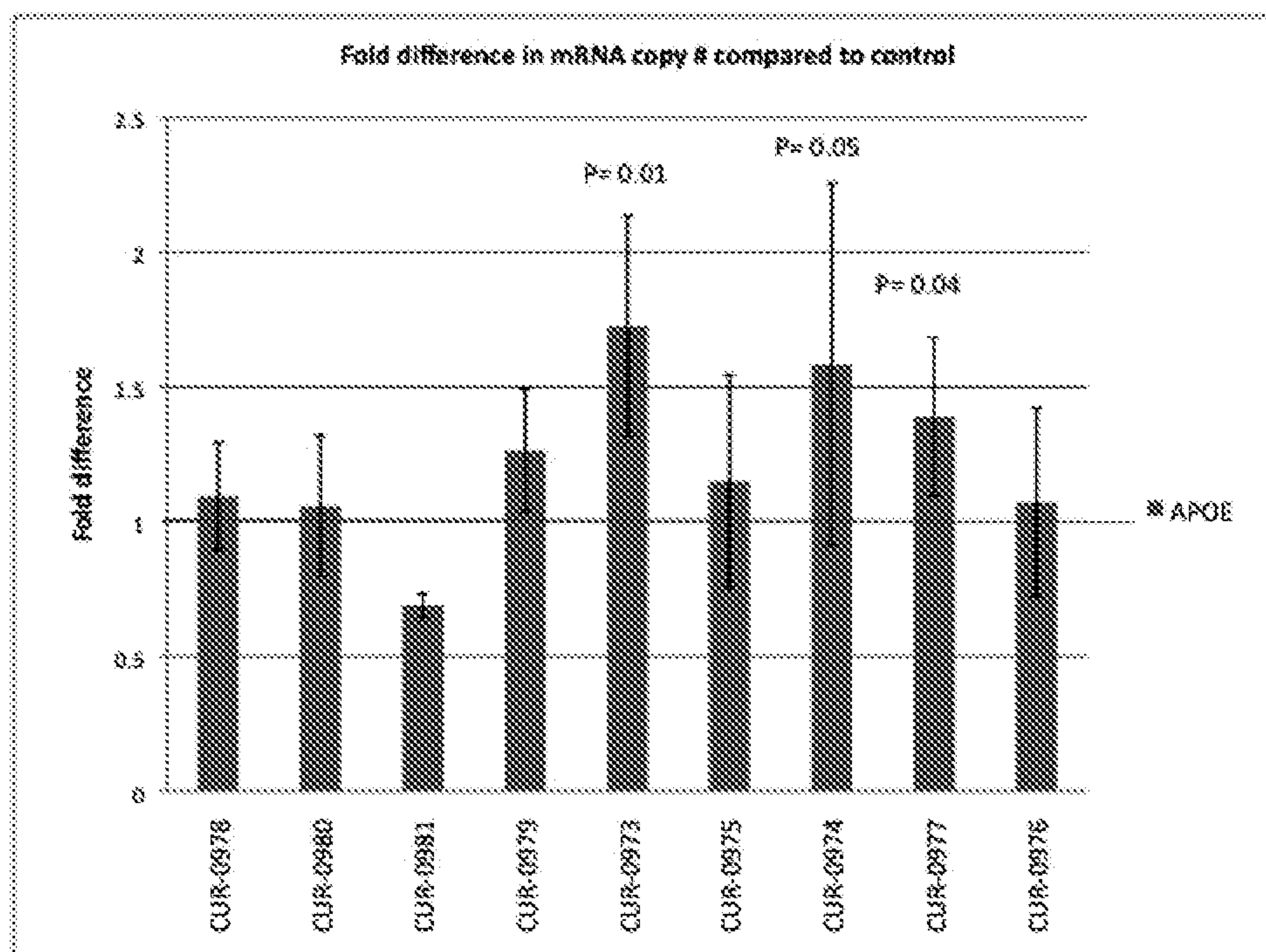


FIGURE 1M



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FIGURE 1N

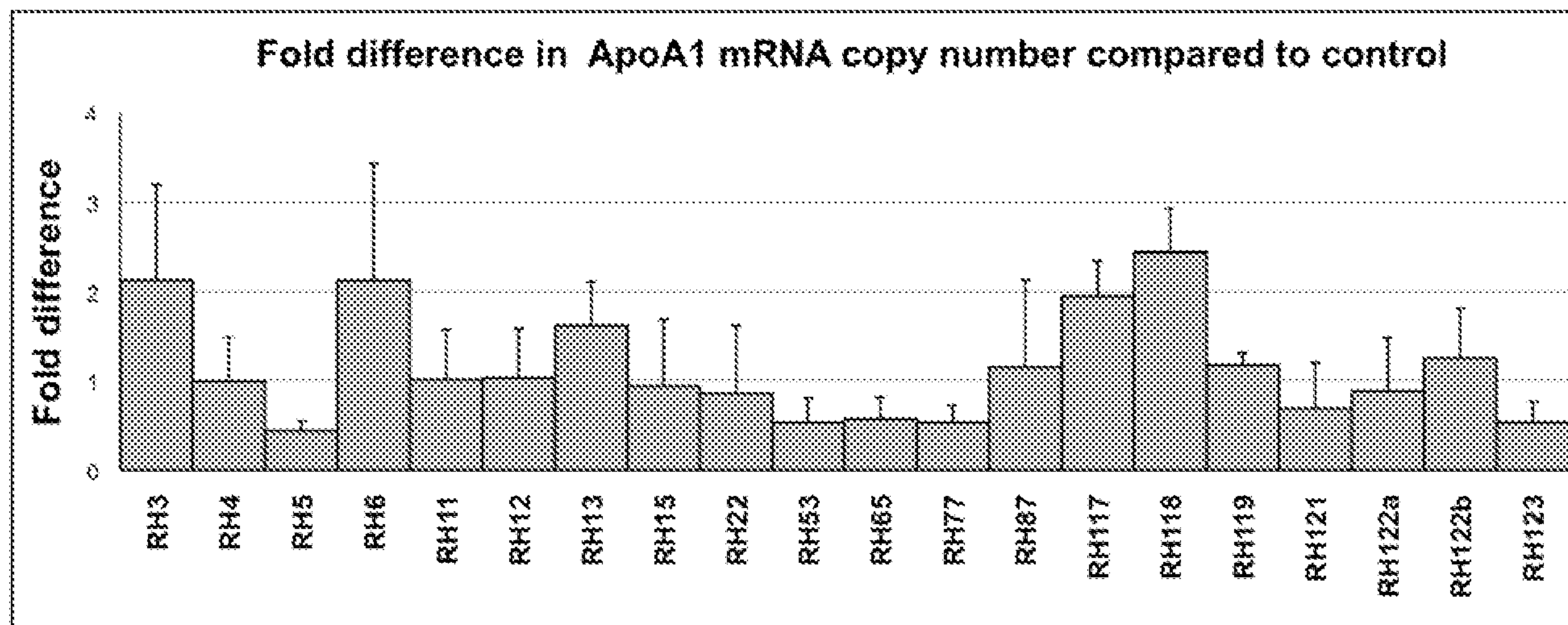


FIGURE 1O

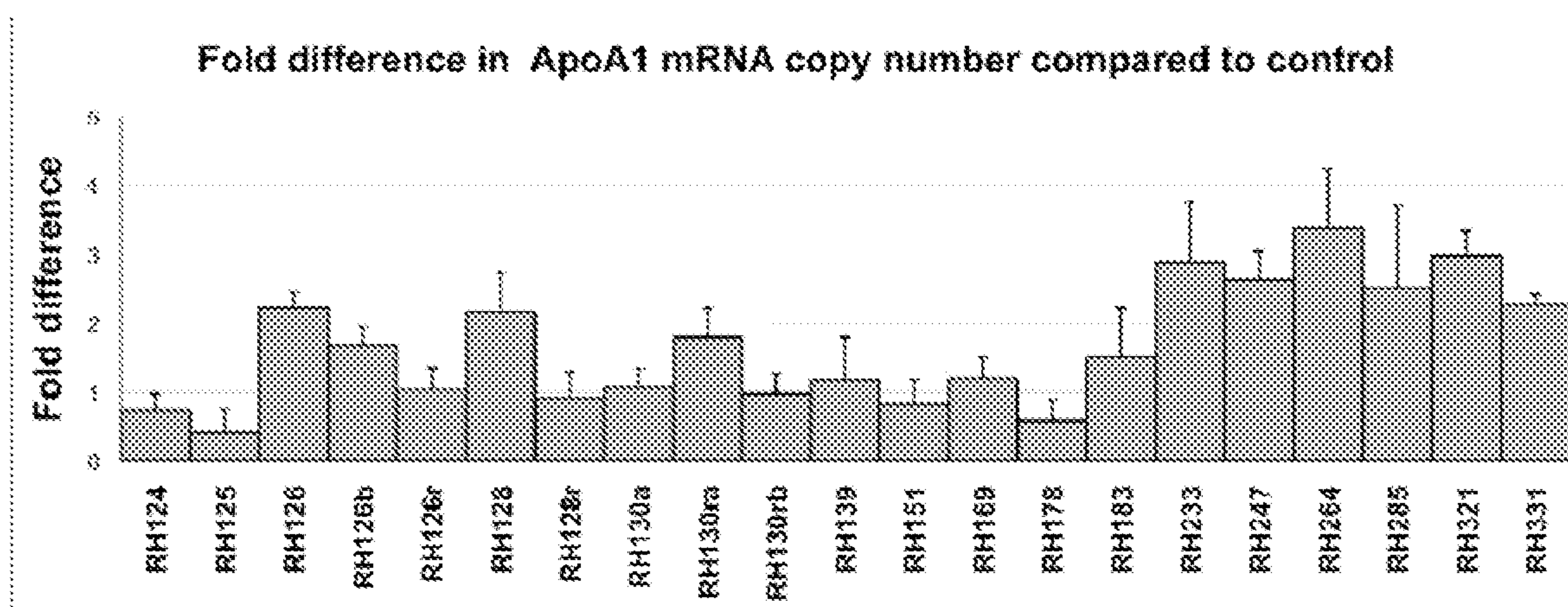
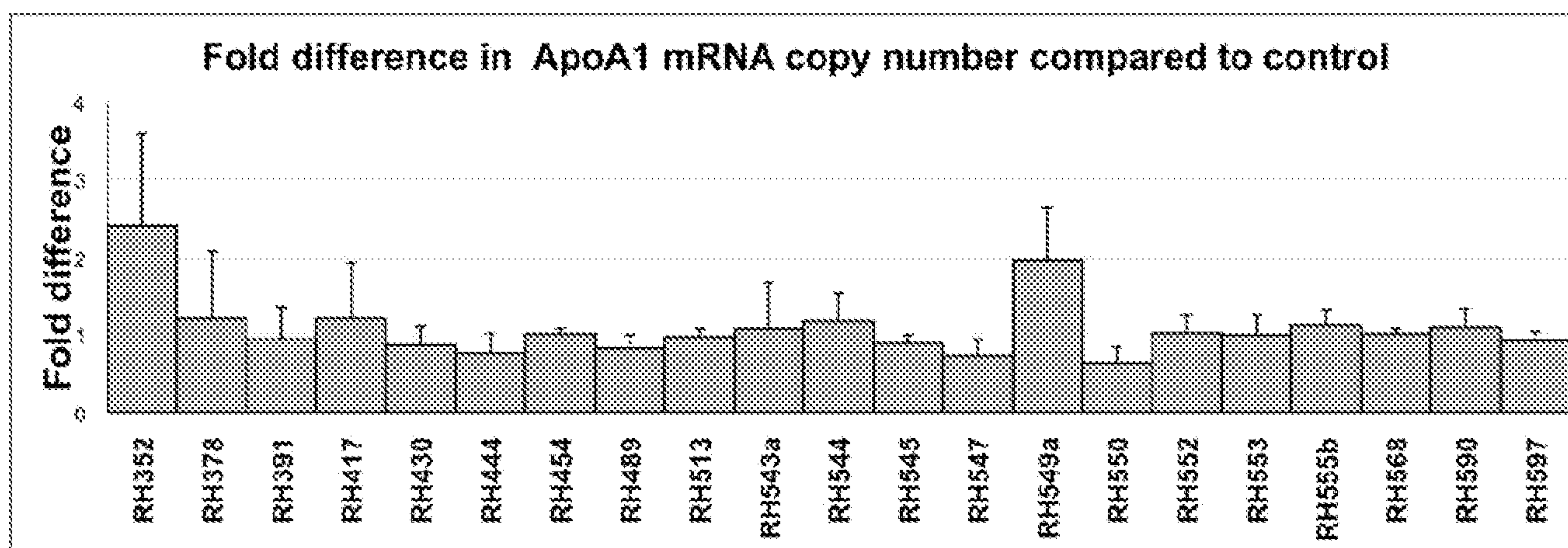


