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(54) **METHODS OF TREATING LIPEDEMA INCLUDING AKR1C1 AS A THERAPEUTIC TARGET**

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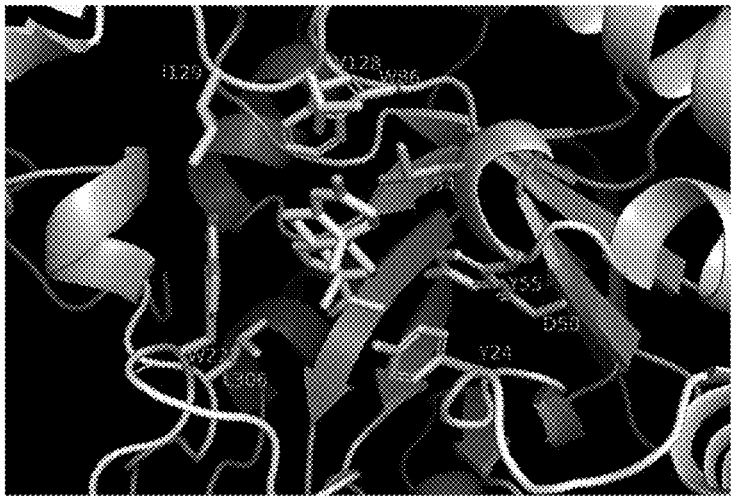
(57) **ABSTRACT**

The present invention identifies AKR1C1 as the first lipedema-associated gene. The invention provides methods for diagnosing or assessing an individual's susceptibility to lipedema by the analysis of the AKR1C1 gene or the expression levels of its product and related metabolites. Also provided are therapeutic methods for treating a patient or methods for prophylactically treating an individual susceptible to lipedema.

Specification includes a Sequence Listing.



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A61K 36/38	(2006.01)
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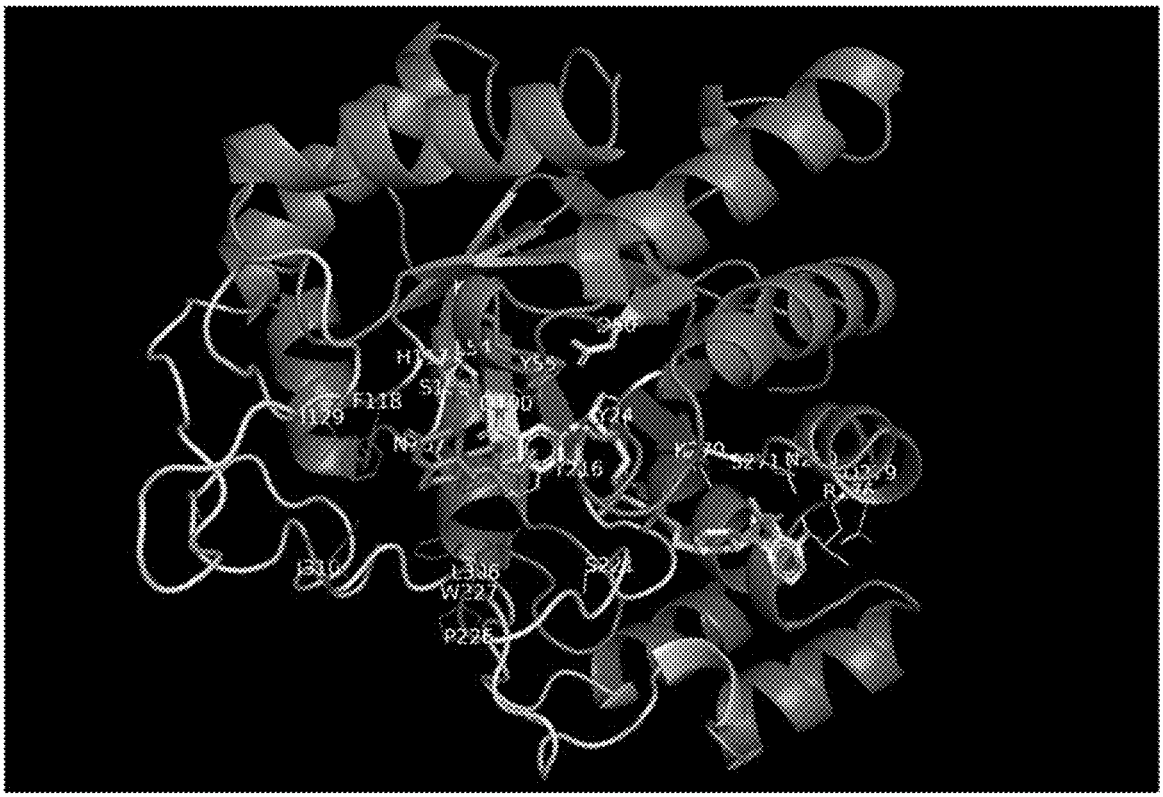
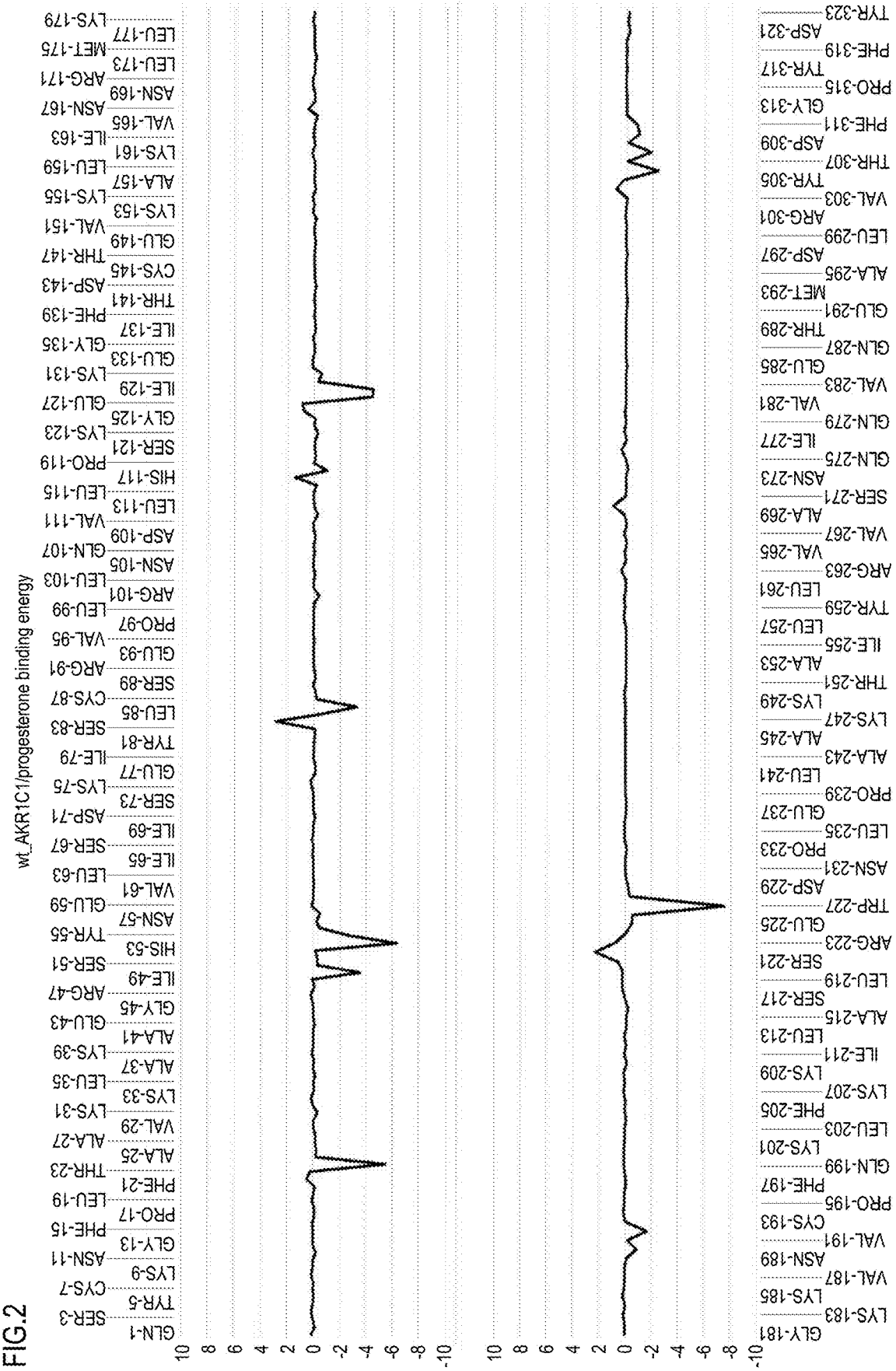