FIGURE 1P



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FIGURE 1Q

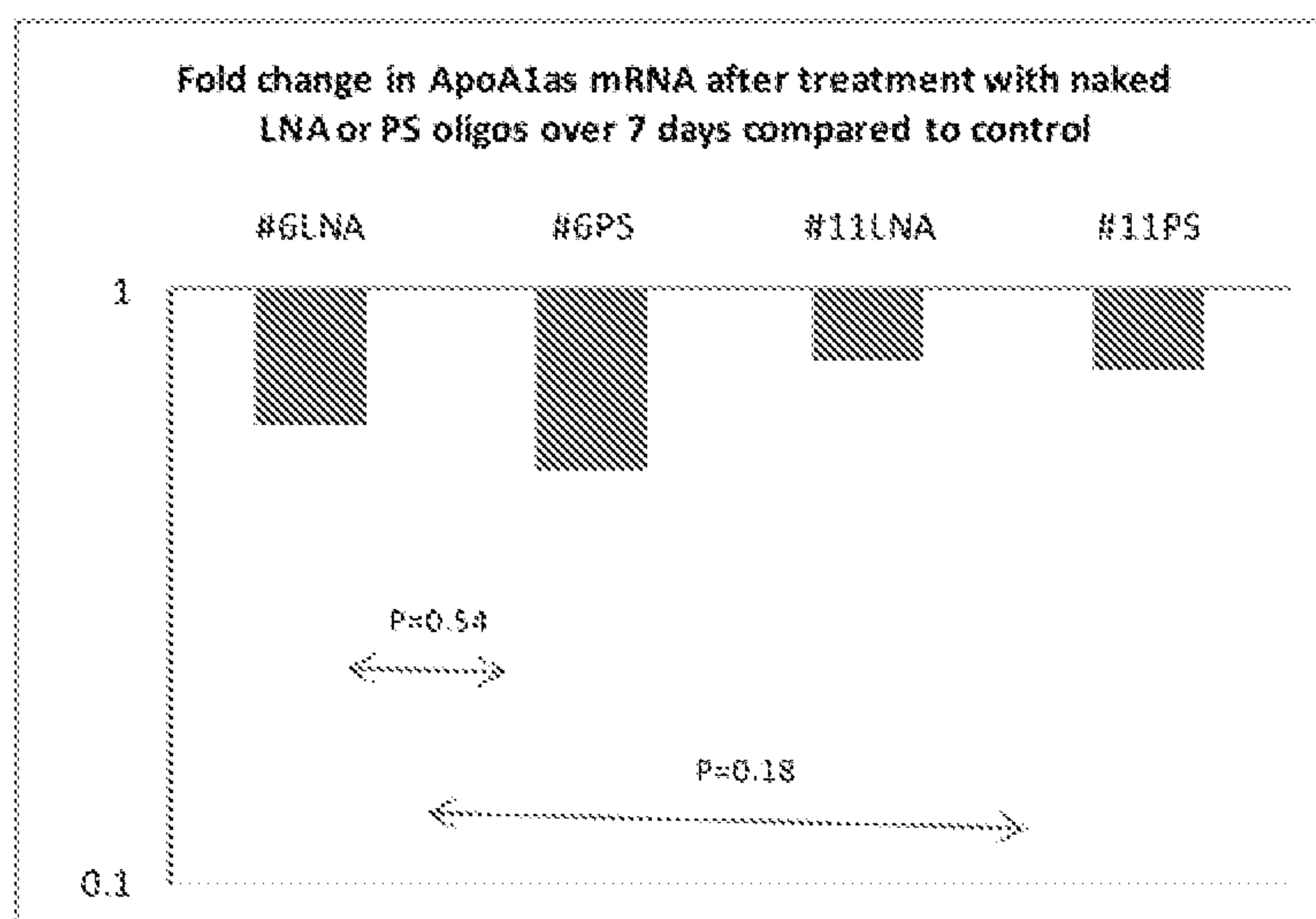
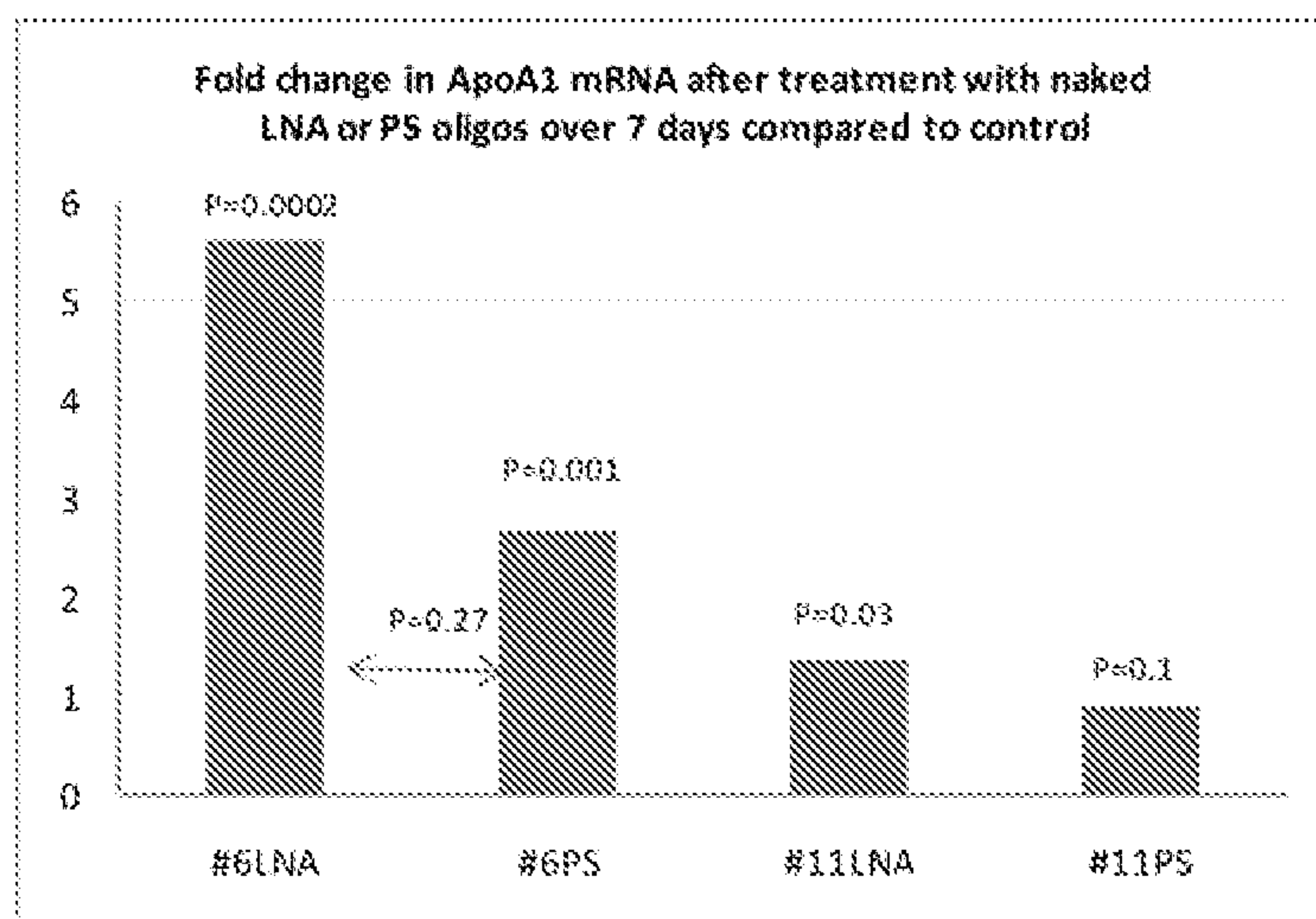
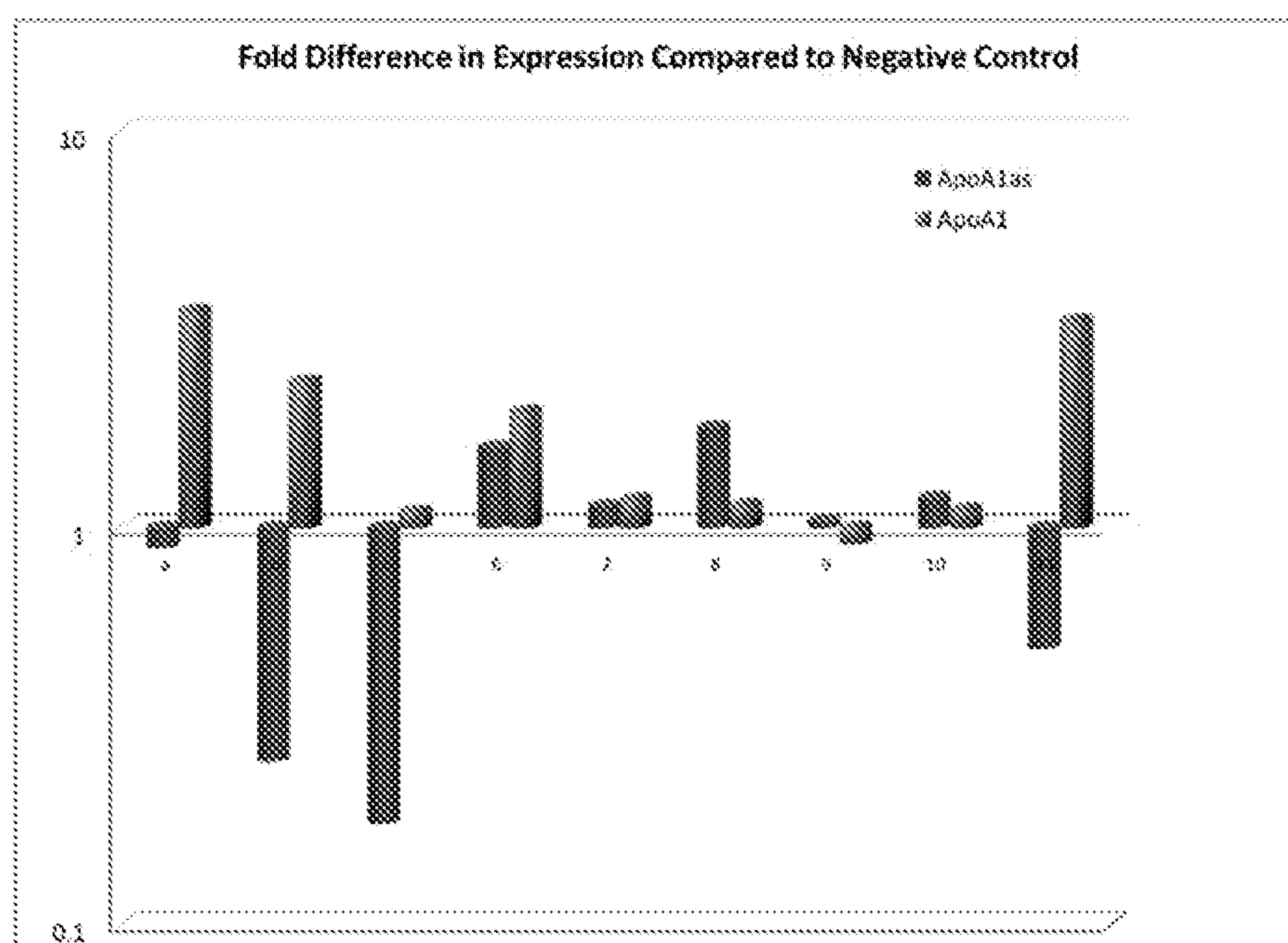


FIGURE 1R



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FIGURE 1S

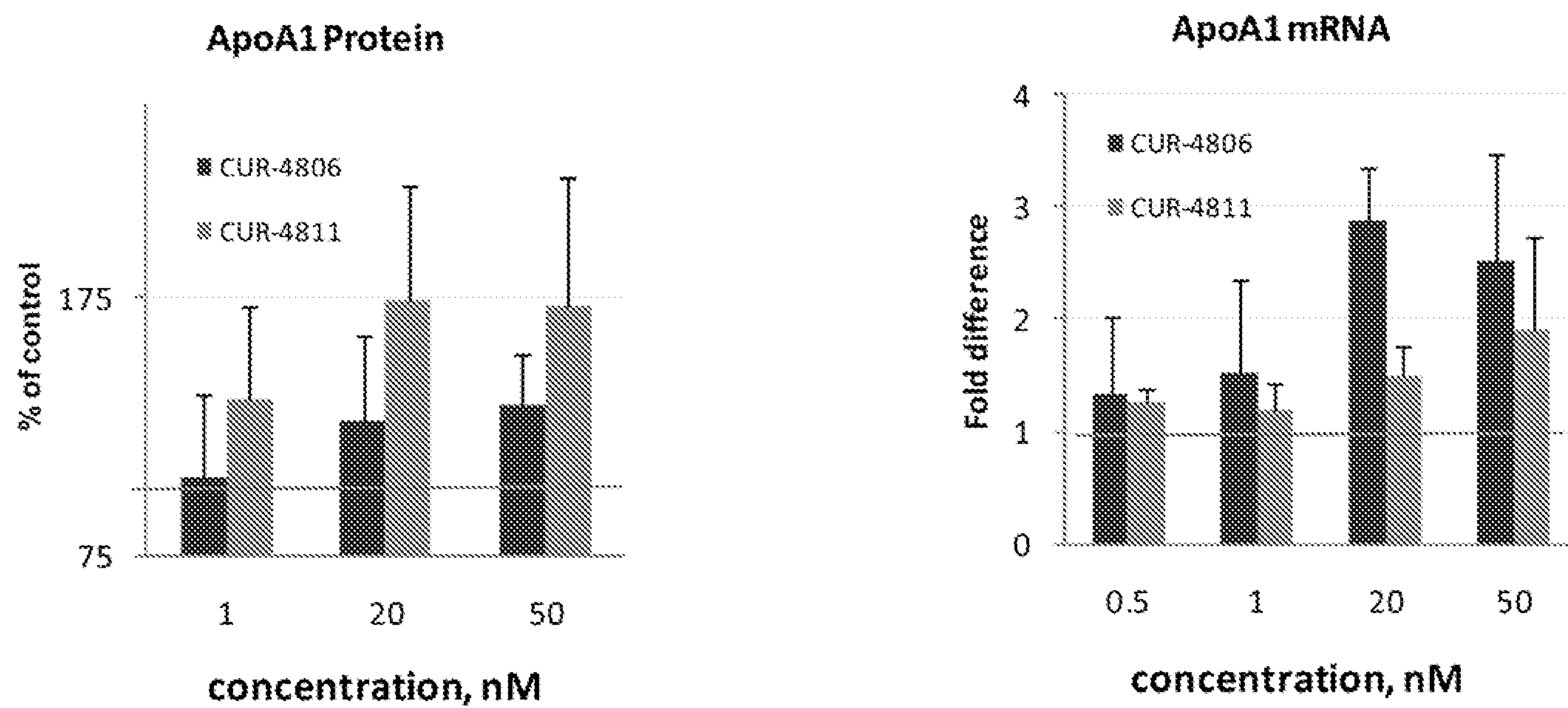


FIGURE 1T

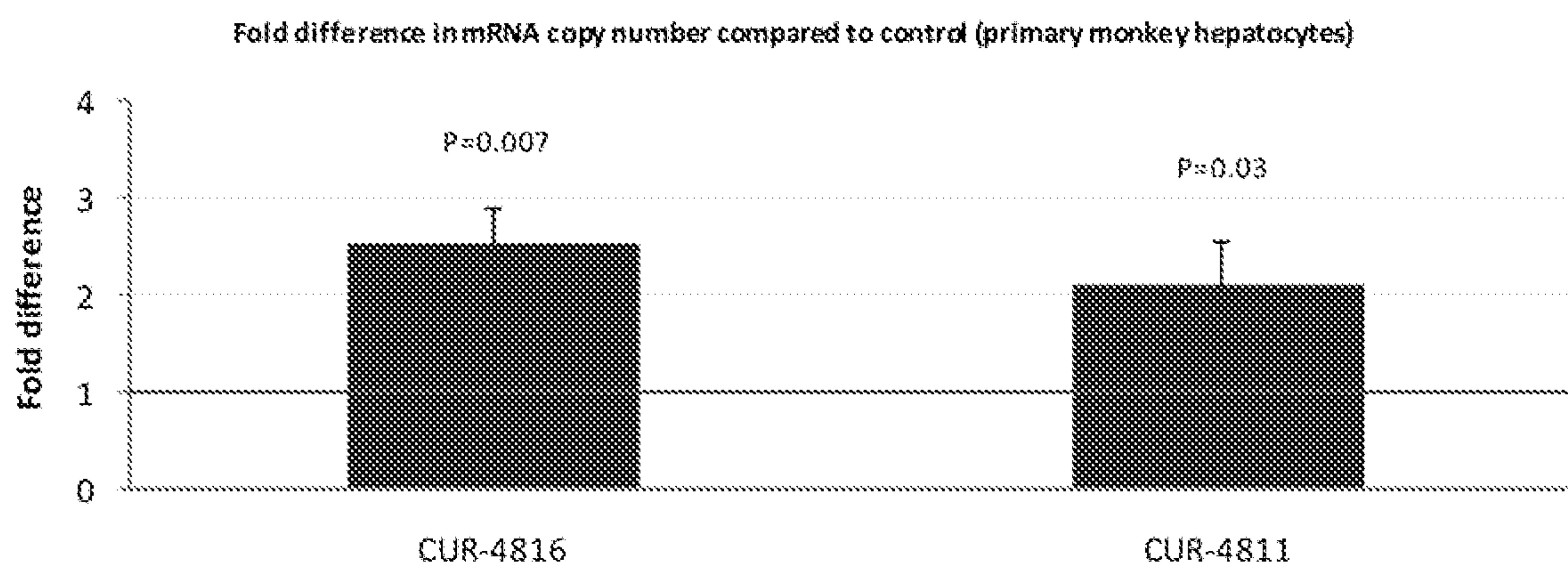


FIGURE 1U

Primate Up-Regulation of ApoA-I mRNA and Protein

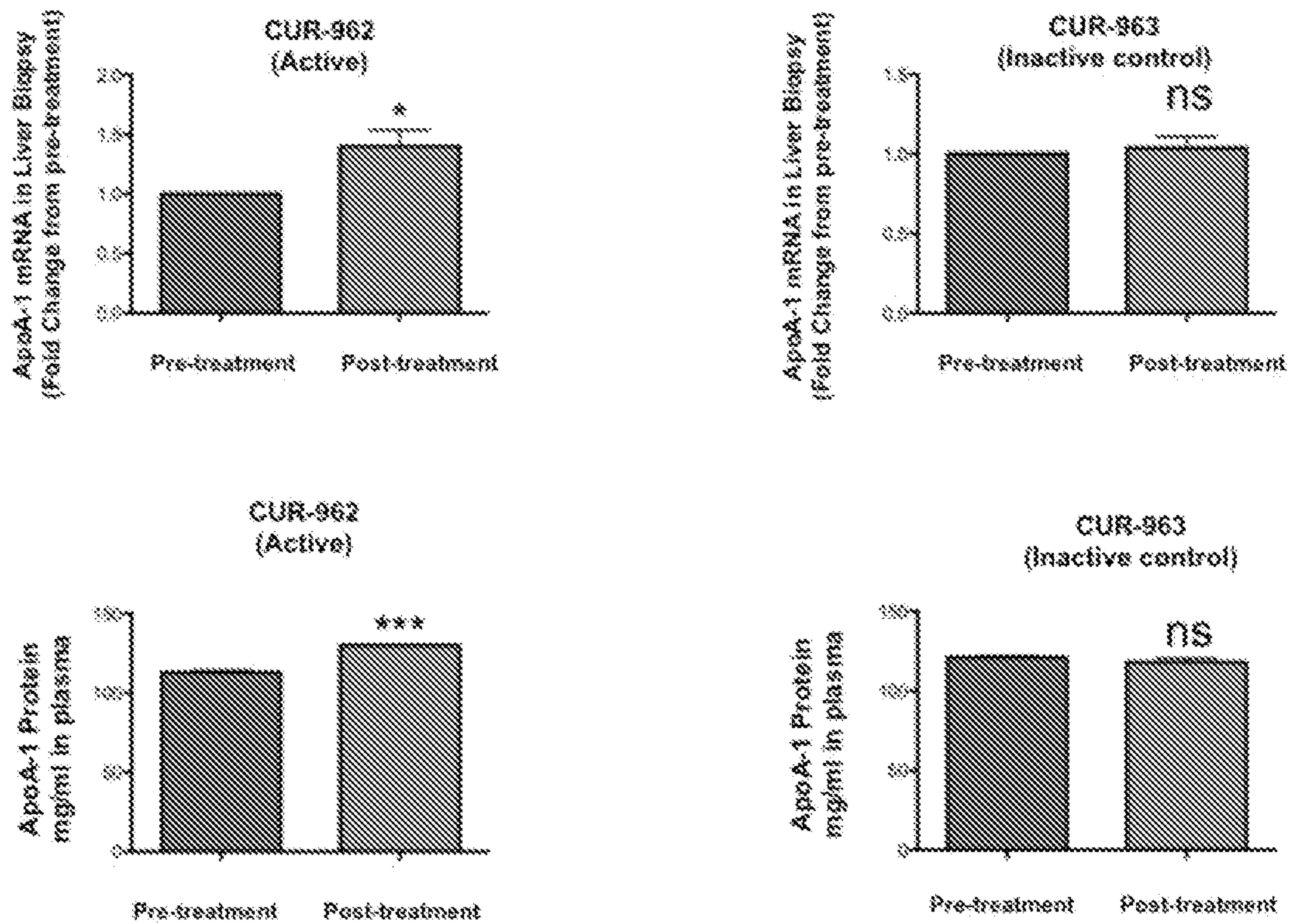


FIGURE 1V

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RH15      RH22
Human  cagcttctcttgcagctcgtgcagcttctgggcggcgccctctcggagct
>>>>> ||| ||||| ||||||| ||||||| ||||||| ||||||| |||||||
Rhesus cagtttctcgtgcagctcgtgcagcttctgggcggcgccctcgtggagct
          RH52      RH65      RH74      RH87
Human  ctggcgcgagcgccctccaccttctggcggttagagctccatctcctcctgc
>>>>> | ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Rhesus cgcgcgcgcagcggtccaccttctggcggttagagctccatctcctcctgc

          RH116 RH118 RH121
          RH112 RH114 RH117 RH120 RH123
          RH113 RH115 RH119 RH122
Human  cacttcttctcgggaagtcgtccag
>>>>> ||||||| ||||||| ||||||| |||||||
Rhesus cacttcttctcgggaagtcgtccag

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RH130a

RH125 RH126b RH128

RH124 RH126a RH128b RH139 RH151 RH169

Human atcttgaatgagggacaccccccacagggtac
>>>>> |||||
Rhesus atcttgaatgagggacaccccccacagggtac
 RH126r RH130ra
 RH128 RH130rb

RH178 RH183

Human ttattgcttggaaccctacaccttccttgggatgaaactaatctctcctc
>>>>> |||||
Rhesus ttattgcttggaaccctacaccttccttgggatgaaactaatctctcctc

RH233 RH247 RH264

Human ctctggtgtgcgccacacagcaacttgcaccaacagtggttactcaacagacta
>>>>> |||||
Rhesus ctctggtgtgtgcgccacacagcaacttgcaccaacagtggttactcaacagacta

RH285 RH321

Human gcatgcctgctgaagaaggggtcctctgacaatcagggggacaatgggg
>>>>> |||||
Rhesus gcatgcctgctgaagaaggggtcctctgacaatcagggggacaatggggaa

RH331 RH352

Human ttttatgctctccagactttctatacattgaagtccacacaggaaggta
>>>>> |||||
Rhesus ttttatgctctccagactttctatacattgaagtccacacaggaaggta

RH378 RH391 RH417

Human aagagaaaactagagaaaaataatttttgaagaaaaaacatttcaggaagtat
>>>>> |||||
Rhesus aagagaaaactagagaaaaataatttttgaagaaaaaacatttcaggaagtat

RH430 RH444 RH454

Human tgaaagtacacggtgacacagggtcggggcaggggtggagggcagagcac
>>>>> |||||
Rhesus tgaaagtacacggtgacacagggtcggggcaggggtggagggcagagcac

RH489 RH513

Human tgtttgctgcagctatgctccttcctcagtgccctgcacaccgggactt
>>>>> |||||
Rhesus tgtttgctgtagctgtgctccttcctcagtgccctgcacaccgggactt

RH556 RH558

RH547 RH550 RH554 RH557 RH561

RH543b RH545 RH549b RH553RH555b RH560

RH543aRH544 RH549aRH552RH555a RH559 RH56B

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Human  gctcgggtgagcatctctcgtctcaggggagcagctagctgagccttat
>>>>  |||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Rhesus gctcagtgagcctctctcttctcagtgacagctagtgtgagctacttat
              RH596          RH597

Human  gttcaggtgcccttgactacctcttggaggggggacaaattacttaac
>>>>  ||||| |||| || | ||||| ||||| ||||| ||||| ||||| |||||
Rhesus gttcaggtgccactcaatacctcttggactttgggggacaaattacttaac

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FIGURE 2

(SEQ ID NO: 1)

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(SEO ID NO: 2)

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(SEO ID NO: 3)

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protein 1 (LRP1), mRNA
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CGGTGTACGGAGAGCACATTTTCTGGACTGACTGGGTGGCGGGGCTGTGACGCGAGCCAAACAAGTATGTGGGCAGCGACATGAAG
CTGCTTGGGTGGACATTTCCCGAGCAACCATGGGCATCATCGGCTGGCCAACGACACCAACAGCTGTGAACCTCTCCCTCTGCCG
TATCAACAATGGAGGCTGCCAGGATCTGTGTCTGCTCACCACCAAGGCCAGCTCAACTGTTCTGTGAGGGGGCCGAGATCCTCC
AGGAGGACTTCACCTGCCGGGCTGTGAACCTCTTGTGCGGGCACAAGATGAGTTTGAAGTGTGCCAATGGGGAATGTATGAGCTTC
AGCTCACCCTGTGATGGGCTCTCCACTGCAAGGACAASTCCGATGAGAAGCCCTCCTACTGCAACTCAGCCGCTGCAAGAAGAC