FIG. 1

FIG.2


wt_AKR1C1/progesterone binding energy
10
8
6
4
2
0
-2
-4
-6
-8
-10
GLN-1
SER-3
TYR-5
CYS-7
LYS-9
ASN-11
GLY-13
PHE-15
PRO-17
LEU-19
THR-23
ALA-25
VAL-29
ALA-27
LYS-31
LYS-33
LEU-35
ALA-37
LYS-39
ALA-41
GLU-43
GLY-45
ARG-47
ILE-49
SER-51
HIS-53
TYR-55
ASN-57
GLU-59
VAL-61
LEU-63
ILE-65
SER-67
ILE-69
ASP-71
SER-73
LYS-75
GLU-77
ILE-79
TYR-81
SER-83
LEU-85
CYS-87
SER-89
GLU-93
ARG-91
VAL-95
PRO-97
LEU-99
ARG-101
LEU-103
ASN-105
GLN-107
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VAL-111
LEU-113
LEU-115
HIS-117
PRO-119
SER-121
LYS-123
GLY-125
GLU-127
ILE-129
LYS-131
GLU-133
GLY-135
ILE-137
PHE-139
THR-141
ASP-143
CYS-145
THR-147
GLU-149
VAL-151
LYS-153
LYS-155
ALA-157
LEU-159
LYS-161
ILE-163
VAL-165
ASN-167
ASN-169
ARG-171
LEU-173
MET-175
LEU-177
LYS-179
GLY-181
LYS-183
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CYS-193
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PHE-197
GLN-199
LYS-201
LEU-203
PHE-205
LYS-207
LYS-209
ILE-211
LEU-213
ALA-215
SER-217
LEU-219
SER-221
ARG-223
GLU-225
TRP-227
ASP-229
ASN-231
PRO-233
LEU-235
GLU-237
PRO-239
LEU-241
ALA-243
ALA-245
LYS-247
LYS-249
THR-251
ALA-253
ILE-255
LEU-257
TYR-259
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ALA-269
SER-271
ASN-273
GLN-275
ILE-277
GLN-279
VAL-281
VAL-283
GLU-285
GLN-287
THR-289
GLU-291
MET-293
ALA-295
ASP-297
LEU-299
ARG-301
VAL-303
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GLY-313
PRO-315
TYR-317
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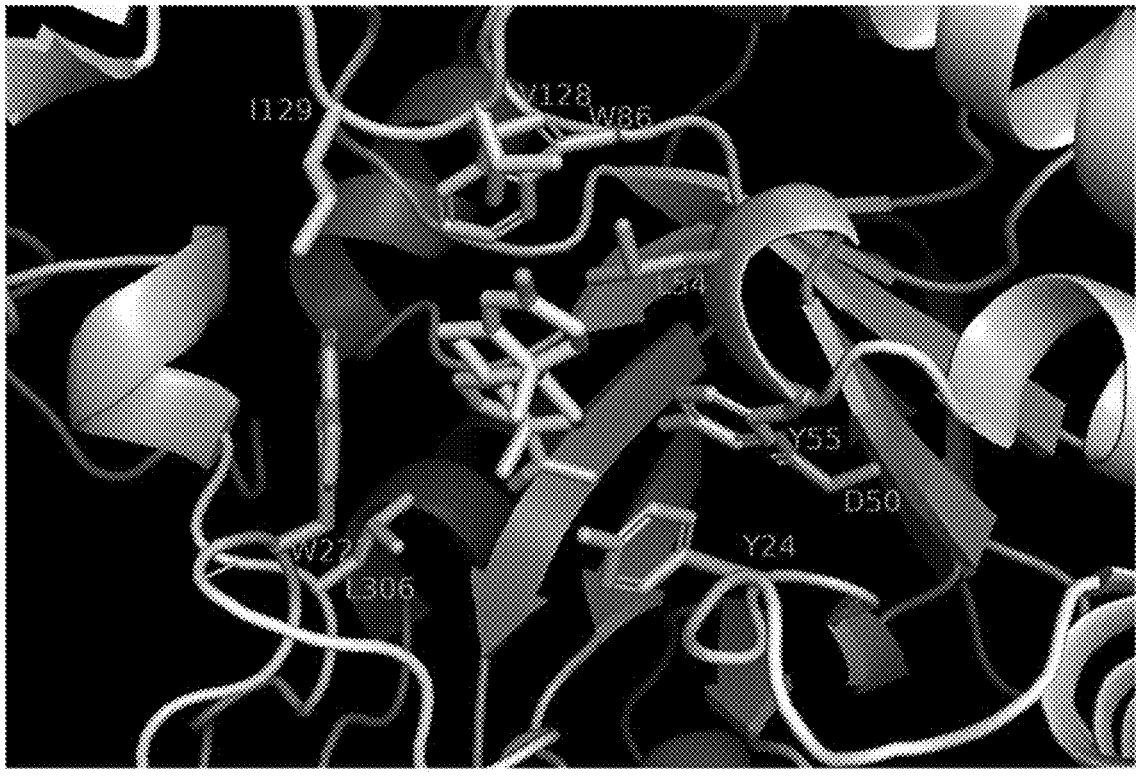
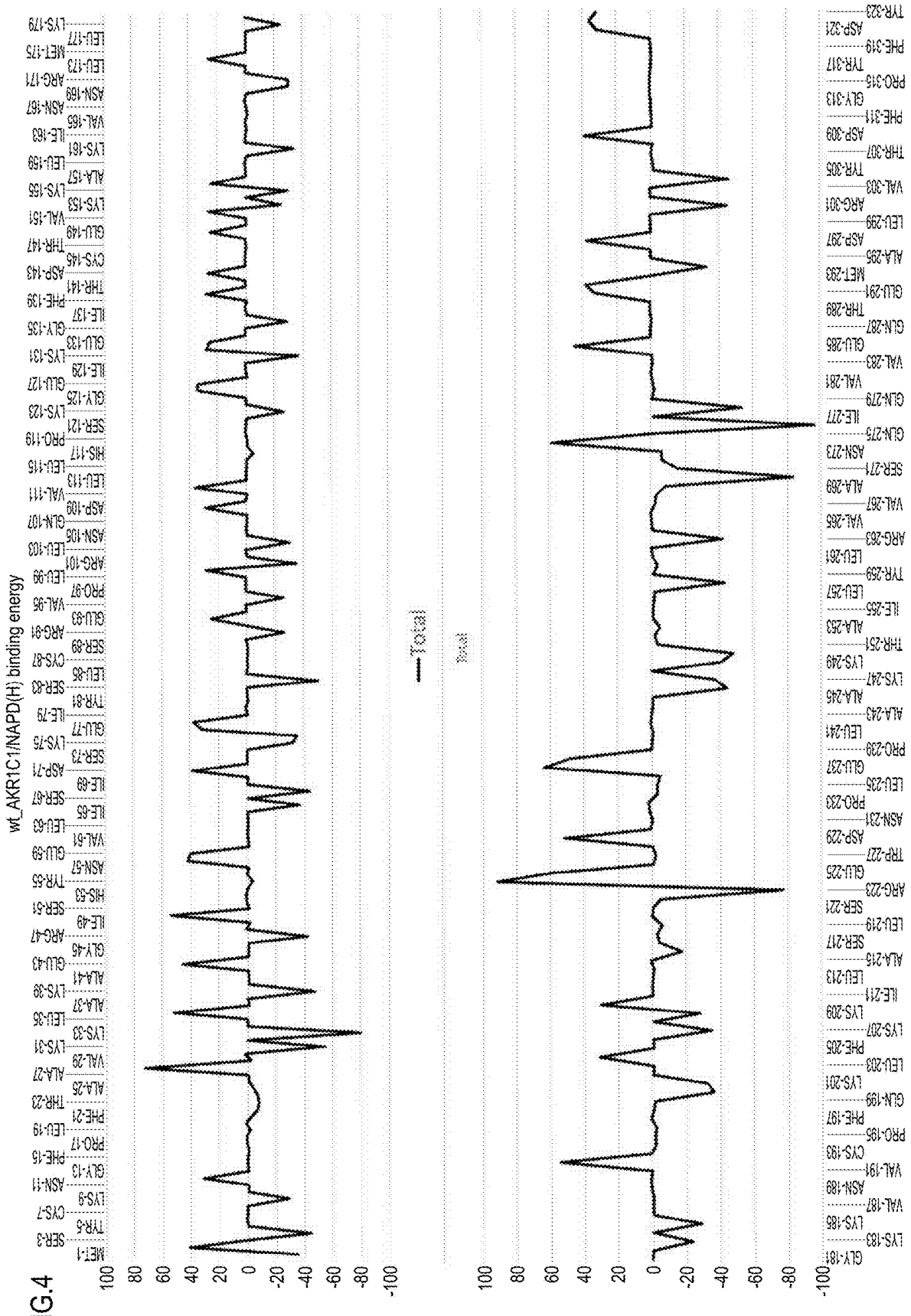


FIG. 3

FIG.4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Total
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80
60
40
20
0
-20
-40
-60
-80
-100
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SER-3
TYR-5
CYS-7
LYS-9
ASN-11
GLY-13
PHE-15
PRO-17
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ALA-37
LYS-39
ALA-41
GLU-43
GLY-45
ARG-47
ILE-49
HIS-53
SER-51
TYR-55
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ARG-101
ASN-105
LEU-103
GLN-107
ASP-109
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LEU-113
LEU-115
HIS-117
PRO-119
SER-121
LYS-123
GLY-125
GLU-127
ILE-129
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GLU-133
GLY-135
ILE-137
PHE-139
THR-141
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THR-147
GLU-149
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ASN-169
ARG-171
LEU-173
MET-175
LEU-177
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GLY-181
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SER-221
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TRP-227
ASP-229
ASN-231
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LEU-235
GLU-237
PRO-239
LEU-241
ALA-243
ALA-245
LYS-247
LYS-249
THR-251
ALA-253
ILE-255
LEU-257
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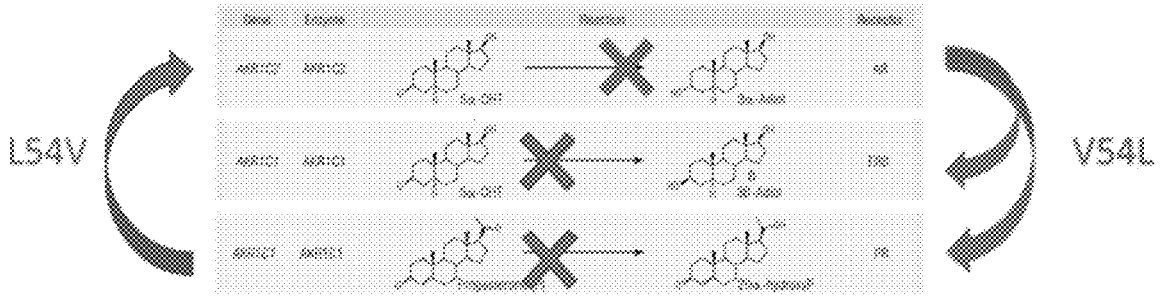

FIG. 5

(a) (b)

FIG. 6

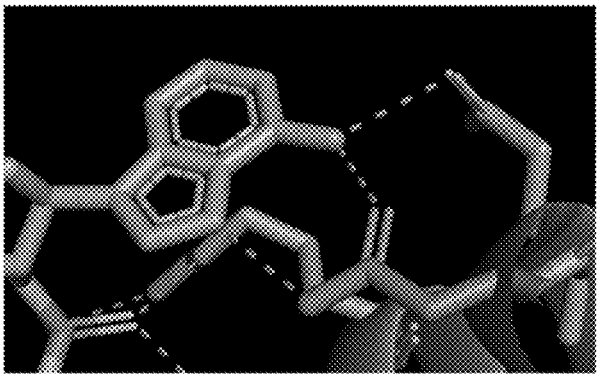

FIG. 7

METHODS OF TREATING LIPEDEMA INCLUDING AKR1C1 AS A THERAPEUTIC TARGET

FIELD OF THE INVENTION

[0001] This invention relates to methods to prevent and treat human lipedema including AKR1C1 as a diagnostic and therapeutic target.

BACKGROUND

[0002] Lipedema is a chronic and progressive pathologic condition mainly characterized by an abnormal body fat distribution. It affects extremities with abnormal fat deposition in thighs and legs and in some cases also the arms, while the trunk, hands and feet remain unaffected (Kruppa et al., 2020). For many years it has been grossly misdiagnosed because its similarity with obesity and lymphedema (Fife et al., 2010). Lipedema patients can be distinguished from these two conditions by a series of features such as body disproportion, bilateral symmetry, hematoma tendency and scarce influence of diet, exercise and bariatric surgery. It has been estimated that about 10% of the woman are affected by lipedema worldwide (Buck D W and Herbst K L, 2016). Male cases have been described in very few reports. For this reason, the involvement of sexual hormones in the etiology of the disease has been postulated several times (Torre et al., 2018; Bauer et al., 2019). In line with this hypothesis, manifestations commonly arise in phases of hormonal changes (puberty, pregnancy or menopause) in females (Torre et al., 2018). There is very strong evidence of a genetic base for the condition, since an autosomal dominant hereditary pattern was found in many families (Buso et al., 2019).

[0003] While genetic factors apparently regulate subcutaneous adipose tissue distribution, so far, no monogenic cause of non-syndromic primary lipedema have been discovered until now.

[0004] Generally, patients with lipedema undergo differential diagnosis from other disorders, and other genes are screened to exclude the patient as having a known diagnosis of another disorder of subcutaneous adipose tissue (ADRA2A, AKT2, ALDH18A1, CIDEC, LIPE, LMNA, MFN2, NSD1, PALB2, PLIN1, POU1F1, PPARG, TBL1XR1) and of localized lipodystrophies (AGPAT2, AKT2, BSCL2, CAV1, CAVIN1 (PTRF), CIDEC, LIPE, LMNA, PLIN1, PPARG, ZMPSTE24).

[0005] To date, no direct or specific treatment of the causes of lipedema has been described. Therapies are performed to help relieve symptoms and prevent frustration. When possible, a conservative management is suggested and this include manual lymph drainage, appropriate compression therapy with custom-made, flat-knitted compressive clothing, psychosocial therapy, patient education on self-management, physiotherapy and exercise therapy (such as low impact, cycling, walking or other exercise or movements), dietary counseling and weight management.

[0006] Up to now, no effective nutritional treatment has been reported for patients with lipedema. Lipedema fat is resistant to diet therapy. Current dietary approaches are aimed at lowering body weight through a hypocaloric diet, inhibiting systemic inflammation with antioxidant and anti-inflammatory components and reducing water retention (Di Renzo et al., 2021).

[0007] In some cases, if symptoms impair quality of life, the potential indication for surgery should be evaluated. Liposuction therapeutic benefit has not yet been evaluated in any randomized, controlled trials. Liposuction can reduce leg circumference, pain, feeling of tightness, tendency to form hematomas, improving quality of life. In highly advanced stages of the disease (i.e. in presence of lymphedema and fibrosis) dermato-fibro-lipectomy may be indicated.

SUMMARY OF THE INVENTION

[0008] This invention provides methods for diagnosing lipedema or identifying agents for treating a patient having lipedema or a predisposition for lipedema. The methods comprise one or more of the following steps:

[0009] detecting step to identify variants in the sequence of AKR1C1 gene from gDNA (genomic DNA). Single nucleotide polymorphism (SNP) analysis is also useful for detecting differences between alleles of AKR1C1 genes, that reside within a region of human chromosome 10. Within this region, about 700 known SNPs have been reported to date;

[0010] detecting step comprises quantifying mRNA encoding an AKR1C1 isoform in a biological sample (blood, urine and adipose tissue specimens);

[0011] detecting increment or reduction of AKR1C1 enzymatic substrate or product (i.e. steroid derivatives and prostaglandins) in a biological sample (blood, urine and adipose tissue specimens) in a lipedema patient compared to controls. The biological sample can be screened with an antibody that specifically binds to AKR1C1 enzymatic substrate or product or the biological sample can be treated or converted by AKR1C1 enzyme;

[0012] identifying natural and synthetic molecules capable of modulating AKR1C1 with possible therapeutic effect on lipedema.

[0013] Only the identification of AKR1C1 as the first lipedema-associated gene rendered the diagnostic and therapeutic approaches herein described possible. The identification of the gene and its linkage to lipedema opened the way to diagnose and treat the disease of lipedema. Since AKR1C1 is the first gene associated with the molecular diagnosis of non-syndromic lipedema, there are currently no molecular diagnostic alternatives.

[0014] Indeed, with their study, the inventors argue in favor of the involvement of AKR1C1 in lipedema (Michelini et al., 2020). AKR1C1 is a gene highly expressed in the subcutaneous tissue and it has been suggested that its activity in the regulation of steroid hormone levels plays an important role in the accumulation of subcutaneous fat depots. The enzyme expressed by this gene, the 20α-hydroxysteroid dehydrogenase (20α-HSD), metabolizes progesterone and causes over production of subcutaneous adipocytes (Blanchette et al., 2005). To date, AKR1C1 has not been implicated in any genetic condition characterized by or including lipedema among its clinical manifestations.

[0015] The association may be due to rare genetic variants or common polymorphisms that alter enzymatic function and can also be caused by epigenetic alterations.

[0016] In one embodiment of the method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to the invention, the variants of step (i) are detected from gDNA, in particular by single nucleotide polymorphism (SNP) analysis for detecting differences between alleles of AKR1C1 genes, that reside within a

region of human chromosome 10, or detected through NGS (Next Generation Sequencing) or Sanger technologies.

[0017] In an advantageous embodiment of the method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to the invention, the variants of step (i) are selected from known loss-of-function (LoF) SNPs indicated in table 1 or from a list of selected SNPs as indicated in table 2 or 4. The SNPs can, for example, be selected on the basis of the following criteria: only missense variants; absent in homozygous state; frequency below 0.1%. The selected variants are subsequently preferably studied by functional modelling to verify their impact, for example in terms of binding affinity to certain compounds. This permits to study for one or more particular variants found in a patient the binding affinity to pharmaceutically active compounds, to find the compound that best fits for the particular variant and thus for the patient being affected by this variant. Preferably, the variants are selected from the group consisting of: c.840C>A (p.Asn280Lys), c.327T>A (p.Asp109Glu), c.928A>C (p.Ile310Leu), or are selected from c.160T>G (p.Leu54Val), c.162A>T (p.Leu54Phe), c.638T>A (p.Leu213Gln), the p.Leu54 and p. Leu213 variants being particularly preferred. The six variants above are particularly interesting as they have been found in lipedema patients.

[0018] In particular, the missense variant p.(Leu213Gln) in AKR1C1, the gene encoding for an aldo-keto reductase catalyzing the reduction of progesterone to its inactive form, 20-α-hydroxyprogesterone, suggests a partial loss-of-function resulting in a slower and less efficient reduction of progesterone to hydroxyprogesterone and an increased subcutaneous fat deposition in variant carriers. The p.(Leu213Gln) variant, to the knowledge of the inventors, is the first one ever identified in a lipedemia family.

[0019] Being an inducible gene (Pallai et al., 2010), AKR1C1 expression in the blood can be a marker of the disease. Similarly, urinary and blood plasma or serum metabolites can be used as disease markers and have diagnostic value.

[0020] In another embodiment of the method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to the invention, the mRNA of step (ii) or the enzymatic substrate or product or metabolite of step (iii) is detected in a biological sample, in particular in blood, urine and/or adipose tissue specimens.

[0021] In a preferred embodiment, the enzymatic substrate or product or metabolite of step (iii) is a steroid derivative or a prostaglandin.

[0022] Preferably, the biological sample of step (iii) is screened with an antibody that specifically binds to the AKR1C1 enzymatic substrate or product or metabolite or the biological sample is treated or converted by AKR1C1 enzyme.

[0023] Preferably, the enzymatic substrate or product in step (iii) is selected among 20α-hydroxysteroid dehydrogenase (20α-HSD); PGF2α and its derivatives, in particular by measurement of 15-keto-13,14-dihydro-PGF2α, the major metabolite of PGF2α in plasma; or isoprostane 8-iso-Prostaglandin F2a (8-iso-PGF2α).

[0024] In a preferred embodiment of the method for the diagnosis of lipedema according to the invention, in step (iii) the levels of at least one of the following metabolites 3α-Hydroxy-5α-pregnan-20-one, 3α-Hydroxy-5β-pregnan-20-one, 3β-Hydroxy-5α-pregnan-20-one, 3β-Hydroxy-5β-pregnan-20-one, 5α-Pregnane-3,20-dione, 5β-Pregnane-3,20-dione, Pregn-4-ene-3,20-dione, 20α-Hydroxy-pregn-4-ene-3-one, 5α-Pregnane-3α,20α-diol, 51-Pregnane-3α,20α-diol, 5α-Androstan-17β-ol-3-one, 5α-androstane-3α,17β-diol, 21-hydroxy-5α-pregnan-20-one, 3α,21-dihydroxy-5α-pregnan-20-one, Pregnanetriol/17-hydroxypregnanolone, 15-keto-13,14-dihydro-PGF2α, in particular 8-iso-Prostaglandin F2a progesterone and/or 5alpha-dihydrotestosterone is determined in a body fluid.

[0025] In another embodiment of the method for the diagnosis of lipedema according to the invention, in step (iii) the ratio (androstanediol$^{1.5}$×20β-DH-cortisone)/(20β-DH-cortisone+[cortisol×log(estriol)] in a body fluid is determined.

[0026] AKR1C1 is a target of natural and synthetic molecules capable of modulating its activity. Benzodiazepines such as medazepam represent a class of non-competitive inhibitors of AKR1C1. Synthetic derivatives of pyrimidine, phthalimide and anthranilic acid potently inhibited AKR1C1 (Brozic et al., 2009). Compounds provided with a core structure of steroid carboxylate and flavones are instead AKR1C1 competitive inhibitors. Among natural compounds, liquiritin has been discovered as a selective and potent AKR1C1 inhibitor capable of reducing the progesterone metabolism in cells (Zeng et al., 2019).

[0027] Prostanoids, acting via peroxisome proliferator-activated receptor gamma (PPARγ), a fundamental receptor in fatty acid storage and glucose homeostasis, have been proposed as potent regulators of fat cell differentiation. Indeed, in vitro studies showed that prostaglandin J2 (PGJ2) binds and activates PPARy acting as a potent adipogenic hormone; inversely, prostaglandin F(2a) (PGF2α), which has PPARy antagonist properties, is a potent antiadipogenic factor (Quinkler et al., 2006; Volat et al., 2012). Another proof of the involvement of prostaglandins (PG) in the regulation of adipocyte differentiation came from the use of PG analogues as hypotensive agents in the treatment of glaucoma, extensively described in literature reports. Indeed, patients treated with topical therapies based on PG analogues showed periorbital fat changes as an adverse effect. These molecules can directly lead to reduced orbital fat by inhibiting adipogenesis (Taketani et al., 2014). Aldo-keto reductases have been reported as major regulators of white adipose tissue development with antiadipogenic properties supported by PGF2α synthase activity (Quinkler et al., 2006; Volat et al., 2012). Indeed, PGF2α can be synthesized from PGD2 and PGE2 by the enzymes AKR1C (1, 2 and 3) (Quinkler et al., 2006; Dozier et al., 2008) and Akr1b7 (Volat et al., 2012). In vitro studies demonstrated that PGD2 enhances adipocyte differentiation while PGE2 and PGF2α suppress adipogenesis (Miller et al., 1996).

[0028] A further aspect of the invention refers to a method of treating and/or preventing of human lipedema in a subject, the method comprising administering or applying to a subject in need thereof a therapeutically effective amount of a compound of natural or synthetic origin, preferably contained in a food supplement, cream or ointment, suitable for modulating the activity of AKR1C1 or of prostaglandins.

[0029] In one embodiment of the method of treating and/or preventing of human lipedema in a subject according to the invention, the compound is an inhibitor of AKR1C1 or modulates the catalytic activity of the AKR1C1 enzyme, and comprises at least one of the compounds indicated in table 6, in particular benzodiazepines, such as medazepam,

derivatives of pyrimidine, phthalimide and anthranilic acid, competitive inhibitors with a core structure of steroid carboxylate and flavones, and liquiritin. Advantegeously, the compound is selected from the group consisting of flavanone, flavone, 3-hydroxyflavone, 5-hydroxyflavone, equilin, diazepam, 20α-hydroxydydrogesterone, coumarin, glycyrrhetinic acid, 7-hydroxyflavone and 3,7-dihydroxyflavone.

[0030] In another embodiment of the method of treating and/or preventing of human lipedema in a subject according to the invention, the compound is suitable for modulating prostaglandins and comprises at least one of the compounds indicated in table 7.

[0031] Variants of the method of treating and/or preventing of human lipedema in a subject according to the invention foresee, that the step of administering or applying to a subject in need thereof a therapeutically effective amount of a compound of natural or synthetic origin is preceded by a step for the diagnosis of lipedema according to the invention that confirmed the tested person is affected by lipedema. Advantageously, the confirmation of the fact that the tested person is affected by lipedema is obtained by the detection of a biomarker in a body fluid in a concentration exceeding a determined limit value.

[0032] A further aspect of the invention relates to a composition for the treatment of human lipedema, in particular in the form of a food supplement, cream or ointment, comprising an inhibitor of AKR1C1 or a compound that modulates the catalytic activity of the AKR1C1 enzyme or of prostaglandins, in particular at least one of the components indicated in tables 6-10.

[0033] An additional aspect of the invention relates to a food supplement comprising the composition according to the invention. Another aspect of the invention relates to a cream comprising the composition according to the invention. A final aspect of the invention refers to an ointment comprising the composition according to the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. **1** depicts in a molecular simulation the structure of AKR1C1.

[0035] FIG. **2** is a plot relating single amino acids of AKR1C1 to their progesterone binding energy.

[0036] FIG. **3** depicts a detail of the structure of AKR1C1 and binding situations to progesterone.

[0037] FIG. **4** is a plot relating single amino acids of AKR1C1 to their NAPD(H) binding energy.

[0038] FIG. **5** illustrates schematically the role of Leu54 in substrate activity for AKR1C1 and AKR1C2.

[0039] FIG. **6** depicts in a molecular simulation the disrupted interaction between the steroid progesterone and the AKR1C1 enzyme due to the replacement of the same Leu54 by phenylalanine.

[0040] FIG. **7** compares in a molecular simulation the cofactor binding contribution of Asn280 and Gln279.