GACCTGCATCGATCACTGTAGCAATGGTGGCTCCTGCACCATGAACAGCAAGATGATGCCTGAGTGGCAGTGCCCCCCCCCATATGA
CAGGACCCCCGGTSCGAGSAGCAGGTTGTTAGTCAGCAACAGCCTGGGCATATGGCCTCCATCCTGATCCCTCTGCTGCTGCTTCTC
CTGCTGCTTCTGCTGGCTGGCGTGGTGTCTGGTATAAGCGGSCGAGTCCGAGSGGCTAAGGGCTTCCAGCACCCAGCGGATGACCAA
TGGGGCCATGAATGTGGAAATTTGGAAACCCCTACCTACAAGATGTATGAAGGTGGAGAGCCCGATGATGTCCGGGGGCCCTACTGGATG
CTGATTTTGGCCTTGACCTTGACAAGCCTACCAACTTCACCAACCCAGTGTATGCCAOGCTCTACATGGGGGGGCCACGGCAGCCGC
CATTCCTTGGCCAGCACCGGAGAGAGCAGAACTGCTGGGCGGGGAGCTGAAGACGAGATAGGAGATCCCTTGGCATAGGGCCC
TGGCCCGACGGATGTCCCCAGAAAGCCCCCTGCCACATGAGTCTTTCAATGAACCCCTTCCCCAGCCGGCCCTTCTCCGGCCCTGC
CSGGTGTACAAATGTAAAAATGAAGGAATTACTTTTTATATGTGAGCGAGCAAGCGGAGCAAGCACAGTATTATCTCTTTGCAATTC
CTTCCTGCTGCTCCTCAGTATCCCCCCCCATGCTGCTTGGAGGGGGCGGGGAGGGCTTTGTGGCTCAAAGGTATGAAGGAGTCCAC
ATGTTCCCTACCGAGCATACCCCTGGAAGCTGGCGGACCGGCTCCCCACCAAGCCTGTGCAAGACACTCAACGGGGCTCCGTGTC
CCAGCTTTCTTTCTTCTTGGCTCTCTGGGTTAGTTTCAGGGGAGGTGGAGTCTCTGCTGACCCCTGTCTGGAAGATTGGCTCTAGC
TGAGGAAGGAGTCTTTTAGTTGAGGGAAGTCACCCCAAACCCAGCTCCCACTTTTCAGGGGACCTCTCAGATGGCCATGCTCAGT
ATCCCTTCCAGACAGGCCCTCCCTCTCTAGCGGCCCTCTCTGTGGCTCCTAGGGCTGAACACATTCTTTGGTAACGTGCCCCAAG
CCTCCCATCCCCCTGAGGGCCAGGAAGAGTCCGGGACACACCAAGGAAGGGCAAGCGGGCAGCCCCATTTTGGGGACGTGAACGTTT
TAATAAATTTTCTGAAATTCCTTTACAACATAAATAACACAGATATTGTATATAAATAAATTTGTAATAAAGGAAAAAAGAAAA
GAAAAGAAAAGAAAAAAGAAAAAAGAAAAA

(SEQ ID NO: 5)

>gi|170932496|ref|NM_000527.3| Homo sapiens low density lipoprotein receptor (LDLR), mRNA

ACATTTGAAAATCACCCCACTGCAAAACCTCCTCCCCCTGCTAGAAACCTCACATTTGAAATGCTGTAAATGACGTGGCCCCCGAGTGC
AATCGCGGGAAGGUCAGGTTTTCCAGCTAGGACACAGCAGGTGCTGATCCGGGTGCGGACACTGCTGGCAGAGGCTGCGAGCATGG
GGCCCTGGGGCTGGAAATTTGGCTGGACCGTCCCTTGCTCCTCGCCGCGGGCGGGGACTGCASTGGGCGACAGATGCCAAAGAAAC
GAGTTCCAGTGCCAAAGACGGGAAATGCATCTCTACAAAGTGGGTCTGCGATGGCAGCCCTGAGTGGCAGGATGGCTCTGATGAGTCT
CCAGGAGACGTGCTTGTCTGTACCTGCCAATCCGGGGACTTCAGCTGTGGGGGGCCGTGTCAACCGCTGCATTCTCAGTTCTGGA
GGTGGATGGCCAAAGTGGACTGGGACAACGGCTCAGACGAGCAAGGCTGTCCCCCAAGACGTGCTCCAGGACGAGTTTCGGCTGC
CAGCATGGGAAGTGCATCTCTCGGCAGTTGCTCTGTGACTCAGACCGGGAAGTCTTGGACGGCTCAGACGAGGCTCCTGCCCCGT
GCTCACCTGTGGTCCCGCCAGCTTCCAGTGCACACAGCTCCACCTGCATCCCCAGCTGTGGGGCTGGGACAACGACCCCCGACTGCG
AAGATGGCTCGGATGAGTGGCCGACCGCTGTAGGGGTCTTTAGCTGTTCCAAGGGGACAGTAGCCCCCTGCTCGGGCTTCGAGTTC
CACTGCCCTAAGTGGCGAGTGCATCCACTCCAGCTGGGCTGTGATGGTGGCCCCGACTGCAAGGACAAATCTGACGAGGAAAACTG
CGCTGTGGCCACCTGTCCCTTGACGAATTCAGTGTCTGTGATGGAACTGCATCCATGGCAGCCGGCAGTGTGACCGGGAAATATG
ACTGCAAGGACATGAGCGATGAAGTTGGCTGGCTTAAATGTGACACTCTGCGAGGGGACCCAAACAGTTCAAGTGTACAGCGGGCGAA
TGCATCACCTTGACAAAAGTCTGCAACATGGCTAGAGACTGCCGGGACTGGTCAAGTGAACCCATCAAAGAGTGGCGGACCAACGA
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TGGTGGCCCCAGCGAAGATGCGAAGATATGATGAGTGTGAGGATCCCGACACCTGCAGCCAGCTCTGCGTGAACCTGGAGGGTGGC
TACAAGTGGCAGTGTGAGGAAGGCTTCCAGCTGGACCCCCACACGAAGGCTTCAAGGCTGTGGGCTCCATCGCTACCTCTTCTT
CACCAACCGGCACGAGGTCAGGAAGATGACGCTGGACCGGAGCGAGTACACCAGCCTCATCCCCAACCTGAGGAACGTGGTGGCTC
TGGACACGGAGGTGGCCAGCAATAGAATCTACTGGTCTGACCTGTCCAGAGAATGATCTGCAGCACCCAGCTTGACAGAGCCCCAC
GGCTCTCTCTCTATGACACCGTCATCAGCAGAGACATCCAGGCCCCCGACGGGCTGGCTGTGGACTGGATCCACAGCAACATCTA
CTGGACCGACTCTGTCTCTGGGCACTGTCTCTGTTGGGATACCAAGGGCGTGAAGAGGAAAAAGCTTATTCAGGGAGAACGGCTCCA
AGCCAAAGGGCCATCGTGGTGGATCCTGTTTATGAGCTTCTATGTAAGTGGCTGAGTGGGGAACTCCCGCCAAAGATCAAAGAAAGGSGGC
CTGAATGGTGTGGAATCTACTGCTGGTGAAGTGAACATTCAGTGGCCCAATGGCATCACCTTAGATCTCCTCAGTGGCCGCT
CTACTGGGTGACTCCAAACTTCACTCCATCTCAAGCATCGATGTCAACGGGGGCAACCGGAAGACCATCTTGGAGGATGAAAAGA
GGCTGGCCCCACCCCTTCTCTTGGCCGTCTTTGAGGACAAAGTATTTTGGACAGATATCATCAACGAAGCCATTTTTCAGTGGCAAC
CGCTTCACAGGTTCCGATGTCAACTTGTGGCTGAAAACCTACTGTCCCCAGAGGATATGGTTCTCTTCCACAACCTCACCCAGCC
AAGAGGAGTGAAGTGGTGTGAGAGGACCACCTGAGCAATGGCGGCTGCCAGTATCTGTGCTCCTGCCCCGAGATCAACCCCC
ACTCGCCCCAAGTTTACCTGCGCTGCCCCGACGGCATGCTGCTGGCCAGGGACATGAGGAGCTGCTTCACAGAGGCTGAGGCTGCA
GTGGCCACCCAGGAGACATCCACCGTCAGGCTAAGGTCAGCTCCACAGCCGTAAAGGACACAGCACACAACCCACCCGACCTGTTC
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CTGGCAGAGGAAATGAGAAGAAGCCAGTAGCGTGAGGGCTCTGTCCATTGTCTCTCCCATCGTGGCTCCTCGTCTTCTTTGCTG
GGGGTCTTCTCTTATGGAAGAACTGGGGCTTAAGAACATCAACAGCATCAACTTTGACAACCCCTCTATCAGAAGACACAGA
GGATGAGGTCCACATTTGCCACAACAGGACGGCTACAGCTACCCCTCGAGACAGATGGTTCAGTCTGGAGGATGACGTGGCTGAA
CATCTGCTGAGTCCCGTCCCTGCCCAGAACCCCTTCTGAGACCTCGCCGGCTTGTGTTTTATTTCAAAGACACAGAGAAGACCAAAGC
ATTGCTGCGCAGAGCTTTGTTTTATATATTTTATTCATCTGGGAGGCAGAACAGGCTTGGACAGTGGCCATGCAATGGCTTGGGT
GGGATTTTGGTTTCTTCTTCTCTCTGTAAGGAATAAGAGAAACAGGCCCCGGGGGACAGGATGACACCTCCATTTCTCTCCAGGA
AGTTTTGAGTTTCTCTCCACCTGACACAATCTCAAACATGGAAGATGAAGGGGGAGGGGATGTAGGCCCCAGAGAAGCAAGTGG
CTTTCAACACACAACAGCAGATGGCACCAACGGGACCCCTGGCCCTGCTCATCCACCAATCTCTAAGCCAAACCCCTAAACTCA
GGAGTCAACGTGTTTACCTCTTCTATGCCAAGCCTTGGTACAGACAGCCAGGTTAGCCTTTGGCCCTGTCAACCCCGAATCATGACCCAC
CCAGTGTCTTTGAGGTGGGTTTGTACCTTCTTAAAGCCAGGAAAGGGATTTCATGGCTCGGAAATGATCTGGCTGAATCCGTGGT
GGCACCGAGACCAAACTCATTCACCAAAATGATGCCACTTCCAGAGGCAGAGCCTGAGTCACTGGTCAACCTTAATAATTTATTAAG
TSCCTGAGACACCCGGTTACCTTGGCCGTGAGGACACGTGGCTGCACCCAGGTGTGGCTGTGAGGACACAGCCTGGTGGCCATC
CTCCCGACCCCTACCCACTTCCATTCCTGCTGGTCTCCTTGCACCTTTCTCAGTTTCAGAGTTGTACACTGTGTACATTGTCATTTGT

GTTATTATTTTGCACCTGTTTTCTGTCTGTGTGTTGGGATGGGATCCCAGGCCAGGGAAAGCCCGTGTCAATGAATGCCGGGGACA
 GAGAGGGGCAGGTTGACCGGGACTTCAAGGCCGTGATCGTGAATATCGAGAACTGCCATTGTCTCTTTATGTCCGCCCCACCTAGT
 GCTTTCACCTTCTATGCAAATGCCTCCAAAGCCATTCACCTTCCCCAATCTTGTCTGTGATGGGTATGTGTTTAAAACATGCACGGTGA
 GGGGGGGGGCAGTGGCTCAGGCTGTAAATCCCAGCACTTTGGGAGGGCCGAGGCGGGTGGATCATGAGGTCAGGAGATCGAGACCAT
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 GTCCGGAGAGACAGTGACAGCCTCCGTGAGACTCCCGCGTGAAGATGTCACAAGGGATTGGCAATTGTCCCCAGGGACAAAACACT
 GTGTCCCCCCCCAGTGCAGGGAACCGTGAAAGCCCTTTCTGGTTTCCGAGCACGGTAAATGCCGTCCCTGTACAGATAGTGGGCATTTT
 TTGTTATGTTTGCACCTTTGTATATTGGTTGAAACTGTTATCACTTATATATATATATATACACACATATATATAAAATCTATTTAT
 TTTTGCAAACCCCTGGTTGCTGTATTTGTTTCAGTACTATTCTCGGGGCCCTGTGTAGGGGGGTTATTGCCTCTGAAATGCCCTCTTCT
 TTATGTACAAAGATTATTTGCACGAACTGACTGTGTGCAACGCTTTTTTGGGAGAATGATGTCCCGCTTGTATGTATGAGTGGCTT
 CTGGGAGATSGGTGTCACTTTTTAAACCACCTGTATASAAGGTTTTTGTAGCCTGAATGTCTTACTGTGATCAATTAAATTTCTTAA
 ATGAACCAATTGTCTTAAA

(SEO ID NO: 6)