DETAILED DESCRIPTION OF INVENTION

[0041] Diagnostic methods can comprise the sequencing (through next generation sequencing [NGS] or Sanger technologies) of the AKR1C1 gene, or portions of it, or through whole genome and whole exome approaches for the diagnosis of lipedema. Single nucleotide polymorphism (SNP) analysis is also useful for detecting differences between alleles of AKR1C1 genes that reside within a region of human chromosome 10. Within this region, about 700 known SNPs have been reported to date. A list of known loss-of-function (LoF) SNPs is shown in table 1. In addition, a series of SNPs to have effect on protein function and an association with lipedema selected on the basis of the following criteria are listed in table 2: only missense variants; absent in homozygous state; frequency below 0.1%.

TABLE 1

AKR1C1 known LoF variants

Transcript Consequence	Protein Consequence	rsID	VEP Annotation	Allele Frequency %
c.84 + 1G > T		rs748912524	splice_donor_variant	0.00039896
c.64C > T	p.Gln22*	rs1430171919	stop_gained	0.000475064
c.90 + 2T > G		rs568245058	splice_donor_variant	0.029441491
c.100delG	p.Ala34Leufs*2	rs763666450	frameshift_variant	0.000399131
c.134delG	p.Gly45Alafs*30	rs1138573	frameshift_variant	0.000397874
c.172G > T	p.Glu58*	rs1302342979	stop_gained	0.000397772
c.81 − 1G > T		rs530323152	splice_acceptor_variant	0.000397779
c.81 − 1G > A		rs530323152	splice_acceptor_variant	0.003580009
c.81 − 1G > C		rs530323152	splice_acceptor_variant	0.000397779
c.196C > T	p.Arg66*	rs201114964	stop_gained	0.013793201
c.252 + 2T > C		rs775284743	splice_donor_variant	0.00122379
c.258G > A	p.Trp86*	rs143557246	stop_gained	0.000801366
c.271C > T	p.Arg91*	rs139089923	stop_gained	0.001775833
c.286C > T	p.Arg96*	rs143132605	stop_gained	0.019841832
c.369 + 2T > C		rs777080970	splice_donor_variant	0.008869274
c.394delG	p.Asp132Metfs*44	rs1188750311	frameshift_variant	0.000600478
c.394_397dupGATG	p.Glu133Glyfs*2	rs1188750311	frameshift_variant	0.000600478
c.403G > T	p.Gly135*	rs763837541	stop_gained	0.000574132
c.448 − 1G > A			splice_acceptor_variant	0.000397766
c.514C > T	p.Gln172*	rs1220725793	stop_gained	0.000397627
c.570 + 1G > A		rs770791176	splice_donor_variant	0.000795494
c.615G > A	p.Trp205*	rs1272520735	stop_gained	0.000416171
c.649dupA	p.Ser217Lysfs*58	rs370014498	frameshift_variant	0.000397864
c.667C > T	p.Arg223*	rs781923069	stop_gained	0.000796768
c.680 + 1G > A		rs142084692	splice_donor_variant	0.00850732
c.680 + 1G > C		rs142084692	splice_donor_variant	0.003899188

TABLE 1-continued

AKR1C1 known LoF variants

Transcript Consequence	Protein Consequence	rsID	VEP Annotation	Allele Frequency %
c.680 + 2T > C		rs757191838	splice_donor_variant	0.00079734
c.681 − 1G > A		rs782472454	splice_acceptor_variant	0.000400352
c.681G > A	p.Trp227*	rs782615031	stop_gained	0.002134426
c.698delC	p.Pro233Argfs*22	rs781955346	frameshift_variant	0.000712728
c.741delG	p.Lys247Asnfs*8	rs781870854	frameshift_variant	0.001989036
c.748C > T	p.Arg250*	rs782207877	stop_gained	0.001988894
c.846 + 1G > A		rs782167092	splice_donor_variant	0.000545756
c.846 + 1G > T		rs782167092	splice_donor_variant	0.003820293
c.910C > T	p.Arg304*		stop_gained	0.005656535
c.929 + 1G > A		rs781944824	splice_donor_variant	0.00209389
c.945delT	p.Asn316Ilefs*15	rs782460823	frameshift_variant	0.001235799
c.962delA	p.Asp321Valfs*10		frameshift_variant	0.000818391
c.969T > G	p.Tyr323*	rs201500205	stop_gained	0.059779068

TABLE 2

AKR1C1 selected variants

Transcript Consequence AKR1C1: NM 001353.6:	Protein Consequence	rsID	VEP Annotation	Allele Frequency %
c.160T + G[1]	p.Leu54Val	rs138675307	missense_variant	0.080147155
c.162A + T[1]	p.Leu54Phe	rs14929564	missense_variant	0.080147155
c.911G > T[2]	p.(Arg304Leu)	—	missense_variant	
c.381A + T[2]	p.(Glu127Asp)	—	missense_variant	
c.664_665delCAinsAT[2]	p.(His222Ile)	—	missense_variant	
c.664_665delCAinsTC[2]	p.(His222Ser)	—	missense_variant	
c.919_920delACinsGT[2]	p.(Thr307Val)	—	missense_variant	
c.925_926delGAinsCT (p.Asp309Leu)[2]	p.(Asp309Leu)	—	missense_variant	
c.914A > T[2]	p.(Tyr305Phe)	—	missense_variant	
c.638T > A[3]	p.Leu213Gln	rs372782197	missense_variant	0.011188627
c.22G > C	p.Val8Leu	rs752938448	missense_variant	0.000397735
c.22G > T	p.Val8Leu	rs752938448	missense_variant	0.000397735
c.32A > G	p.Asn11Ser	rs1446558895	missense_variant	0.000397772
c.5G > A*	p.Gly2Glu	rs1405103238	missense_variant	0.000475638
c.82A > G*	p.Met28Val	rs1187727403	missense_variant	0.003225598
c.97A > G	p.Lys33Glu	rs1177376359	missense_variant	0.000399109
c.104T > C	p.Leu35Ser	rs1174379434	missense_variant	0.000397988
c.139C > A	p.Arg47Ser	rs748193660	missense_variant	0.000397791
c.163T > C	p.Tyr55His	rs1564314801	missense_variant	0.000397725
c.168T > A	p.Asn56Lys		missense_variant	0.000397747
c.184G > A	p.Gly62Arg	rs1274415938	missense_variant	0.000397829
c.272G > T	p.Arg91Leu	rs375752583	missense_variant	0.000399304
c.274C > A	p.Pro92Thr	rs763383627	missense_variant	0.000399081
c.290C > G	p.Pro97Arg	rs756379873	missense_variant	0.000398594
c.298G > C	p.Glu100Gln	rs1564315232	missense_variant	0.000398318
c.338T > C	p.Leu113Pro	rs1344076147	missense_variant	0.000398362
c.355C > A	p.Pro119Thr	rs752532298	missense_variant	0.000398314
c.392A > T	p.Cys131Ile	rs369662093	missense_variant	0.000602736
c.394G > T	p.Asp132Tyr	rs1364894460	missense_variant	0.000601214
c.566A > G	p.Asn189Ser	rs771829414	missense_variant	0.00039769
c.584A > G*	p.Asp195Gly	rs1407820595	missense_variant	0.000417011
c.607C > A*	p.Pro203Thr	rs962503713	missense_variant	0.000415866
c.607C > G*	p.Pro203Ala	rs962503713	missense_variant	0.000415866
c.575A > C	p.Glu192Ala	rs1564317029	missense_variant	0.000399683
c.616T > G	p.Cys206Gly	rs782505662	missense_variant	0.00039807
c.698C > T	p.Pro233Leu	rs370027719	missense_variant	0.000401068
c.715C > A	p.Pro239Thr	rs1554769975	missense_variant	0.000398889
c.755C > G	p.Pro252Arg	rs1303247012	missense_variant	0.00039776
c.764T > C	p.Ile255Thr	rs1554770000	missense_variant	0.000397782
c.773G > T	p.Arg258Eeu	rs138128200	missense_variant	0.000397807
c.787C > G	p.Arg263Gly	rs782766545	missense_variant	0.000397842

TABLE 2-continued

AKR1C1 selected variants

Transcript Consequence AKR1C1: NM 001353.6:	Protein Consequence	rsID	VEP Annotation	Allele Frequency %
c.788G > C	p.Arg263Pro	rs535110977	missense_variant	0.000397905
c.788G > T	p.Arg263Leu	rs535110977	missense_variant	0.003183091
c.797T > C	p.Val266Ala	rs1554770013	missense_variant	0.003184105
c.962A > G	p.Asp321Gly	rs1185288451	missense_variant	0.000408243

*Further studies showed that these variants do not find a unique match between the nucleotide sequence and the amino acid sequence among all the queried databases.
The above variants are, if not stated otherwise, extracted from the following database: https://gnomad.broadinstitute.org/gene/ENSG00000187134?dataset=gnomad_r2_1
[1]identified in lipedema patients.
[2]These variants have been created in a mutagenesis experiment described by Couture et al.
[3]The enzyme activity parameters described by Couture et al. were used to calculate those of the first variant identified by Michelini et al. in a family with lipedema, p.(Leu213Gln).

[0042] The complete sequence of the AKR1C1 gene is well known and documented in literature. The following links take to a database that discloses details about the gene and the whole sequence: https://www.ensembl.org/*Homo sapiens*/Gene/Summary?db=core;g=ENSG00000187134;r=10:4963253-4983283 https://www.ensembl.org/*Homo sapiens*/Transcript/Exons?db=core;g=ENSG00000187134;r=10:4963253-4983283;t=ENST00000380872 The sequence listing reports the complete sequence of the AKR1C1 gene (*Homo sapiens*) as SEQ ID NO 1, the corresponding coding sequence (cDNA) as SEQ ID NO 2 and two isoform corresponding proteins as SEQ ID NO 3 and SEQ ID NO 4. The DNA and corresponding protein sequence of the variant c.928A>C (p.(Ile310Leu)) are depicted as SEQ ID NO 5 and SEQ ID NO 6, respectively.

[0043] Further details regarding the identification of missense AKR1C1 variants in lipedema patients, sequencing, molecular modelling etc. are described in Michelini S, Chiurazzi P, Marino V, Dell'Orco D, Manara E, Baglivo M, Fiorentino A, Maltese P E, Pinelli M, Herbst K L, Dautaj A, Bertelli M., Aldo-Keto Reductase 1C1 (AKR1C1) as the First Mutated Gene in a Family with Nonsyndromic Primary Lipedema. Int J Mol Sci. 2020 Aug. 29; 21(17):6264. doi: 10.3390/ijms21176264. PMID: 32872468; PMCID: PMC7503355.

[0044] From structural analysis and molecular dynamics it was found that (FIG. **1**): Human AKR1C1 three-dimensional structure shows an (αβ)8-barrel motif. Two more β-sheets B1 (7-9), B2 (15-17), and two more α-helices, H1(239-248) and H2 (290-298), not taking part in the core barrel structure. Three large loops complete the structure: loop A is located at 117-143, loop B is located at 217-238, and loop C is located at 299-322.

[0045] The NADP(H)-binding residues are highly conserved and include Thr23, Asp50, Ser166, Asn167, Gln190, Tyr216, Leu219, Ser221, Arg270, Ser271, Phe272, Arg276, Glu279 and Asn280, which contribute toward the binding affinity and specificity of the cofactor (see FIG. **1**). Residues involved in substrate binding are: Tyr24, Leu54, Phe118, Phe129, Thr226, Trp227, Asn306 and Tyr310, while those involved in catalysis are: Asp50, Tyr55, Lys84 and His117 (see FIG. **1**).