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>gi148762938|ref|NM_000041.2| Homo sapiens apolipoprotein E (APOE), mRNA
GGGATCCTTGAGTCTACTCAGCCCCAGCGGAGGTGAAGGACGTCTTCCCCAGGAGCCGACTGGCCAATCACAGGCAGGAAGATG
AAGGTTCTGTGGGCTGCTTGCTGCTCATTCTTGGCAGGATGCCAGGCCAAGGTGGAGCAAGCGGTGGAGACAGAGCCCGGAGCC
CGAGCTGCCGCCAGCAGACCGAGTGGCAGAGCGGCCAGCGCTGGGAAGTGGCACTGGGTGCTTTTGGGATTACCTGCGCTGGGTGC
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GGCGGCGCAGGCCCGGCTGGGCGCGGACATGGAGGACGTGTGCGGCGCGCTGGTGCAGTACCGCGGGCGAGGTGCAGGCCATGCTCG
GCCAGAGCAACCGAGGAGCTGCGGGGTGCGGCTCGGCTCCACCTGGGCAAGCTGGGTAAGCGGCTCCTCUGCGATGCCGATGACCTG
CAGAAAGCGCTTGCAGTGTACAGGCCCGGGGCGCGCGAGGGCGCGGAGCGCGGCTCAGCGCCATCCGCGAGCGGCTGGGGCGGCT
GGTGGAAACAGGGCGCGGTGCGGGCGCGCACTGTGCGGCTCCCTGGCGCGGCGAGCGCTACAGGAGCGGGCGGCGAGGCTGGGGCGAGC
GGCTGCGCGCGCGGATGGAGGAGATGGGCGAGCGGACCGCGACCGGCTGGAGGAGGTGAAGGAGCAGGTGGCGGAGGTGCGCGGCG
AAGCTGGAGGAGCAGGCCCCAGCAGATACGCTGCAGGCCGAGGGCTTCCAGGCCCGGCTCAAGAGCTGGTTCCAGCCCCCTGGGTGGA
AGACATGCAGCGCCAGTGGGCGCGGCTGCTGGAGAAAGGTGCAAGGCTGCCGTGGGCAACAGCGCGCGGCTGTGCCACAGCGACAATC
ACTGAACGCGGAAGCTGCAGCCATGCGACCCACGCGCACCGGCTGCTCCTGCTCCTCGCGCGAGCCTGCAGCGGGAGACCCCTGTCC
CCGCCCCAGCCGCTCCTCTGGGGTGGACCCCTAGTTTAAATAAAGATTTCACCAAGTTTCAAGCAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAA
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(SEO ID NO: 7)

>gi| 4557320|ref|NM_000039| Homo sapiens apolipoprotein A-I (APOA1), mRNA

AGAGACTGCGAGAAGGAGGCTCCCCACGGCCCTTCAGGATGAAAGCTGCGGTGCTGACCTTGGCCGTGCTCTTCTGACGGGGAGC
 CAGGCTCGGCATTTCTGGCAGCAAGATGAACCCCCCAGAGCCCCCTGGGATCGAGTGAAGGACCTGGCCACTGTGTACGTGGATGT
 GCTCAAAGACAGCGGCAGAGACTATGTGTCCCACTTTGAAGGCTCCGCCTTGGGAAAACAGCTAAACCTAAAGCTCCTTGACAAC
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 GTGGCAGGAGGAGATGGAGCTCTACCGCCAGAASGTGGAGCCGCTGCGCGCAGAGCTCCAAGAGGGCGCGCGCCAGAAGCTGCACG
 AGCTGCAAGAGAAAGCTGAGCCCACTGGGCGAGGAGATGCGCGACCGCGCGCGCGCCCATGTGGACGCGCTGGGCACGCATCTGGCC
 CCTACAGCGACGAGCTGCGCCAGCGCTTGGCCGCGCGCCTTGAGGCTCTCAAGGAGAAACGGCGCGCGCCAGACTGGCCGAGTACCA
 CGCCAAGGCCACCGAGCATCTGAGCACCGCTCAGCGAGAAGGCCAAGCCCGCGCTCGAGGACCTCCGCCAAGGCCCTGCTGCCCGTGC
 TGGAGAGCTTCAGAGCTCAGCTTCTTGAGGCTCTCGAGGAGTACACTAAGAAGCTCAACACCCAGTGAAGCGCCCGCGCGCCCC
 CCTTCCCGGTGCTCAGAATAAACGTTTTCCAAAGTGGG

FIGURE 3

Natural antisense sequence (AK311445): SEQ ID NO: 8

ACGCCCAGGGAACCCCGACCCCTCTGAGTCCGGGGTACTGCGCCCGGGTCTCCACGCCCAGAGATGCTCCCGGGTCTCCACCGTCCG
GGCAAGCCCCAAGCGCAGCAGCGCAGAGTCTCTGGGTCAACAGAGCTCGTACTAGGACATCGTCTCCCCATTTAACACCGCCTCCG
GTCCCATCTGAGTTGCAAGTGGTGGGGATGTGGGGCTCCGGATCAAAGTCCCGGAAACCGAGGCACTTCCCGAAGCCTCCTTGGGCT

CGAAACAAAACAATAACGCCCAACTCCATCATATTTCCAGAACTCCCAACCACCTGCATACAGACATTTCAGCTGCACAAGCCCCCTCC
 ATGCTACAGTCAACAGGATCTCCAGGCCACGGCTCAAGGCCAGGTACTCACATCAGTGGTTCTATCAACACTCAGGACAGACCCAT
 AGAAGAGGCCCAAGCAGGCCCTGGAAGTGCATGTGGAGGCCACCAGGCCAAGGAATTCTGGAGTCCCAGGTACTCATAACTCTGGGT
 GGCATGGCCCCCTTTGCACCAATGGACTGTTTTGCCCTTAGAAAGGGATGGATCTGAGCTGGGCGCAGTGGCTCATGCTTGAATCCCA
 GCACCTTTGGGAGGCCAAGGTGGGCAGCTACCTEAGGTTCAGGTTGGTCTCAAACCTCCTGACCTCAGGCGATCCACCTCAGCCTCTC
 AAAGTGCCTGGAATTATAGGTGTGAGCCACTGTGCCAGGCCAAAATCAATCTTTTGGAAATTTTGAAGCATATAATTCCAAAAGGT
 ATGAAGGTAAATCATTAGATTGCTCTAATAAGGGAAATGGGAACAGTTAAGTCTTATACAAATAAGACAAAGATAAGATACTACAAA
 AAGGGGATGAGCCCAAGAAAAAATCAAAGTCCCAGAGAGAGAACAGCCATTGATTCTAAATACACAAGTCTATGGCCCCAACCCA
 AACTTGTTCACATAAGAACAACCTGTGGTTCGAGAATCTGGTCATCCCCCAGAGTGAATACATGAACACATTGTAATGTTTGAAA
 TGTTTATTTTTCTTGTTGATTCTTACTGTTAGAAGAGCTAAGTGATTGCGCCCAAAGTGGCTAAGTGATTGCGCCAGTTTGATACA
 CAGGGATATAAATTTGCTGACACCAAGCTCATACTTTACAAATGTAATATCTTCATAAAACAAAATACTGGGCGGGCGCGGTGG
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 GAAgCCCCG

Natural antisense sequence (BF133827): SEQ ID NO: 9

CAAAATGGCGTGCTACCCCTGTCCAACCTTGTCTGTAGACAGAGTCAATTGAACACTGTCTTTGGGACTTCCGTGCAACTGAGGTGG
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 ACAGGAAAGGCCAAGAGACATGTGACACTGTGTCTGATGGTTCACAAGTCAAGCTTTTAAAAAGCAGCCTGATATTGTGAGCTAAC
 ATGGCTTTCTGTAATTGAATGCAATGTATTTTCTATGCTTGTCTGGGTAAAGTTGACCTTGGTTTGATTAGCTCAAGCAATATTT
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 TCAAAACTTTTGACAAGCACACACAGGACATGAATTACAATCACCCGAAGATTTTTLACAGGCTTCTCAATTTTAATGACATGCTG
 ACACGtGTCTATCAGATCTCACAACAAGATGACACATGGGTGTGAGGTATGgcgCAGAAGACTAGAGTGGGGTGTAAccaatgagc
 atgtgtgtgttgacacagggcgaattcggcgaacggacagtgtgtggaggcagaagggtttaagaagggcaggaaagcccatgtttaa
 cagaatggggtgacgaagagggtatgggaagggtctaactcaccgggggtgggggycaccaggggggcccacggacacagaaaaaccac
 gcaggtcaggcaccctacaaagaccgaaggaaaaagggaaccacgcagaatcactcacg

Natural antisense sequence (Hs.668679): SEQ ID NO: 10

AAAGTCCAAGGTGGGAACAGATAGGTCTGGGGSCATGGGGGCTGGGCCTAATAGGGGCCGGGCATGGATGGGGCTCTCCTGTCTCAC
 CGATCCTGGGCTGGGCATCTTGTCTCTTGGTGGGTGGGGGCCAAGGAAGGAGTGGTGGGGCTTGGGCCAGAGTCTGGTGTGGCTGTG
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 TCACAGTGTGGTGGGAGAAAGGACGTCAATCTCTAAGGGACAAGCTTTTGGCCCCCTCCCCACACCAGGGCAGGTACTTATGTGG
 GGCTTATGCAGGSCAGAAGGGCTTTGGCAGGTGAGCTGCCAGGGCTGGGGCCAGGCTCCCCAGGGTCTGGCGTGGTGCATCAG
 GGGCCTGGTGGGGGCTTACCGAGGATGACGGGGCCGTGTGTGGTTACTGAGCTCAGCCCTTGGGCGTGGTGTGCGGGGGGAAGAGCAC
 ATTGAGGAGCCAGAAGGGGGCCGAGGAGGGAGCAGCAGCCCCAGCAGCAGGCTCACCCTACTGCCATGGGGAGCCGGGGCGGCCCA
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 CAGTTCCGCTTCTTCTCTTGGCGCCAGGGGAAACAGAGCCGGGGCAGCAGGAGGCCCAGAACTACACAATGTTTTATTGAAAAG
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 CGCGCAACCAACAGCACCCGCTCAAGGCCCTCGGTGAGCACCTCGAGGAAGTCCATGTAGTTCTCTGTCGCCCCCTCACTGTTCTGGGT
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Natural antisense sequence (Hs.593769): SEQ ID NO: 11

tttaangTTTGATGTTTATTGGTGGTGTCTGATGAGCGTTTCTCTTGTCCAGACTGTGTTTTCTCTCTCAGACCAGCTCCAGGGT
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 TCTGGCCCTAGCTCTGGCAGATCCATCCTCAGTGAAGCACATCCCTGGGgCAAAGGCACCTCTGAGGCCAAGACCAGCATGGGCTT
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Natural antisense sequence (Hs.387239): SEQ ID NO: 12

TGTCACTCTGACCTCAGTGTAGGCACTGCCTCCTCTGGGAAGTCTTTGCTGACCTGAAAGGCTCAGCCTCTTGTGCTTCTTAAGCT
 TTTCTCAGAGCAATTTAGCTTCATTAGTAATTAAGCTTCCATTAGTGAATGATCTGATTAAATGGTTGTCACTCCAGATTTTAATT
 CTAACCTTTTTTTTTTTTTTTTTTTTTTTTGAGAGCCAGTCTCTTTTTTTTTTTTGAGACAGTCTCATTCCTGCGGCCAGTCTGGAGTGCAA
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Natural antisense sequence (Hs.711951): SEQ ID NO: 13

TgTTTTTTTTTTTTTTTTTTTAAAGAGATGGAGTCCCTGATCTGTGCCCCAGGCTGGAGTTCAGTGGCACAATATTGGCCCCACTGCCAA
 CCGTTGAACCTTCCCGGTTCAAGTGAATCTCTCTGCTTAGGTCTTCTGAGTAGCTGGGATTACAGGTGCCACCCACCACGCCCCAGCT
 AATTTTTGTGGTFTTAGTAGAGACGGAGTTTTGGCATGTTGGCCAAAGCTAGTCTCAAACCTCTGACCTCAAGTGATCGGCCGGCT
 CAGCCTCCCAAAGTGTCTGGGATTACAGGCTTGAGCCACTGGGCTGGCCCAAGTTTTCCCATGTCTTGAGGCACCCTACCCATGCA
 CCTCAGAATCCTCCCTTGCCTTTATCCCTTTGATACAGCACATCCCAAAGTGAATCCCCACATGGTCCCTGGTTGCTCAGACTCAG
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 TGAGAAGTTCTGCAGCACAA

Natural antisense sequence (DC401271): SEQ ID NO: 14

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 TTCAGACACTGTCCAGCACCTTCAGGCTTCGGTGGGAGAGCAGGGGGAGGGGAGCACCATCTTGCAACACATCAGCACCCCTTGAGA
 GCATATATCAGAGGGGACCCCTGAAGCTGGTGGCCACTTTTCAGACAAATACTTCAAGGAGAGAAAAAGCTGTTATGGAACAGTTC
 CGCCACTTSCCAATGCCCTTCCACTGGAAGCAGGAAGAACTCAAGTTTAAGACAGGCTTGGGGAGGCTGCAGCACCCAGTGGGGGA
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Natural antisense sequence (BM933147): SEQ ID NO: 15