[0046] To describe the interaction of the enzyme with cofactor and substrate in energetic terms, thus to furnish an energy landscape of binding, molecular dynamics simulations were run on the AKR1C1/steroid/NADP(H) ternary complex, and binding energy was calculated as well by use of the MMPBSA (Genheden and Ryde, 2015) method and GROMACS molecular dynamics software (Abraham et al., 2015). The overall energies of binding for the two are (Table 3):

TABLE 3

Steroid (STR)	−115.4 kJ/mol	+/− 10.6 kJ/mol
NADP(H) (NPD)	−337.6 kJ/mol	+/− 53.9 kJ/mol

[0047] The MMPBSA method also allowed for quantification of the contribution to binding of each amino acid, allowing the impact of an amino acidic missense substitution to be evaluated as follows (see also Table 4). MMPSA profile of STR binding shows three amino acids account for 50% of the binding energy: Tyr24, Leu54, and Trp227; another significant contribution is given by Asp50, Tyr55, Trp86, Val128, Ile129, Leu306, showing an overall hydrophobic nature of the binding (see FIGS. **2** and **3**).

[0048] MMPBSA profile of NADP(H) binding is dominated by charge pairs giving prominent repulsion/attraction peaks between charged amino acids of the protein and the phosphate groups of the cofactor. The four most prominent negative binding energy peaks derive from Lys33, His222+Arg223, Lys270, Arg276, all neighboring the phosphate group on the 2' position of the ribose ring that carries the adenine moiety (see FIG. **4**). Such evidence accounts for the significant difference in affinity for NADP(H) vs NAD(H) cofactors in binding AKR enzymes, with the former showing a mid-nanomolar value (100 nM) whereas the latter binds with mid-micromolar affinity (200 mM).

[0049] Multiple alignments of protein sequences produce a matrix of aminoacids; by elaborating the columns as vectors, entropy of aminoacidic positions can be calculated according to Shannon, describing the amount of variability through a column in the alignment. The lower the value, the lower the variability accepted by the position. The inventors aligned 120 sequences from the AKR1C family to derive Shannon entropy (Strait & Dewey, 1996) of each position; values for each missense mutation from Table 2 (AKR1C1 selected variants) are reported in Table 4.

[0050] In silico mutagenesis of AKR1C1 and molecular dynamics simulations, entropy evaluation, binding energy for cofactor and for substrate allowed for the determination of the structural impact of variants, thus the structural consequence prediction on AKR1C1 that are conducive of

loss function for many of the selected mutations in Table 2. Mutations are reported alongside their predicted effect in Table 4.

TABLE 4

Transcript Consequence AKR1C1: NM_001353.6:	Protein Consequence	Shannon Entropy (natural value/normalized value to 4.32 max)	Interaction with substrate	Interaction with cofactor	Predicted structural consequence
c.160T > G	p.(Leu54Val)*	2.11/0.49	•		Known to acquire the function of AKR1C2‡
c.162A > T	p.(Leu54Phe)*	2.11/0.49	•		Disruption of substrate binding
c.911G > T[1]	p.(Arg304Leu)	0.61/0.14			Disruption of folding
c.381A > T[1]	p.(Glu127Asp)	1.72/0.40			—
c.664_665delCAinsAT[1]	p.(His222Ile)	1.78/0.41		•	Disruption of cofactor binding
c.664_665delCAinsTC[1]	p.(His222Ser)	1.78/0.41		•	Disruption of cofactor binding
c.919_920delACinsGT[1]	p.(Thr307Val)	3.21 /0.74			—
c.925_926delGAinsCT (p.Asp309Leu)[1]	p.(Asp309Leu)	2.95/0.68			—
c.914A > T[1]	p.(Tyr305Phe)	0.19/0.04			—
c.638T > A[2]	p.(Leu213Gln)*	0.26/0.06			Disruption of folding
c.22G > C	p.(Val8Leu)	1.07/0.25			—
c.22G > T	p.(Val8Leu)	1.07/0.25			—
c.32A > G	p.(Asn111Ser)	0.47/0.11			—
c.97A > G	p.(Lys33Glu)	1.95/0.45		•	Disruption of cofactor binding
c.104T > C	p.(Leu35Ser)	2.98/0.69			—
c.139C > A	p.(Arg47Ser)	0.51/0.12			Disruption of folding
c.163T > C	p.(Tyr55His)	0/0	•		Disruption of catalysis
c.168T > A	p.(Asn56Lys)	1.88/0.44			—
c.184G > A	p.(Gly62Arg)	0/0			Disruption of folding
c.272G > T	p.(Arg91Leu)	1.17/0.27			Disruption of folding
c.274C > A	p.(Pro92Thr)	0.33/0.08			Disruption of folding
c.290C > G	p.(Pro97Arg)	1.39/0.32			Disruption of folding
c.298G > C	p.(Glu100Gln)	0.14/0.03			—
c.338T > C	p.(Leu113Pro)	0/0			Disruption of folding
c.355C > A	p.(Pro119Thr)	0/0			Disruption of folding
c.392A > T	p.(Lys131Ile)	2.16/0.5			—
c.394G > T	p.(Asp132Tyr)	0.73/0.17			—
c.566A > G	p.(Asn189Ser)	0.14/0.03			Disruption of folding
c.575A > C	p.(Glu192Ala)	0/0			Disruption of folding
c.616T > G	p.(Cys206Gly)	0/0			Disruption of folding
c.698C > T	p.(Pro233Leu)	0.07/0.016			Disruption of folding
c.715C > A	p.(Pro239Thr)	0.12/0.03			Disruption of folding
c.755C > G	p.(Pro252Arg)	0.43/0.10			Disruption of folding
c.764T > C	p.(Ile255Thr)	0.97/0.22			Disruption of folding
c.773G > T	p.(Arg258Leu)	0.07/0.016			Disruption of folding
c.787C > G	p.(Arg263Gly)	0.24/0.06			Disruption of folding
c.788G > C	p.(Arg263Pro)	0.24/0.06			Disruption of folding
c.788G > T	p.(Arg263Leu)	0.24/0.06			Disruption of folding
c.797T > C	p.(Val266Ala)	0.12/0.03			Disruption of folding
c.962A > G	p.(Asp321Gly)	1/0.23			—
c.840C > A	p.(Asn280Lys)*	0.21/0.05		•	Disruption of cofactor binding
c.327T > A	p.(Asp109Glu)*	0.38/0.09			—
c.928A > C[3]	p.(Ile310Leu)*	2.93/0.68			—

Legend.

*Variants found in lipedema families are marked with an asterisk and a detailed description of structural consequences is reported below;

‡references: (Penning et al., 2019; Hara et al., 1996; Matsuura et al., 1997).

The above variants are, if not stated otherwise, extracted from the following database: https://gnomad.broadinstitute.org/gene/ENSG00000187134?dataset=gnomad_r2_1

[1]These variants have been created in a mutagenesis experiment described by Couture et al.

[2]The enzyme activity parameters described by Couture et al. were used to calculate those of the first variant identified by Michelini et al. in a family with lipedema, p.(Leu213Gln).

[3]This variant is not described in the above database, the respective DNA and protein sequences are reflected by SEQ ID NO 5 and 6, respectively.

[0051] In the following the Applicant reports a detailed descriptions of structural consequences of variants found in lipedema families.

[0052] In the inventors' patients, six missense mutations were found, namely Leu54Val, Leu54Phe, Asp109Glu, Asn280Lys, Ile310Leu and Leu213Gln. The effects of such mutations on enzyme folding, stability, and biological activity have been studied with structural biology, and molecular dynamics approach to evaluate their involvement in lipedema development.

[0053] Starting with Leu54Val and Leu54Phe, the role of Leu54 in substrate selectivity has been already elucidated (Penning et al., 2019; Hara et al., 1996; Matsuura et al., 1997), and can be summarized as follows (see also FIG. **5**). Human AKR1C1 and AKR1C2 differ in that AKR1C1 exhibits 20α-HSD activity, whereas AKR1C2 exhibits 3α-HSD. The two enzymes differ for seven amino acids, and only one is located at the active site at position 54: leucine

for C1 and valine for C2. The replacement of Leu54 by the less bulky valine changes the 20a activity to 3α. Consistently, the reverse mutation Val54Leu converts the 3α-HSD into 20α-HSD regarding its activity (Zhang et al., 2014). Evidence that enzymes work in the reduction direction in mammalian cells (Byrns et al., 2010; Byrns et al., 2012; Rizner et al., 2003; Rizner et al., 2006) lead the Leu54Val mutation to hamper the processing of progesterone.

[0054] Similarly, the interaction between the steroid and the enzyme is disrupted by the replacement of the same Leu54 by phenylalanine, as shown by the molecular dynamics simulation. In the wildtype, Leu54 and Trp227 play a significant role in binding the steroid by interacting with opposite faces of the polycyclic ring of the ligand and contribute as much as 33% of the overall binding energy. Mutation of Leu54 to Phe, although enhancing the hydrophobic nature of the interaction, introduce a second large, aromatic sidechain in place hampering the ligand entrance in the site and conducive of binding disruption (see FIGS. **6** (*a*) and (*b*)). Indeed, from the molecular dynamics simulations, we noticed that the steroid was unstable, and phenylalanine was pushed back.

[0055] Interestingly, phenylalanine is present at position 54 in the wildtype, non-human AKR1C8P, but here the steric hindrance with the opposite amino acid 227 is compensated by the presence of the smaller asparagine. At the same time, the cumbersome tryptophan is 'shifted' to position 228. Nonetheless, 1C8 preserve the same 20α-HSD activity of 1C1. As previously mentioned, this may indicate coevolution between positions 54 and 227.

[0056] Referring now to Asn280Lys, it can be said that asparagine 280 takes part in cofactor binding; together with Gln279 it is responsible for adenine group binding through a hydrogen bond to the amine group (see FIG. **7**). The molecular simulation showed how Asn is the stronger binder of the two. Such finding is also confirmed by the molecular mechanics' energy contributions to the cofactor binding resulting from MMPBSA, showing 6-fold higher interaction energy for Asn280 with respect to Gln279 (17 kJ/mol vs. 3 kJ/mol).

[0057] Although such variant involved the replacement of a small side chain with a bulky one, molecular modelling showed how hydrophobic moiety of lysine can be easily accommodated by displacement of water molecules. MD simulation confirmed a small effect is exerted on the protein structure, while the missing H-bond acceptor capability of Lys led to the loss of interaction with the adenine ring, resulting in the aromatic ring flipping away from its position, also because of the attraction of Lys280 to the phosphate group. The optimal binding geometry is then disrupted rather than folding.

[0058] On the other hand, Asp109Glu is a small structural change. Furthermore, the conservation at this position is very high, with a relatively low amino acid entropy.

[0059] Finally, Ile310Leu is a small structural change since Leu and Ile are isoforms. Furthermore, amino acid entropy is large, implying that position 310 is not conserved throughout evolution.