TGTGACACCCCTTCCCTCAGGCTTACAGCAGCAAAAGTTGCCCTCGGAAGAGAAAGAGAAAGCTGTCATGGGAATGGCCAGCACATCCCTAAAT
 GCTCCAGTGGCCCGTGGTCTCTCCCTTCGTCTCATTGACTGCCACAGACAGGAAGTAGGCTCAGGGACTTGGCACCTACCCAAACAG
 CAGGACGCTCCTTTCTGGCCATACTCCTGAGGGTAACAAAATCACATGGAAGCCCAAAGCAAGCCAGGCTCAGGCTCCTCTGTCCCTC
 TGCTACTTAACAATGTCCGTCTTCCCGAGCCGCCCTGCGAGATGCTTCTGTATGGGAAAGCCCTCTGCATCTAATGACACTCTGC
 TTTCAAAGACGGGACAGTCCCTGGTCTCTGGAGAGTGAACATTGCTGGCCTTCTCAGTTGACACTTCTCCGCTGAGGCATCCCTTA
 GCCCTGAACCAGAAATGAAAGAGCCGGCTCAGAGTGAAAAGGAAGAATAGCCATCAATCTGCTCCTGTGTGCAAGGAGCACAGACC
 TGGTCTCAGACTCTGCCGTCTCCCCGCTTCCCTGCCCCCTCTGAGTGACTCAAGGTGCAGGCTGAGGGAGATGTTGATGGTATGCT
 CATCCACAAAGCCCTTCAGGCCAGGCATCCGGGCAACTTGAGCTGTGTCTGGGCACACTGTCCCAAAGTG

Natural antisense sequence (CK626173): SEQ ID NO: 16

GACTTTTATCTGCACTGTTTCAACASCAGGTAGCCAGCCGTUTTTTTTACTGCCCTGCCCTCTGGCTGAAGCTCGGCCACACTATCAG
 GACTCAGCCCTGTAGGGATGACTCTGCCACACAGCTACAGCACCAGCTGGCACAAATGGCTTTCTCTCCAACCTTCCCTCAGGCTTCC
 CTGAGTCACTGCCCAGCCCTAGGACTGGCAACACCCCTGGCCCTGCTCACCCATCCACCCCTGGCAAGAGGGAAAGAGGAAGAAGCC
 TGCAGAGAGCTGGTGGCTGTCTCCAGATGCTGCTCCATTCTCAGGCCAAGCCTCAAGATGGGGGAAACCTGAGTGGGAGCCTCTC
 TCCCTGGCTTGCCTTCCCTCCCACTTCTGGGAAAGCAGGGCAGTACAGTCCCTGTTCTCATGTGTCTGCCCTTGGCTGGGCTCCCC
 TCACCTCCCCAAAGACCCAGGCAGGGTCCCATTCAGCAGACCTGAUTGTAAGGAATTGGCAAGAAATGACGTCCCTAGCCAGCCTGG
 COTCCCCCTTGGTATTTTTCAGCTGGAGATTATTASTCTCAAGCAAAACTCTTTTGTATTCCAAGCCCACTCCACACATTATTT
 TCCCTCTCTCTAAA

Natural antisense sequence (AW544265): SEQ ID NO: 17

GACAGGGTCTTATTTCTGCAGCTCGGGTTGACCTGGATTTCTUCAGGCAGACCATTTCTGGCCTTACGTTCACTGACATCCACCTGCC
 TCTGCCCTCCTGAGTGTCTGTGTTAAGGAGTAGTTCCAGCCTATAGTGTCTGAAATACTGTTATTTTACTGTAATGATAGCCAAAG
 CTAAAATGAGTTAAGAAAGTTCCTTTCTTACTGCTGTCTCGTTCCCTCATTCACTTGCCTCATCTTCTGCTGCTCAGAACTACCC
 CACCCCCAATCCTTCTTAGCCCCAGAGCCTTCTCTSAACCTAACCCTTGCTTCTCTCAGCATCTCAGGGGCCCTCTTTGCTT

CGTTAATCTCTACTGGAAACACAGAGAACTCCCTGCCCCTCTGCCATTCTTCTGCTGGAGCTACCTTCCCACCCCTTGTGCAAGCCA
GGCCCCCTCATACCCCAAGCAGGTGACACCATCTGTGTCCAC

Natural antisense sequence (bloflor.aApr07): SEO ID NO: 18

gagctgogctcaacogctgttgcoctgattttgggtotaagtgaaggcttgoggttcagattccaacsaacttcoctttgttaaagggaat
ggacaagaaaactccccctggatatgcocttgaagccagctacagcgtgaggtgggtgcagctagaaaagtgcctagaaacacacacacag
ctctcagaaggtotggaggaaaacatcaggggtgtagtctcocttgacaaacagaggaaaacatcacattctcagccatcccgaggagaga
gaaactaaagtgtatgaacaaacaaagggcocttgcoctaagaacttccctaacattttctcttaagggaagaggttgatttgaggaaaaatcgc
ccgcttggacagctgaacogaagccattccacagcoctctgaagaagcagaggccaccccaggggggtcgggtuccgggggatatagctgccc
caccgtggctgaagatctcggctgcagaccaaaggagggggggggagattctgaggccaaactttcaacctccetaaggaacccagggcc
ttgaggggtggccggggggggccctctgtgaaacttgatcggggctgggtggggggcagggggcccccagggacaaggtgggggacggaggtg
tcggccacagagcacagcgggaacccggggacttcggccaggagggcccaggataaacggaggccgactcgtgtatgtcggcgaggccgc
tccgggggacccgggacttg

Natural antisense sequence (sherflor.aApr07): SEQ ID NO: 19

ggGGCTCCCTCTCAACCTATTCTGGCGCCTGGAGCAAGCCTTACCTGCACTCCCGCCCGCGGCGAGGAGCAAGGGCGACGGTCCAGC
GCAATTTCCAGCCCCAGGGCCCCATGCTCGCAGCCTCTGCCAGGCAGTGTCCCGACCCGATCACGACCTGCTGTCTCTAGCTGG
AAACCTTGGCTTCCCGCGATTGCACTCGGGGCCACGTCATTTACAGCATTTCATGTGAGGTTTCTAGCAGGGGGAGGAGTTTGC
AGTGGGGTGATTTTCAAATGCTTCACTCACTGCAAGAGGAGGAGTTTGGAAACGGCCGATGTGACatgggatttttaaccagtga
agctctgattccactccagtccttcgaaagtgtgcgagggcagggcgacttgatttgttgtatttgggtctccgggtgaagagctg
acgccccctcaaaaattggaaaacgcacattcttgaaagatcctcctgaaatttctcgatgtttaactgttaacatuttgtgtgttg
tcacagaaggataacaacagccttcaagatcctccaatagcctaattgcatttgtcctctctgctcaaaaaggaaaaacactaaaa
atgttggggaactccgcgcacttctataatttgcccttttccttctaggaattgtgtatagatttttagctccttctogttgpatat
tgtttttacattgttattccaaatcacctaataagacactcatcaatcaggaat

Natural antisense sequence (Hs.626623): SEO ID NO: 20

CCACGTAATTTTTTGTGTTTTTTTGTGTTTTTTTCTGTAAATGTCCTCCGGTTCTTCCATAACTTATAAACATGATTTATACCGAG
GAGATGGGAAAGTGGACGGGGCAGGGTGGACTGACCGGGGATGGGGAAGTCTCTCTCGCTGCCCCCTCGGGGCGGGCCAGGCCCT
TTGGAGGATGGGGACGCCAGGACACTCCTCCCTGAGGTTGCTGGCCGCTCTGCCCTGGGCGCTGGGAAGTCAGAGCCCGATACTC
CCCGTCCACCTGCCAGTTCACAAACTTCGACTGCTGGGTCTGGATGGCCATCTTGGACCTGAGACCGGGGCCAGCTGGTGAATGA
CC

Natural antisense sequence (Hs.714236): SEQ ID NO: 21

accatccttttttttttttttttttttATGCGTGAAACTTGGTGAATCTTTATTAAACTAGGGTCCACCCAGGAGGACGGCTGGGGCGG
GGACAGGGTCTCCCGCTGCAGGCTGCGCGGAGGCAGGAGGCACGGGGTGGCGTGGGGTGCATGGCTGCAGGCTTCGGCGTTCACT
GATTGTCCGCTGGGCACAGGGGCGGGCGCTGGTGGCCACGGCAGGCTGCACCTTCTCCACCAGCCCCGGGCCACTGGCGCTGCATGTCT
TCCACCAGGGGCTCGAAGCAGCTCTTGASGCGGGGCTTGAAGGGCTCGGGCTGCAGGCGTATCTGCTGGGGCTGCTCCTCCAGCTT
GGCGCGCACCTCCGCGACCTGCTCCTTCACCTCGTCCAGGCGGGTCCGCGGTCCGGCTGCCCATCTCCTCCATCCGCGCGCGCAGCC
GCTCGCCCCAGGCTTGGGCGCGCTGTAGCGGCTGGCGGGCCAGGGAGCCACAGTGGCGGGCCCCGACGCGGCGCTGTTCCACC
AGGGGCCCCAGGCGCTCCCGGATGGCGCTGAGGGCGCGGCTCGGCGGCCCTGCGGGGCCCCGGCGCTGGTACACTGCCAGGCGCTTCTG
CAGGTCATCGGCATCGCGGAGGAGCCGCTTACGCGAGCTTGCACAGGTGGGAGGCGAGGCGCACCGCGCAGCTCCTCGGTGCTCTGGC
CGAGCATGGGCTGCACCTCGCGCGGCTACTGCACCAGGCGGGCGCACAGCTCCTCCATGTCCGCGGCCGAGCGGGCTCGCGCGCC
TGCAGCTCCTTGGACAGCGTGGCGCGCTCTCCTCGGCCACCGGGGTCACTTGTCTCCTCCAGTTCGGATTTGTAGGCCCTTCAACTC
CTTCATG

Natural antisense sequence (DA327409 extended): SEQ ID NO: 22

CAGCTTCTCTTGCAGCTCGTSCAGCTTCTGGCGCGCGCCCTCTTGGAGCTCTGCGCGCAGCGGCTCCACCTTCTGGCGGTAGAGCT
CCATCTCCTCCTGCCACTTCTTCTGGAAGTCTGTCAGATCCAAATGGCAAACCTTCTTTCATCCACCAGGACCCCAACCCACAGGCTA
CTTATTGCTGGAAACCTACGTTTCTTCTTGGATTGAAGTAATCTCTCCCTCTTCTGGTGGCGCCACAGCACTTGCACCAACASTGG
GTAC-CAACAGACTAGCGTGCCTGCCGAAGAAGGGGTCTCTGACAATCAGGGGACAATGGGGGAATTATGCTCTCCAGACTTTCTA
CACACACAAGTCAACACAGGAAGGAAGGTAAAGAGAAAAC TAGAGAAAATAATTTTGAAGAAAAACATTTTCAGGAGGTATTGAAAGT
ACACGGTAACTCAGCCTSGGGGCAGGGGTSGAGGSCAGCAGCACTGTTTGCTGCAGCTATGCTCCTTCCTCASTGCCCTGCACACCC
GGGACTTGGCTCGGTGAGCATCTCTCGTGTCAGTGACAGCTAGTGTGA

FIGURE 4

Sequence ID	Sequence Name	Sequence
SEQ ID NO:23	CUR-0521	rUrUsGrUArAaArGrUnAsUrGArGrCGrUrGrGrUrGrUrCrAr GrCrA

SEQ ID NO:24	CUR-0519	rArArUrCrArArUrGrGrCrUrGrUrUrCrUrCrUrCrUrCrUrGrGrGrArC
SEQ ID NO:25	CUR-0523	rArCrCrUrArUrArArUrUrCrCrArGrCrArCrUrUrUrGrArGrArGrGrC
SEQ ID NO:26	CUR-1214	C*C*C*A*C*C*A*C*T*T*G*C*A*A*C*T*C*A*G*A
SEQ ID NO:27	CUR-1215	T*T*C*G*G*G*A*A*G*T*G*C*T*C*G*G*T*T*T*C*G
SEQ ID NO:28	CUR-1216	C*C*C*A*G*C*T*C*A*G*A*T*C*C*A*T*C*C*C*T*T
SEQ ID NO:29	CUR-1217	G*C*C*T*C*T*T*C*T*A*T*G*G*G*T*C*T*G*T*C
SEQ ID NO:30	CUR-1218	G*C*C*A*T*G*C*C*A*C*C*A*G*A*G*T*T*A
SEQ ID NO:31	CUR-1219	G*C*T*G*T*T*C*T*C*T*C*T*C*T*G*G*G*A*C*T*T
SEQ ID NO:32	CUR-1220	G*T*T*C*C*C*A*T*T*C*C*C*T*T*A*T*T*A*G
SEQ ID NO:33	CUR-1221	C*G*A*A*T*C*A*C*T*T*A*G*C*C*A*C*T*T*T*G
SEQ ID NO:34	CUR-1222	C*T*G*G*G*A*T*T*A*C*A*G*G*C*G*T*G*A*G*C
SEQ ID NO:35	CUR-1087	C*A*T*G*T*C*T*C*C*T*G*C*C*T*T*T*C*C*T*G*T
SEQ ID NO:36	CUR-1088	G*C*T*C*A*C*A*A*T*A*T*C*A*G*G*C*T*G*C*T*T
SEQ ID NO:37	CUR-1089	T*C*A*A*C*T*T*T*A*C*C*C*A*G*A*C*A*A*G*C*A
SEQ ID NO:38	CUR-1090	G*G*A*C*A*G*G*G*T*A*G*C*A*A*C*G*C*C*A*T*T
SEQ ID NO:39	CUR-1091	C*C*A*C*C*T*C*A*G*T*T*G*C*A*C*G*G*A*A
SEQ ID NO:40	CUR-1092	T*T*G*G*G*C*A*T*A*G*A*G*T*T*T*G*G*T*T*C