[0060] AKR1C1 is a member of the AKR1C family of enzymes that share a high percentage of amino acid sequence identity (from 84 to 98%). This family catalyzes NADPH dependent oxydoreductions either for the biosynthesis or inactivation of steroid hormones, bile acids and neurosteroids. All AKR1C enzyme catalyze a sequential ordered Bi—Bi substrate enzyme reaction. In particular, AKR1C1 in involved in the "alternative pathway" of androgen biosynthesis inactivating the most potent androgen 5alpha-dihydrotestosterone (5alpha-DHT) to 5alpha-androstane-3beta,17beta-diol, a potent agonist of ERbeta which exerts anti-proliferative effect. Androgens play an important role in regulation of body fat distribution in humans. They exert direct effects on adipocyte differentiation in a depot-specific manner, via the androgen receptor (AR), leading to modulation of adipocyte size and fat compartment expansion. AKR1C1 can also regulate the cellular concentration of allopregnanolone by preventing its formation from progesterone and by catalyzing its inactivation. Indeed, AKR1C1 catalyzes progesterone reaction to form the less potent progestogen 20alpha-hydroxy-4-pregnen-3-one, reduce 5alpha-pregnane-3,20-dione (5alpha-DHP) to form 20alpha-hydroxy-5alpha-pregnan-3-one or 3alpha-hydroxy-5alpha-pregnan-20-one (allopregnanolone) to a less neuroactive 5alpha-pregnane-3alpha,20alpha-diol. AKR1C1 therefore is involved in the inactivation of allopregnanolone, that acts in the central nervous system as positive allosteric modulator of gamma aminobutyric acid receptor A (GABAA). As other enzyme of the family can reduce also 20alpha-hydroxy-5alpha-pregnan-3-one to 5alpha-pregnane-3alpha,20alpha-diol. Progesterone has lipogenic action on adipose tissue by upregulating adipocyte determination and differentiation through 1/sterol regulatory element-binding protein 1c (ADD1/SREBP1c) expression in primary cultured preadipocyte from rat parametrial adipose tissue (Lacasa et al., 2001). ADD1/SREBP1c promotes adipocyte differentiation and gene expression linked to fatty acid metabolism (Kim and Spiegelman, 1996). The levels of progesterone and 5alpha-dihydrotestosterone can be detected in body fluids. Levels of progesterone ranges during normal menstrual cycles from 0 ng/ml (follicular phase) to 28 ng/ml (central luteal phase), values range from 11 to 422 ng/ml during pregnancy, while in post menopause or in males, levels of progesterone are less than 1.2 ng/ml. Levels of 5alpha-DHT range from 250-990 pg/ml in males, from 24-368 in pre menopause females and from 10-181 in post menopause females.

[0061] A recent study revealed that the best combination to diagnose polycystic ovary syndrome (PCOS), including up to four steroids, was a ratio comprising androstanediol, estriol, 20βDHcortisone and cortisol accordingly to the following formula: (androstanediol$^{1.5}$×20β-DH-cortisone)/(20β-DH-cortisone+[cortisolxlog(estriol)]. This ratio was significantly increased in PCOS compared to controls at a threshold value of ≥435 (Dhayat et al., 2018). Considering the activity of the AKR1C1 enzyme, this ratio reasonably has diagnostic value in lipedema.

[0062] AKR1C1 is also involved in catalyzing the synthesis of prostaglandins in humans (Dozier et al., 2008). It has been shown that prostaglandin 2 alpha (PGF2α) inhibited adipogenesis by activating at its specific receptor on preadipocytes (Lepak and Serrero, 1995; Taketani et al., 2014). In mice, a decrease in intra-adipose tissue PGF2α levels following Akr1b7 ablation leads to increased adiposity, a phenotype that is reversed by the chronic administration of Cloprostenol, a PGF2α agonist (Volat el al., 2012). PGF2α and its derivatives can therefore be used as molecular diagnostic/prognostic markers and therapeutic agents also in lipedema. PGF2α can be reliably quantified by measurement of 15-keto-13,14-dihydro-PGF2α, the major metabolite of

PGF2α in plasma (Helmersson et al., 2005). The isoprostane 8-iso-Prostaglandin F2α (8-iso-PGF2α), a prostaglandin-like molecule, is a quantitative ROS biomarker used to measure oxidative stress in vivo which correlates positively with BMI, intra-abdominal fat and waist circumference (Milne et al., 2015; Jia et al., 2019). Both molecules can be easily quantified in different body fluids such as plasma, serum or urine.

[0063] A list of AKR1C1 metabolites for use in diagnostics is reported in table 5.

TABLE 5

AKR1C1 metabolites for use in diagnostics

Molecule	Common name
3α-Hvdroxy-5α-pregnan-20-one	Allopregnanolone (allo)
3α-Hydroxy-5β-pregnan-20-one	Pregnanolone (preg)
3β-Hvdroxy-5α-pregnan-20-one	Isopregnanolone (iso)
3β-Hydroxy-5β-pregnan-20-one	Epipregnanolone (epi)
5α-Pregnane-3,20-dione	5α-Dihydroprogesterone (5α-DHP)
5β-Pregnane-3,20-dione	5β-Dihydroprogesterone (5β-DHP)
Pregn-4-ene-3,20-dione	Progesterone (P)
20α-Hydroxy-pregn-4-ene-3-one	20α-dihydroprogesterone (20α-OHP)
5α-Pregnane-3α,20α-diol	Allopregnanediol
5β-Pregnane-3α,20α-diol	Pregnanediol
5α-Androstan-17β-ol-3-one	5α-Dihydrotestosterone (5α-DHT)
5α-androstane-3α,17β-diol	3α-Androstanediol (3α-Adiol)
21-hydroxy-5α-pregnan-20-one	5α-Dihydrodeoxycorticosterone (5αDHDOC)
3a,21-dihydroxy-5α-pregnan-20-one	3α,5α-Tetrahydrodeoxycorticosterone
Pregnanetriol/17-hydroxypregnanolone	(alloTHDOC) (P3)/(17HP)
15-keto-13,14-dihydro-PGF2α	PGFM
8-iso-Prostaglandin F2α	8-iso-PGF2α

[0064] In the literature, a number of natural and synthetic compounds are known to exert a modulatory action on the key human progesterone-metabolizing enzyme, AKR1C.

[0065] A list of compounds for treatments for lipedema comprising the use of natural molecules or chemicals that modulate the catalytic activity of the AKR1C1 enzyme are shown in table 6.

TABLE 6

Natural and synthetic compounds that modulate AKR1C1

Compound	Main sources	Enzyme activity (inhibition/activation)
2,3-dimethoxynaphthalene-1,4-dione (DMNQ)	Synthetic	activation
20α-hydroxydydrogesterone	Synthetic	inhibition
3,5-dichlorosalycilic acid	Synthetic	inhibition
3,5-diiodosalycilic acid	Synthetic	inhibition
3,7-dihydroxyflavone	Synthetic	inhibition
3-bromo-5-phenylsalicylic acid	Synthetic	inhibition
3-Hihydroxy flavone	Synthetic	inhibition
5-Hihydroxy flavone	Synthetic	inhibition
5,7-Dihydroxyflavone	*Passiflora coerulea*	inhibition
5-Metoxy flavone	Synthetic	inhibition
7-Hydroxy flavone	Synthetic	inhibition
Abietic acid	Pine wood	inhibition
AKR1C1 Inhibitor, 5-PBSA	Synthetic	inhibition
AKR1C1-IN-1	Synthetic	inhibition
Apigenin	Snapdragon, chamomille	inhibition
Benzodiazepines (diazepam, medazepam, estazolam, flunitrazepam, nitrazepam, cloxazolam, bromazepam, oxazolam and oxazepam)	Synthetic	inhibition
Biochanin A	Red clover, soy, alfalfa sprouts, peanuts, chickpea (*Cicer arietinum*) and in other legumes	inhibition
Chrysin	Scutellaria baicalensis	inhibition
Coumarin	Woodruff, vanilla, lavender oil, tonka bean, minor constituent in cherries, strawberries, apricots	inhibition
Coumestrol	Soybeans, brussels sprouts, spinach and a variety of legumes, clover, Kala Chana, Alfalfa sprouts	inhibition

TABLE 6-continued

Natural and synthetic compounds that modulate AKR1C1

Compound	Main sources	Enzyme activity (inhibition/activation)
Cyclopentanone	Synthetic	inhibition
Curcumin	Curcuma longa	Unknown
Daidzein	Soybeans, beer	inhibition
Diethylstilbestrol	Synthetic	inhibition
Dydrogesterone	Synthetic	inhibition
Equilin	Horse estrogen; estrogen replacement therapy	inhibition
Ethacrynic acid	Synthetic	activation
Flavanone	yellow/red fruits, vegetables	inhibition
Flavone	yellow/red fruits, vegetables	inhibition
Genistein	Soybeans, beer	inhibition
Glycyrrhetinic acid	Licorice	inhibition
Hydrogen peroxide	Synthetic	activation
Kaempferol	Tea, grapes, berries and cruciferous vegetables	inhibition
Liquiritin	Licorice	inhibition
Luteolin	Parsley, artichoke, basil, celery	inhibition
Mangosteen extract	Mangosteen	inhibition
Medroxyprogesterone acetate	Synthetic	inhibition
Methyl jasmonate	Derived from jasmonic acid as found in many plants	inhibition
Naringenin	Grapefruit	inhibition
Nonsteroidal Anti-Inflammatory Drugs (mefenamic acid, indomethacin, celecoxib, diclofenac, naproxen, ibuprofen, ketoprofen, paracetamol, acetylsalicylic acid, etodolac, 3-phenoyxbenzoic acid, sulindac, meclofenamic acid, zomepirac,	Synthetic	inhibition
Norethinodrone	Synthetic	inhibition
Quercetin	Chamomille, red onions, apples, tea, endive	inhibition
Resveratrol	Skins of certain red, grapes, in peanuts, blueberries, pines, roots and stalks of knotweed	inhibition
Steroidal Inhibitors (medroxyprogesterone acetate, bethamethasone, steroidal lactones, cholanic acid derivatives	Synthetic	inhibition
t-butylhydroquinone	Synthetic	activation
Tamoxifen	Synthetic	inhibition
Wagonin	Scutellaria baicalensis	inhibition
Zearalenone	Mold-infected grain and feeds	inhibition

[0066] PGE2 and PGF2α and its analogue (viprostol, latanoprost, isopropyl unoprostone, bimatoprost) can exhibit antiadipogenic properties. Some active constituents from Chinese herbs as ricinoleic acid, acteoside, amentoflavone, quercetin-3-O-rutinoside and hinokiflavone were predicted to be prostaglandin D2 synthase (PTGDS) inhibitors (Fong et al., 2015). Inversely, other natural supplements such as chlorella and green tea are proposed be used to decrease PGE2 and PGF2α levels (Koeberle et al., 2009; Haidari et al., 2018).

[0067] A list of compounds for treatments of lipedema comprising the use of natural molecules or chemicals that modulate prostaglandins are shown in table 7.

TABLE 7

Natural and synthetic compounds that modulate prostaglandins

Compound	Main sources	Activity
Acteoside	Rehmannia glutinosa	PTGDS inhibitors
Amentoflavone	*Biota orientalis*	PTGDS inhibitors
Chlorella	Chlorella	decrease PGE2 and PGF2α levels
Green tea	Green tea	decrease PGE2 levels
Hinokiflavone	*Platycladus orientalis*	PTGDS inhibitors
Quercetin-3-O-rutinoside	*Platycladus orientalis*	PTGDS inhibitors
Ricinoleic acid	*Ricinus communis*	PTGDS inhibitors
Sennosides	*Cassia* species	increase PGE2 formation
Viprostol, latanoprost, isopropyl unoprostone, bimatoprost	Syntetic	PGF2α analogues

[0068] Natural and synthetic compounds that modulate AKR1C1 and listed in Table 6 were submitted to molecular docking procedure by using Autodock Vina 1.2 with the following parameters: AKR1C1 and NADPH coordinate from PDB entry 1MRQ; amino acids Tyr24, Leu54 and Trp227 set as flexible sidechain: docking box set centered at x=4.29 y=33.9 z=17.06 with size x=17.39 y=11.16 z=12.67, vina scoring function. Results are reported as binding affinity in Kcal/mol (the lowest, the better) in Table 8. Taking 2 Kcal/mol as the common threshold for binding energy significance, we have 11 top compounds (in bold); significantly 6 out of 11 are simple flavones/flavonones (in bold and italics). The double stacking interaction of B ring with Tyr24 phenol and Trp227 indole rings is the driving force of the interaction.

TABLE 8

Docking analysis of natural and synthetic compounds that modulate AKR1C1

Compound	Binding affinity (Kcal/mol)
Flavanone	−17.45
Flavone	−17.25
3-Hydroxyflavone	−16.12
5-Hydroxyflavone	−16.09
Equilin	−15.84
Diazepam	−15.70
20α-Hydroxydydrogesterone	−15.69
Coumarin	−15.58
Glycyrrhetinic Acid	−15.57
7-Hydroxyflavone	−15.54
3,7-Dihydroxyflavone	−15.48
Coumestrol	−15.20
Apigenin	−15.16
Flurbiprofen	−15.06
Abietic Acid	−15.04
Mefenamic Acid	−15.03
Beta-Mangostin	−15.00
Cholanic Acid	−14.98
Alpha-Mangostin	−14.96
5,7-Dyhydroxyflavone	−14.94
Naringenin	−14.93
Ketoprofen	−14.91
Naproxen	−14.70
Luteolin	−14.70
Quercetin	−14.65
Gamma-Mangostin	−14.62
Norethindrone	−14.60
Betamethasone	−14.51
Biochanin A	−14.43
Oxazolam	−14.42
Medazepam	−14.42
3-Bromo,5-Phenylsalicylic Acid	−14.32
5-Pbsa	−14.32
Genistein	−14.28
Liquiritin	−14.18
Meclofenamic Acid	−14.17
Sulindac	−14.09
5-Methoxyflavone	−14.06
Zearalenone	−13.98
Nitrazepam	−13.96
Estazolam	−13.85
Kaempferol	−13.85
Spironolactone	−13.75
Wagonin	−13.74
Bromazepam	−13.73
Indomethacin	−13.71
Daidzein	−13.68
Oxazepam	−13.66
Paracetamol	−13.66
Resveratrol	−13.50

TABLE 8-continued

Docking analysis of natural and synthetic compounds that modulate AKR1C1

Compound	Binding affinity (Kcal/mol)
Medroxyprogesterone Acetate	−13.46
2,3-Dimethoxynaphthalene-1,4-Dione	−13.43
Cloxazolam	−13.40
Medroxyprogesterone Acetate	−13.33
Diethylstilbestrol	−13.31
Ibuprofen	−13.23
Cyclopentanone	−13.20
Zomepirac	−13.03
3,5-Dichlorosalicylic Acid	−12.97
Flunitrazepam	−12.93
Tamoxifen	−12.89
Hydroxytyrosol	−12.75
T-Butylhydroquinone	−12.60
Curcumin	−12.52
Ethacrynic Acid	−12.37
Methyl Jasmonate	−11.84
3,5-Diiodosalicylic Acid	−11.76

[0069] The analysis has been repeated for AKR1C1 mutant Leu54Val by using the same parameters (Table 9). Such mutation is known to convert enzymatic activity of AKR1C1 into that of AKR1C2, which might eliminate androgen inhibitory effects on adipogenesis favouring progression of adipogenesis (Kiani et al., 2021), thus selective targeting of such mutation would modulate its possible effect on fat deposition. Again, flavones are among the favorites, but with lower affinity and competing with natural steroids like equilin or with large pentacyclic molecules glycyrrhetinic acid; this is due to the lower steric hindrance of valine vs. leucine resulting in less selective active site.

TABLE 9

Docking analysis of natural and synthetic compounds interacting with AKR1C1 mutant Leu54Val

Compound	Binding affinity (Kcal/mol)
Glycyrrhetinic Acid	−14.41
Equilin	−14.22
Flavone	−14.18
Flavanone	−14.11
3-Hydroxyflavone	−14.10
Abietic Acid	−13.91
Coumestrol	−13.63
Nitrazepam	−13.59
Betamethasone	−13.52
5-Hydroxyflavone	−13.49
20α-Hydroxydydrogesterone	−13.46
Diazepam	−13.42
Cholanic Acid	−13.41
Alpha-Mangostin	−13.41
Gamma-Mangostin	−13.38
Beta-Mangostin	−13.34
Estazolam	−13.29
Genistein	−13.24
Sulindac	−13.08
5-Pbsa	−13.04
3-Bromo-5-Phenylsalicylic Acid	−13.04
3,7-Dihydroxyflavone	−13.00
Oxazepam	−12.93
Norethindrone	−12.84
7-Hydroxyflavone	−12.84
Naringenin	−12.84
Daidzein	−12.80

TABLE 9-continued

Docking analysis of natural and synthetic compounds interacting with AKR1C1 mutant Leu54Val	
Compound	Binding affinity (Kcal/mol)
5-Methoxyflavone	−12.72
5,7-Dyhydroxyflavone	−12.62
Flunitrazepam	−12.61
Bromazepam	−12.59
Celecoxib	−12.59
Zomepirac	−12.58
Apigenin	−12.57
Luteolin	−12.56
Kaempferol	−12.51
Quercetin	−12.47
Medroxyprogesterone-Acetate	−12.41
Medroxyprogesterone-Acetate	−12.41
Coumarin	−12.40
Wagonin	−12.39
Zearalenone	−12.35
Indomethacin	−12.30
Meclofenamic Acid	−12.24
Biochanin-A	−12.17
Flurbiprofen	−12.11
Ketoprofen	−12.07
Liquiritin	−12.06
Diclofenac	−12.05
Naproxen	−12.04
Mefenamic Acid	−11.94
Medazepam	−11.69
3-Phenoxybenzoic Acid	−11.55
Resveratrol	−11.50
Oxazolam	−11.46
Cloxazolam	−11.42
Diethylstilbestrol	−11.39
Spironolactone	−11.33
Etodolac	−11.33
Tamoxifen	−11.18
Hydroxytyrosol	−11.08
Methyl-Jasmonate	−10.83
T-Butylhydroquinone	−10.79
Cyclopentanone	−10.69
Curcumin	−10.61
Ethacrynic Acid	−10.57
2,3-Dimethoxynaphthalene-1,4-Dione	−10.42
Ibuprofen	−10.24
Paracetamol	−10.15
Acetylsalicylic Acid	−10.12
3,5-Diiodosalicylic Acid	−10.02
3,5-Dichlorosalicylic Acid	−9.69

[0070] AKR1C1 Leu54Phe mutant is the other variant affecting substrate binding site accessibility presently analyzed. Oppositely but coherently with Leu54Val flavones are the tighter binders due to the incremented steric hindrance of phenylalanine which is able to stacking interact with A/C rings of the binder (Table 10).

TABLE 10

Docking analysis of natural and synthetic compounds interacting with AKR1C1 mutant Leu54Val	
Compound	Binding affinity (Kcal/mol)
Flavone	−18.35
Flavanone	−18.21
Medroxyprogesterone-Acetate	−18.04
Cholanic Acid	−17.96
Equilin	−17.81
Estazolam	−17.48
5-Hydroxyflavone	−17.29

TABLE 10-continued

Docking analysis of natural and synthetic compounds interacting with AKR1C1 mutant Leu54Val	
Compound	Binding affinity (Kcal/mol)
Nitrazepam	−17.29
Diazepam	−17.11
7-Hydroxyflavone	−16.94
20α-Hydroxydydrogesterone	−16.81
Zearalenone	−16.76
Spironolactone	−16.45
3-Hydroxyflavone	−16.42
Medazepam	−16.35
Cloxazolam	−16.33
Norethindrone	−16.30
Sulindac	−16.24
Glycyrrhetinic Acid	−16.17
Ketoprofen	−16.15
5,7-Dyhydroxyflavone	−16.13
5-Pbsa	−16.05
3-Bromo-5-Phenylsalicylic Acid	−16.05
Betamethasone	−15.93
Daidzein	−15.91
Gamma-Mangostin	−15.77
Naproxen	−15.72
3-7-Dihydroxyflavone	−15.72
Coumestrol	−15.70
Apigenin	−15.68
T-Butylhydroquinone	−15.66
Luteolin	−15.60
Naringenin	−15.56
Genistein	−15.54
Celecoxib	−15.51
Bromazepam	−15.51
Resveratrol	−15.46
Oxazolam	−15.46
Liquiritin	−15.46
Abietic Acid	−15.44
Coumarin	−15.43
Alpha-Mangostin	−15.27
3-Phenoxybenzoic Acid	−15.24
Etodolac	−15.23
3,5-Dichlorosalicylic Acid	−15.23
Biochanin-A	−15.22
Flurbiprofen	−15.21
Diethylstilbestrol	−15.12
Wagonin	−15.09
Oxazepam	−15.02
Flunitrazepam	−15.01
Kaempferol	−14.96
5-Methoxyflavone	−14.87
Mefenamic Acid	−14.82
Zomepirac	−14.57
Beta-Mangostin	−14.56
3,5-Diiodosalicylic Acid	−14.46
Acetylsalicylic Acid	−14.34
Methyl-Jasmonate	−14.09
Paracetamol	−13.97
Diclofenac	−13.92
Quercetin	−13.88
Hydroxytyrosol	−13.86
Indomethacin	−13.80
Meclofenamic Acid	−13.78
2,3-Dimethoxynaphthalene-1,4-Dione	−13.73
Ibuprofen	−13.58
Cyclopentanone	−13.29
Curcumin	−13.00
Tamoxifen	−12.89
Ethacrynic Acid	−12.19

[0071] The molecules of tables 9 and 10 have been analyzed considering the interaction with two specific variants, both on nucleotide 54. For every substance indicated in table 8, it is possible to identify through a study determining the

affinity to AKR1C1 the most efficient one for a patient with a specific variant, as done for a patient with a variant on nucleotide 54.

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<210> SEQ ID NO 3
<211> LENGTH: 323
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

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Pro Val Leu Gly Phe Gly Thr Tyr Ala Pro Ala Glu Val Pro Lys Ser
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Lys Ala Leu Glu Ala Thr Lys Leu Ala Ile Glu Ala Gly Phe Arg His
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Ile Asp Ser Ala His Leu Tyr Asn Asn Glu Glu Gln Val Gly Leu Ala
    50                  55                  60

Ile Arg Ser Lys Ile Ala Asp Gly Ser Val Lys Arg Glu Asp Ile Phe
65                  70                  75                  80

Tyr Thr Ser Lys Leu Trp Cys Asn Ser His Arg Pro Glu Leu Val Arg
                85                  90                  95

Pro Ala Leu Glu Arg Ser Leu Lys Asn Leu Gln Leu Asp Tyr Val Asp
            100                 105                 110

Leu Tyr Leu Ile His Phe Pro Val Ser Val Lys Pro Gly Glu Glu Val
        115                 120                 125

Ile Pro Lys Asp Glu Asn Gly Lys Ile Leu Phe Asp Thr Val Asp Leu
    130                 135                 140

Cys Ala Thr Trp Glu Ala Val Glu Lys Cys Lys Asp Ala Gly Leu Ala
145                 150                 155                 160

Lys Ser Ile Gly Val Ser Asn Phe Asn Arg Arg Gln Leu Glu Met Ile
                165                 170                 175

Leu Asn Lys Pro Gly Leu Lys Tyr Lys Pro Val Cys Asn Gln Val Glu
            180                 185                 190

Cys His Pro Tyr Phe Asn Gln Arg Lys Leu Leu Asp Phe Cys Lys Ser
        195                 200                 205

Lys Asp Ile Val Leu Val Ala Tyr Ser Ala Leu Gly Ser His Arg Glu
    210                 215                 220

Glu Pro Trp Val Asp Pro Asn Ser Pro Val Leu Leu Glu Asp Pro Val
225                 230                 235                 240

Leu Cys Ala Leu Ala Lys Lys His Lys Arg Thr Pro Ala Leu Ile Ala
                245                 250                 255

Leu Arg Tyr Gln Leu Gln Arg Gly Val Val Val Leu Ala Lys Ser Tyr
            260                 265                 270

Asn Glu Gln Arg Ile Arg Gln Asn Val Gln Val Phe Glu Phe Gln Leu
        275                 280                 285