FIGURE 5

Sequence ID	Antisense Sequence Name	Sequence
SEQ ID NO:41	CUR-0476	rGrUrGrGrUrCrArGrUrCrArCrArGrCrCrArCrArCrCrArGrArCrUrU
SEQ ID NO:42	CUR-0478	rGrUrCrCrUrUrUrCrUrCrCrArCrCrArCrArCrUrGrUrGrArCrUrC
SEQ ID NO:43	CUR-0480	rCrArGrCrArCrUrGrArCrArCrArGrCrUrArCrCrArCrUrArCrUrC
SEQ ID NO:44	CUR-0444	rUrGrUrGrCrUrUrCrArCrUrGrArGrGrArUrGrGrArUrCrUrGrCrCrA
SEQ ID NO:45	CUR-0446	rArGrArGrArArArCrGrCrUrCrArUrCrArGrArCrArCrCrArCrArA
SEQ ID NO:46	CUR-0448	rCrUrUrArGrGrArArGrCrArCrArArGrArGrGrCrUrGrArGrCrCrUrU
SEQ ID NO:47	CUR-0450	rCrCrUrGrGrCrUrCrCrCrUrGrUrCrCrCrArCrCrUrUrGrCrUrCrCrA
SEQ ID NO:48	CUR-0452	rGrUrUrCrArUrUrUrCrCrArCrArCrCrCrArGrCrUrUrCrArArCrUrA
SEQ ID NO:49	CUR-0541	A*G*A*C*C*T*A*T*C*T*G*T*T*C*C*C*A*C*C
SEQ ID NO:50	CUR-0542	G*T*C*A*G*T*C*A*C*A*G*C*C*A*C*A*C*C*A*G
SEQ ID NO:51	CUR-0815	C*C*T*A*T*C*T*G*T*T*C*C*C*A*C*C*T*T*G
SEQ ID NO:52	CUR-0816	G*T*C*A*C*A*G*C*C*A*C*A*C*C*A*G*A*C*T*C*T
SEQ ID NO:53	CUR-0817	T*C*C*C*T*T*C*T*C*C*C*A*C*C*A*C*A*C*T*G*T
SEQ ID NO:54	CUR-0818	C*T*C*C*T*C*A*A*T*G*T*G*C*T*C*T*T*C*C*C
SEQ ID NO:55	CUR-0819	C*C*C*A*C*T*C*C*C*A*C*A*C*C*A*G*A*T*A
SEQ ID NO:56	CUR-0820	A*A*C*A*G*C*C*T*C*T*T*C*C*C*A*G*C*A*C*C
SEQ ID NO:57	CUR-0821	C*A*C*C*A*T*C*T*A*C*T*C*C*T*G*A*G*C*C*C

SEQ ID NO:58	CUR-0822	T*C*A*T*T*G*T*C*A*C*G*C*G*C*T*C*C*C*A*C
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FIGURE 6

Sequence ID	Antisense Sequence Name	Sequence
SEQ ID NO:59	CUR-0767	T*C*C*C*A*G*C*T*A*C*T*C*A*G*A*A*G*A*C*C*T
SEQ ID NO:60	CUR-0768	T*G*G*G*C*G*A*C*A*G*A*T*C*A*G*G*A*C*T*C
SEQ ID NO:61	CUR-0769	C*T*G*A*G*G*T*G*C*A*T*G*G*G*T*A*G*T*G*G*T
SEQ ID NO:62	CUR-0770	G*C*C*G*A*T*C*A*C*T*T*G*A*G*G*T*C*A*G*G*A
SEQ ID NO:63	CUR-0771	A*T*T*A*G*C*T*G*G*G*C*G*T*G*G*T*G*G*T*G
SEQ ID NO:64	CUR-0772	G*C*T*C*T*G*C*G*T*G*T*G*G*G*C*T*C*A*T*T
SEQ ID NO:65	CUR-0773	G*T*C*C*C*T*C*T*G*A*T*A*T*A*T*G*C*T*C*T*C
SEQ ID NO:66	CUR-0774	C*T*T*G*A*G*T*T*C*T*T*C*C*T*G*C*T*T*C*C*A
SEQ ID NO:67	CUR-0775	T*C*T*C*C*A*G*C*C*A*G*T*C*A*C*C*C*A*G*A
SEQ ID NO:68	CUR-1017	C*C*A*T*C*A*A*C*A*T*C*T*C*C*C*T*C*A*G*C*C
SEQ ID NO:69	CUR-1018	C*T*T*C*T*T*C*C*T*C*T*T*T*C*C*C*T*C*T*T
SEQ ID NO:70	CUR-1019	A*C*A*G*C*A*G*C*A*C*T*C*A*G*G*A*G*G*C*A
SEQ ID NO:71	CUR-1020	G*G*G*T*A*G*T*T*C*T*G*A*G*G*G*C*A*C*G*A
SEQ ID NO:72	CUR-1021	A*G*G*C*A*G*G*T*G*G*A*T*G*T*C*A*G*T*G
SEQ ID NO:73	CUR-1022	G*G*G*T*A*G*G*G*T*T*C*A*G*A*G*A*A*G*G*C

FIGURE 7

Sequence ID	Antisense Sequence Name	Sequence
SEQ ID NO:74	CUR-1054	A*C*T*C*C*T*C*C*T*C*T*T*G*C*A*G*T*G*A*G
SEQ ID NO:75	CUR-1055	G*C*T*G*T*T*G*T*T*A*T*C*C*T*T*C*T*G*T*G
SEQ ID NO:76	CUR-1056	A*T*C*G*C*G*G*G*A*A*G*C*C*A*G*G*G*T*T*T
SEQ ID NO:77	CUR-1057	G*C*C*A*G*A*A*T*A*G*G*T*T*G*A*G*A*G*G*G*A
SEQ ID NO:78	CUR-1058	G*C*C*G*T*T*C*G*A*A*A*C*T*C*C*T*C*C*T*C*T
SEQ ID NO:79	CUR-1059	G*G*T*T*T*C*C*G*C*T*G*T*G*C*T*C*T*G*T*G
SEQ ID NO:80	CUR-1060	A*C*G*T*T*G*C*C*T*C*A*G*A*A*T*C*T*C*C*C
SEQ ID NO:81	CUR-1061	T*T*C*C*T*C*A*A*T*C*A*A*C*C*T*C*T*T*C*C*T
SEQ ID NO:82	CUR-1062	G*T*G*A*T*G*T*T*T*C*C*T*C*T*G*T*T*G*T*C
SEQ ID NO:83	CUR-1063	C*A*C*T*T*T*C*T*A*G*C*T*G*C*A*C*C*A*C*C

FIGURE 8

Sequence ID	Antisense Sequence Name	Sequence
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SEQ ID NO:84	CUR-0973	C*C*G*T*C*C*A*C*T*T*T*C*C*C*A*T*C*T*C*C*T
SEQ ID NO:85	CUR-0974	C*C*C*G*T*C*C*A*C*T*T*T*C*C*C*A*T*C*T*C
SEQ ID NO:86	CUR-0975	C*T*T*C*A*C*A*G*C*A*C*C*A*G*G*G*C*A*G*A
SEQ ID NO:87	CUR-0976	C*C*C*A*G*U*A*G*T*C*G*A*A*G*T*T*T*G*T*G*A
SEQ ID NO:88	CUR-0977	C*C*G*G*T*C*T*C*A*G*G*T*C*C*A*A*G*A*T*G
SEQ ID NO:89	CUR-0978	T*G*G*T*G*G*A*G*A*A*G*G*T*G*C*A*G*G*C*T
SEQ ID NO:90	CUR-0979	G*A*T*T*C*A*C*C*A*A*G*T*T*T*C*A*C*G*C*A*T
SEQ ID NO:91	CUR-0980	G*A*C*G*A*G*G*T*G*A*A*G*G*A*G*C*A*G*G*T
SEQ ID NO:92	CUR-0981	T*C*G*G*A*A*C*T*G*G*A*G*G*A*A*C*A*A*C*T*G

FIGURE 9

SEQ ID NO: 93- TGGAGCTCAGTTT
 SEQ ID NO: 94- TTTCTCGTGCAGCT
 SEQ ID NO: 95- TTCTGGCGCGTGCC
 SEQ ID NO: 96- CCCTCGTGGAGCTC
 SEQ ID NO: 97- GCGCGCGCGCAGCG
 SEQ ID NO: 98- CGGCTCCACCTTCT
 SEQ ID NO: 99- CTGGCGGTAGAGCT
 SEQ ID NO: 100- GCTCGTGCAGCTTC
 SEQ ID NO: 101- CAGCTTCTGCGCG
 SEQ ID NO: 102- GCGCGCAGCGGCTC
 SEQ ID NO: 103- TCCACCTTCTGGCG
 SEQ ID NO: 104- CGGTGTAGAGCTCC
 SEQ ID NO: 105- CCACTCTCCTCTGC
 SEQ ID NO: 106- GAAGTCGTCCAGATCCAAA
 SEQ ID NO: 107- AAGTCGTCCAGATCCAAAT
 SEQ ID NO: 108- AGTCGTCCAGATCCAAATG
 SEQ ID NO: 109- GTCGTCCAGATCCAAATGG
 SEQ ID NO: 110- TCGTCCAGATCCAAATGGC
 SEQ ID NO: 111- CGTCCAGATCCAAATGGCA
 SEQ ID NO: 112- GTCCAGATCCAAATGGCAA
 SEQ ID NO: 113- TCCAGATCCAAATGGCAA
 SEQ ID NO: 114- CAGATCCAAATG
 SEQ ID NO: 115- AGATCCAAATGGCAA
 SEQ ID NO: 116- AGATCCAAATGG
 SEQ ID NO: 117- GATCCAAATGGC
 SEQ ID NO: 118- ATCCAAATGGCA
 SEQ ID NO: 119- TCCAAATGGCAA
 SEQ ID NO: 120- CCAAATGGCAA
 SEQ ID NO: 121- CCAAATGGCAAACCTTCTT
 SEQ ID NO: 122- ATGGCAAATCTTCTTCATC
 SEQ ID NO: 123- AAATGGCAAACCTTCTTCA
 SEQ ID NO: 124- AAATGGCAAATCTTCTTCA
 SEQ ID NO: 125- ATGGCAAACCTTCTTCATC
 SEQ ID NO: 126- CCAAATGGCAAATCTTCTT
 SEQ ID NO: 127- ATGGCAAACCTTCTT
 SEQ ID NO: 128- ATGGCAAATCTTCTT
 SEQ ID NO: 129- CTTCTTCATCC
 SEQ ID NO: 130- CCAGGACCCCAACCCACA
 SEQ ID NO: 131- GCTACTTAFTGCTG
 SEQ ID NO: 132- TGCTGGAAACCTAC
 SEQ ID NO: 133- TTCCTTGGATTGAA
 SEQ ID NO: 134- GCGCACAGCACTTGCA
 SEQ ID NO: 135- CACCAACAGTGGGTAC
 SEQ ID NO: 136- CAACAGACTAGC
 SEQ ID NO: 137- GAAGAAGGGTCTT
 SEQ ID NO: 138- GAATTATGCTCTCC

SEQ ID NO: 139- CTCCAGACTTTCTA
SEQ ID NO: 140- AAGTCACACAGGAAGG
SEQ ID NO: 141- GAAACTAGAGAAAA
SEQ ID NO: 142- AATAATTTTGTGAAG
SEQ ID NO: 143- GAAGTATTGAAAGT
SEQ ID NO: 144- TGAAAGTACACGGT
SEQ ID NO: 145- CTGGGGCAGGGGTG
SEQ ID NO: 146- GGGGTGGAGGGCAG
SEQ ID NO: 147- TGCTCCTTCCTCAG
SEQ ID NO: 148- ACCCGGGACTTGCTC
SEQ ID NO: 149- TGTCAGTGACAGCT
SEQ ID NO: 150- TGTCAGTGACAGCTAGTGT
SEQ ID NO: 151- GTCAGTGACAGCTAGTGTG
SEQ ID NO: 152- TCAGTGACAGCTAGTGTGA
SEQ ID NO: 153- AGTGACAGCTAGTGTGAGT
SEQ ID NO: 154- TGACAGCTAGTGTGA
SEQ ID NO: 155- TGACAGCTAGTGTGAGTAC
SEQ ID NO: 156- GACAGCTAGTGTGAGTACT
SEQ ID NO: 157- CAGCTAGTGTGAGTACTCT
SEQ ID NO: 158- AGCTAGTGTGAGTACTCTT
SEQ ID NO: 159- GCTAGTGTGAGTACTCTTA
SEQ ID NO: 160- CTAGTGTGAGTACTCTTAT
SEQ ID NO: 161- CTAGTGTGAGTACT
SEQ ID NO: 162- TASTGTGAGTACTCTTATG
SEQ ID NO: 163- AGTGTGAGTACTCTTATGT
SEQ ID NO: 164- GTGTGAGTACTCTTATGTT
SEQ ID NO: 165- TGTGAGTACTCTTATGTTG
SEQ ID NO: 166- GTGAGTACTCTTATGTTCA
SEQ ID NO: 167- TGAGTACTCTTATGTTGAG
SEQ ID NO: 168- ACTCTTATGTTGAG
SEQ ID NO: 169- TACCTCTTGACTTT
SEQ ID NO: 170- GACTTTGGGGACAA
SEQ ID NO: 171- A*A*A*C*T*G*A*G*C*T*C*C*A
SEQ ID NO: 172- A*G*C*T*G*C*A*C*G*A*G*A*A*A
SEQ ID NO: 173- G*G*C*A*C*G*C*G*C*C*A*G*A*A
SEQ ID NO: 174- G*A*G*C*T*C*C*A*C*G*A*G*G*G
SEQ ID NO: 175- C*G*C*T*G*C*G*C*G*C*G*G*G*C
SEQ ID NO: 176- A*G*A*A*G*G*T*G*G*A*G*C*C*G
SEQ ID NO: 177- A*G*C*T*C*T*A*C*C*G*C*C*A*G
SEQ ID NO: 178- G*A*A*G*C*T*G*C*A*C*G*A*G*C
SEQ ID NO: 179- C*G*C*G*C*C*A*G*A*A*G*C*T*G
SEQ ID NO: 180- G*A*G*C*C*G*C*T*G*C*G*C*G*C
SEQ ID NO: 181- C*G*C*C*A*G*A*A*G*G*T*G*G*A
SEQ ID NO: 182- G*G*A*G*C*T*C*T*A*C*A*C*G
SEQ ID NO: 183- G*C*A*G*G*A*G*G*A*G*A*T*G*G
SEQ ID NO: 184- T*T*T*G*G*A*T*C*T*G*G*A*C*G*A*C*T*T*C
SEQ ID NO: 185- A*T*T*T*G*G*A*T*C*T*G*G*A*C*G*A*C*T*T
SEQ ID NO: 186- C*A*T*T*T*G*G*A*T*C*T*G*G*A*C*G*A*C*T
SEQ ID NO: 187- C*C*A*T*T*T*G*G*A*T*C*T*G*G*A*C*G*A*C
SEQ ID NO: 188- G*C*C*A*T*T*T*G*G*A*T*C*T*G*G*A*C*G*A
SEQ ID NO: 189- T*G*C*C*A*T*T*T*G*G*A*T*C*T*G*G*A*C*G
SEQ ID NO: 190- T*T*G*C*C*A*T*T*T*G*G*A*T*C*T*G*G*A*C
SEQ ID NO: 191- T*T*T*G*C*C*A*T*T*T*G*G*A*T*C*T*G*G*A
SEQ ID NO: 192- C*A*T*T*T*G*G*A*T*C*T*G
SEQ ID NO: 193- T*T*T*G*C*C*A*T*T*T*G*G*A*T*C*T
SEQ ID NO: 194- C*C*A*T*T*T*G*G*A*T*C*T
SEQ ID NO: 195- G*C*C*A*T*T*T*G*G*A*T*C
SEQ ID NO: 196- T*G*C*C*A*T*T*T*G*G*A*T
SEQ ID NO: 197- T*G*C*C*A*T*T*T*G*G*A*T
SEQ ID NO: 198- T*T*T*G*C*C*A*T*T*T*G*G
SEQ ID NO: 199- A*A*G*A*A*G*G*T*T*T*G*C*C*A*T*T*T*G*G
SEQ ID NO: 200- G*A*T*G*A*A*G*A*A*G*A*T*T*T*G*C*C*A*T
SEQ ID NO: 201- T*G*A*A*G*A*A*G*G*T*T*T*G*C*C*A*T*T*T

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SEQ ID NO: 202- T*G*A*A*A*G*A*A*G*A*A*T*T*T*G*C*C*A*T*T*T
SEQ ID NO: 203- G*A*T*G*A*A*A*G*A*A*G*G*T*T*T*G*C*C*A*T
SEQ ID NO: 204- A*A*G*A*A*G*A*A*T*T*T*G*C*C*A*T*T*T*G*G
SEQ ID NO: 205- A*A*G*A*A*G*G*T*T*T*G*C*C*A*T
SEQ ID NO: 206- A*A*G*A*A*G*A*A*T*T*T*G*C*C*A*T
SEQ ID NO: 207- G*G*A*T*G*A*A*G*A*A*G
SEQ ID NO: 208- T*G*T*G*G*G*T*T*G*G*G*T*C*C*T*G*G
SEQ ID NO: 209- C*A*G*C*A*A*T*A*A*G*T*A*G*C
SEQ ID NO: 210- G*T*A*G*G*T*T*T*C*C*A*G*C*A
SEQ ID NO: 211- T*T*C*A*A*T*C*C*A*A*G*G*A*A
SEQ ID NO: 212- T*G*C*A*A*G*T*G*C*T*G*T*G*G*G*C
SEQ ID NO: 213- G*T*A*C*C*C*A*C*T*G*T*T*G*G*T*G
SEQ ID NO: 214- G*C*T*A*G*T*C*T*G*T*T*G
SEQ ID NO: 215- A*G*G*A*C*C*C*C*T*T*C*T*T*C
SEQ ID NO: 216- G*G*A*G*A*G*C*A*T*A*A*T*T*C
SEQ ID NO: 217- T*A*G*A*A*A*G*T*C*T*G*G*A*G
SEQ ID NO: 218- C*C*T*T*C*C*T*G*T*G*T*G*A*C*T*T
SEQ ID NO: 219- T*T*T*T*C*T*C*T*A*G*T*T*T*C
SEQ ID NO: 220- C*T*T*C*A*A*A*A*A*T*T*A*T*T
SEQ ID NO: 221- A*C*T*T*T*C*A*A*T*A*C*T*T*C
SEQ ID NO: 222- A*C*C*G*T*G*T*A*C*T*T*T*C*A
SEQ ID NO: 223- C*A*C*C*C*C*T*G*C*C*C*A*G
SEQ ID NO: 224- C*T*G*C*C*C*T*C*A*C*C*C
SEQ ID NO: 225- C*T*G*A*G*G*A*A*G*G*A*G*C*A
SEQ ID NO: 226- G*A*G*C*A*A*G*T*C*C*G*G*G*T
SEQ ID NO: 227- A*G*C*T*G*T*C*A*C*T*G*A*C*A
SEQ ID NO: 228- A*C*A*C*T*A*G*C*T*G*T*C*A*C*T*G*A*C*A
SEQ ID NO: 229- C*A*C*A*C*T*A*G*C*T*G*T*C*A*C*T*G*A*C
SEQ ID NO: 230- T*C*A*C*A*C*T*A*G*C*T*G*T*C*A*C*T*G*A
SEQ ID NO: 231- A*C*T*C*A*C*A*C*T*A*G*C*T*G*T*C*A*C*T
SEQ ID NO: 232- T*C*A*C*A*C*T*A*G*C*T*G*T*C*A
SEQ ID NO: 233- G*T*A*C*T*C*A*C*A*C*T*A*G*C*T*G*T*C*A
SEQ ID NO: 234- A*G*T*A*C*T*C*A*C*A*C*T*A*G*C*T*G*T*C
SEQ ID NO: 235- A*G*A*G*T*A*C*T*C*A*C*A*C*T*A*G*C*T*G
SEQ ID NO: 236- A*A*G*A*G*T*A*C*T*C*A*C*A*C*T*A*G*C*T
SEQ ID NO: 237- T*A*A*G*A*G*T*A*C*T*C*A*C*A*C*T*A*G*C
SEQ ID NO: 238- A*T*A*A*G*A*G*T*A*C*T*C*A*C*A*C*T*A*G
SEQ ID NO: 239- A*G*T*A*C*T*C*A*C*A*C*T*A*G
SEQ ID NO: 240- C*A*T*A*A*G*A*G*T*A*C*T*C*A*C*A*C*T*A
SEQ ID NO: 241- A*C*A*T*A*A*G*A*G*T*A*C*T*C*A*C*A*C*T
SEQ ID NO: 242- A*A*C*A*T*A*A*G*A*G*T*A*C*T*C*A*C*A*C
SEQ ID NO: 243- G*A*A*C*A*T*A*A*G*A*G*T*A*C*T*C*A*C*A
SEQ ID NO: 244- T*G*A*A*C*A*T*A*A*G*A*G*T*A*C*T*C*A*C
SEQ ID NO: 245- C*T*G*A*A*C*A*T*A*A*G*A*G*T*A*C*T*C*A
SEQ ID NO: 246- C*T*G*A*A*C*A*T*A*A*G*A*G*T
SEQ ID NO: 247- A*A*A*G*T*C*A*A*G*A*G*G*T*A
SEQ ID NO: 248- T*T*G*T*C*C*C*A*A*A*G*T*C
SEQ ID NO: 249- +A*+A*+G*A*A*G*G*T*T*T*G*C*+C*+A*+T
SEQ ID NO: 250- +T*+C*+A*C*A*C*T*A*G*C*T*G*+T*+C*+A
SEQ ID NO: 251- A*A*G*A*A*G*G*T*T*T*G*C*C*A*T*
SEQ ID NO: 252- T*C*A*C*A*C*T*A*G*C*T*G*T*C*A*
SEQ ID NO: 253- CTCCTCTGCCACTTCTTCTG
SEQ ID NO: 254- CTGGTGGATGAAGAAGGTTTGC
SEQ ID NO: 255- TTTGGATCTGGACGACTTC
SEQ ID NO: 256- +C*+A*+A*T*A*A*G*T*A*G*C*C*+T*+G*+T
SEQ ID NO: 257- +C*+A*+C*G*C*T*A*G*T*C*T*G*+T*+T*+G
SEQ ID NO: 258- +C*+T*+T*C*C*T*T*C*C*T*G*T*+G*+T*+G
SEQ ID NO: 259- +C*+A*+G*G*C*T*G*A*G*T*T*A*+C*+C*+G
SEQ ID NO: 260- +G*+C*T*A*G*T*C*T*G*+T*+T*+G
SEQ ID NO: 261- +G*+T*C*T*G*A*T*G*G*+A*+G*+A
SEQ ID NO: 262- GCTAGT
SEQ ID NO: 263- T*G*C*C*A*T*T*T*G*G*A*T*C*T*G*G*A*C*G

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FIGURE 10

Sequence ID	Sense Sequence Name	Sequence
SEQ ID NO:264	CUR-0521	rCrUrGrArCrArCrCrArArGrCrUrCrArUrArCrUrUrUrArCAA
SEQ ID NO:265	CUR-0519	rCrCrCrArGrArGrArGrArGrArArCrArGrCrCrArUrUrGrATT
SEQ ID NO:266	CUR-0523	rCrUrCrUrCrArArArGrUrGrCrUrGrGrArArUrUrArUrArGGT

FIGURE 11

Sequence ID	Antisense Sequence Name	Sequence
SEQ ID NO:267	CUR-0476	rGrUrCrUrGrGrUrGrUrGrGrCrUrGrUrGrArCrUrGrArCrCAC
SEQ ID NO:268	CUR-0478	rGrUrCrArCrArGrUrGrUrGrGrUrGrGrGrArGrArArGrGrGAC
SEQ ID NO:269	CUR-0480	rGrUrArGrGrUrGrGrUrArGrCrUrGrUrGrUrCrArGrUrGrCTG
SEQ ID NO:270	CUR-0444	rGrCrArGrArUrCrCrArUrCrCrUrCrArGrUrGrArArGrCrACA
SEQ ID NO:271	CUR-0446	rGrGrUrGrGrUrGrUrCrUrGrArUrGrArGrCrGrUrUrUrCrUCT
SEQ ID NO:272	CUR-0448	rGrGrCrUrCrArGrCrCrUrCrUrUrGrUrGrCrUrUrCrCrUrAAG
SEQ ID NO:273	CUR-0450	rGrArGrCrArArGrGrUrGrGrGrArCrArGrGrGrArGrCrCrAGG
SEQ ID NO:274	CUR-0452	rGrUrUrGrArArGrCrUrGrGrGrUrGrUrGrGrArArArUrGrAAC

FIGURE 12

SEQ ID NO: 275- TTTGGATCTGGACGACTTC
 SEQ ID NO: 276- CTCCTCCTGCCACTTCTTCTG
 SEQ ID NO: 277- CTGGTGGATGAAGAAGGTTTGC
 SEQ ID NO: 278- +G*+C*T*A*G*T*C*T*G*+T*+T*+G (CUR-962)
 SEQ ID NO: 279- +G*+T*C*T*G*A*T*G*G*+A*+G*+A (CUR-963)