Thr Ser Glu Glu Met Lys Ala Ile Asp Gly Leu Asn Arg Asn Val Arg
    290                 295                 300

Tyr Leu Thr Leu Asp Ile Phe Ala Gly Pro Pro Asn Tyr Pro Phe Ser
305                 310                 315                 320

Asp Glu Tyr


<210> SEQ ID NO 4
<211> LENGTH: 297
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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Pro Val Leu Gly Phe Gly Thr Tyr Ala Pro Ala Glu Val Pro Lys Ser
            20                  25                  30

Lys Ala Leu Glu Ala Thr Lys Leu Ala Ile Glu Ala Gly Phe Arg His
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Ile Asp Ser Ala His Leu Tyr Asn Asn Glu Glu Gln Val Gly Leu Ala
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Ile Arg Ser Lys Ile Ala Asp Gly Ser Val Lys Arg Glu Asp Ile Phe
65                  70                  75                  80

Tyr Thr Ser Lys Leu Trp Cys Asn Ser His Arg Pro Glu Leu Val Arg
                85                  90                  95

Pro Ala Leu Glu Arg Ser Leu Lys Asn Leu Gln Leu Asp Tyr Val Asp
            100                 105                 110

Leu Tyr Leu Ile His Phe Pro Val Ser Val Lys Ala Val Glu Lys Cys
        115                 120                 125

Lys Asp Ala Gly Leu Ala Lys Ser Ile Gly Val Ser Asn Phe Asn Arg
    130                 135                 140

Arg Gln Leu Glu Met Ile Leu Asn Lys Pro Gly Leu Lys Tyr Lys Pro
145                 150                 155                 160

Val Cys Asn Gln Val Glu Cys His Pro Tyr Phe Asn Gln Arg Lys Leu
                165                 170                 175

Leu Asp Phe Cys Lys Ser Lys Asp Ile Val Leu Val Ala Tyr Ser Ala
            180                 185                 190

Leu Gly Ser His Arg Glu Glu Pro Trp Val Asp Pro Asn Ser Pro Val
        195                 200                 205

Leu Leu Glu Asp Pro Val Leu Cys Ala Leu Ala Lys Lys His Lys Arg
    210                 215                 220

Thr Pro Ala Leu Ile Ala Leu Arg Tyr Gln Leu Gln Arg Gly Val Val
225                 230                 235                 240

Val Leu Ala Lys Ser Tyr Asn Glu Gln Arg Ile Arg Gln Asn Val Gln
                245                 250                 255

Val Phe Glu Phe Gln Leu Thr Ser Glu Glu Met Lys Ala Ile Asp Gly
            260                 265                 270

Leu Asn Arg Asn Val Arg Tyr Leu Thr Leu Asp Ile Phe Ala Gly Pro
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Pro Asn Tyr Pro Phe Ser Asp Glu Tyr
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<210> SEQ ID NO 5
<211> LENGTH: 19869
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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Lys Ala Leu Glu Ala Thr Lys Leu Ala Ile Glu Ala Gly Phe Arg His
        35                  40                  45

Ile Asp Ser Ala His Leu Tyr Asn Asn Glu Glu Gln Val Gly Leu Ala
    50                  55                  60

Ile Arg Ser Lys Ile Ala Asp Gly Ser Val Lys Arg Glu Asp Ile Phe
65                  70                  75                  80

Tyr Thr Ser Lys Leu Trp Cys Asn Ser His Arg Pro Glu Leu Val Arg
                85                  90                  95

Pro Ala Leu Glu Arg Ser Leu Lys Asn Leu Gln Leu Asp Tyr Val Asp
            100                 105                 110

Leu Tyr Leu Ile His Phe Pro Val Ser Val Lys Pro Gly Glu Glu Val
        115                 120                 125

Ile Pro Lys Asp Glu Asn Gly Lys Ile Leu Phe Asp Thr Val Asp Leu
    130                 135                 140

Cys Ala Thr Trp Glu Ala Val Glu Lys Cys Lys Asp Ala Gly Leu Ala
145                 150                 155                 160

Lys Ser Ile Gly Val Ser Asn Phe Asn Arg Arg Gln Leu Glu Met Ile
                165                 170                 175
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Leu Asn Lys Pro Gly Leu Lys Tyr Lys Pro Val Cys Asn Gln Val Glu
            180                 185                 190

Cys His Pro Tyr Phe Asn Gln Arg Lys Leu Leu Asp Phe Cys Lys Ser
        195                 200                 205

Lys Asp Ile Val Leu Val Ala Tyr Ser Ala Leu Gly Ser His Arg Glu
    210                 215                 220

Glu Pro Trp Val Asp Pro Asn Ser Pro Val Leu Leu Glu Asp Pro Val
225                 230                 235                 240

Leu Cys Ala Leu Ala Lys Lys His Lys Arg Thr Pro Ala Leu Ile Ala
                245                 250                 255

Leu Arg Tyr Gln Leu Gln Arg Gly Val Val Val Leu Ala Lys Ser Tyr
            260                 265                 270

Asn Glu Gln Arg Ile Arg Gln Asn Val Gln Val Phe Glu Phe Gln Leu
        275                 280                 285

Thr Ser Glu Glu Met Lys Ala Ile Asp Gly Leu Asn Arg Asn Val Arg
    290                 295                 300

Tyr Leu Thr Leu Asp Leu Phe Ala Gly Pro Pro Asn Tyr Pro Phe Ser
305                 310                 315                 320

Asp Glu Tyr
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1. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof with molecules capable of modulating the activity of AKR1C1 comprising at least one of the following steps:

(i) detecting step to identify rare and polymorphic variants in the sequence of AKR1C1 gene, copy number variants (CNV), complex rearrangements and epigenetic modifications;

(ii) detecting step to quantify mRNA encoding an AKR1C1 isoform or to verify the presence of mRNA encoding an AKR1C polypeptide or fragment thereof;

(iii) detecting an increment or reduction of AKR1C1 enzymatic substrate or product or metabolites, in a biological sample of a lipedema patient compared to controls;

(iv) identifying natural and synthetic molecules capable of modulating AKR1C1 with possible therapeutic effect on lipedema.

2. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the variants of step (i) are detected from gDNA, in particular by single nucleotide polymorphism (SNP) analysis for detecting differences between alleles of AKR1C1 genes, that reside within a region of human chromosome 10, or detected through NGS or Sanger technologies.

3. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the mRNA of step (ii) or the enzymatic substrate or product or metabolite of step (iii) is detected in in a biological sample.

4. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **3** wherein the biological sample is blood, urine and/or adipose tissue specimens.

5. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the enzymatic substrate or product or metabolite of step (iii) is a steroid derivative or a prostaglandin.

6. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **3** wherein the biological sample of step (iii) is screened with an antibody that specifically binds to the AKR1C1 enzymatic substrate or product or metabolite or the biological sample is treated or converted by AKR1C1 enzyme.

7. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the variants of step (i) are selected from known loss-of-function (LoF) SNPs selected from the group consisting of c.84+1G>T, c.64C>T, c.90+2T>G, c.100delG, c.134delG, c.172G>T, c.81-1G>T, c.81-1G>A, c.81-1G>C, c.196C>T, c.252+2T>C, c.258G>A, c.271C>T, c.286C>T, c.369+2T>C, c.394delG, c.394_397dupGATG, c.403G>T, c.448-1G>A, c.514C>T, c.570+1G>A, c.615G>A, c.649dupA, c.667C>T, c.680+1G>A, c.680+1G>C, c.680+2T>C, c.681-1G>A, c.681G>A, c.698delC, c.741delG, c.748C>T, c.846+1G>A, c.846+1G>T, c.910C>T, c.929+1G>A, c.945delT, c.962delA, c.969T>G or selected from c.160T>G, c.162A>T, c.911G>T, c.381A>T, c.664_665delCAinsAT, c.664_665delCAinsTC, c.919_920delACinsGT, c.925_926delGAinsCT (p.Asp309Leu), c.914A>T, c.638T>A, c.22G>C, c.22G>T, c.32A>G, c.5G>A, c.82A>G, c.97A>G, c.104T>C, c.139C>A, c.163T>C, c.168T>A, c.184G>A, c.272G>T, c.274C>A, c.290C>G, c.298G>C, c.338T>C, c.355C>A, c.392A>T, c.394G>T, c.566A>G, c.584A>G, c.607C>A, c.607C>G, c.575A>C, c.616T>G, c.698C>T, c.715C>A, c.755C>G, c.764T>C, c.773G>T, c.787C>G, c.788G>C, c.788G>T, c.797T>C, c.962A>G.

8. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the variants of step (i) are selected from the group consisting of: c.840C>A (p.Asn280Lys), c.327T>A (p.Asp109Glu), c.928A>C (p.Ile310Leu).

9. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the variants of step (i) are selected from c.160T>G (p.Leu54Val), c.638T>A (p.Leu213Gln), and c.162A>T (p.Leu54Phe).

10. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the SNPs are selected on the basis of the following criteria: only missense variants; absent in homozygous state; frequency below 0.1%.

11. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **10** wherein the selected variants are studied by functional modelling to verify their impact in terms of binding affinity to certain pharmaceutically active compounds.

12. Method for the diagnosis of lipedema and/or for the individuation of treatments thereof according to claim **1** wherein the enzymatic substrate or product in step (iii) is selected among 20α-hydroxysteroid dehydrogenase (20α-HSD); PGF2α and its derivatives, in particular by measurement of 15-keto-13,14-dihydro-PGF2α, the major metabolite of PGF2α in plasma; or isoprostane 8-iso-Prostaglandin F2α (8-iso-PGF2α).

13. Method for the diagnosis of lipedema according to claim **1** wherein in step (iii) the levels of at least one of the following metabolites 3α-Hydroxy-5α-pregnan-20-one, 3α-Hydroxy-5β-pregnan-20-one, 3β-Hydroxy-5α-pregnan-20-one, 3β-Hydroxy-5β-pregnan-20-one, 5α-Pregnane-3,20-dione, 5β-Pregnane-3,20-dione, Pregn-4-ene-3,20-dione, 20α-Hydroxy-pregn-4-ene-3-one, 5α-Pregnane-3α,20α-diol, 5β-Pregnane-3α,20α-diol, 5α-Androstan-17β-ol-3-one, 5α-androstane-3α,17β-diol, 21-hydroxy-5α-pregnan-20-one, 3α,21-dihydroxy-5α-pregnan-20-one, Pregnanetriol/17-hydroxypregnanolone, 15-keto-13,14-dihydro-PGF2α, in particular 8-iso-Prostaglandin F2α progesterone and/or 5alpha-dihydrotestosterone is determined in a body fluid.

14. Method for the diagnosis of lipedema according to claim **1** wherein in step (iii) the ratio (androstanediol$^{1.5}$× 20β-DH-cortisone)/(20β-DH-cortisone+[cortisolxlog(estriol)] in a body fluid is determined.

15. A method of treating and/or preventing of human lipedema in a subject, the method comprising administering or applying to a subject in need thereof a therapeutically effective amount of a compound of natural or synthetic origin, preferably contained in a food supplement, cream or ointment, suitable for modulating the activity of AKR1C1 or of prostaglandins.

16. The method according to claim **15** wherein the compound is an inhibitor of AKR1C1 or modulates the catalytic activity of the AKR1C1 enzyme, and comprises at least one the compounds indicated in table 6, in particular benzodiazepines, such as medazepam, derivatives of pyrimidine, phthalimide and anthranilic acid, competitive inhibitors with a core structure of steroid carboxylate and flavones, and liquiritin or at least one of the compounds indicated in table 8, preferably flavanone, flavone, 3-hydroxyflavone, 5-hydroxyflavone, equilin, diazepam, 20α-hydroxydydrogesterone, coumarin, glycyrrhetinic acid, 7-hydroxyflavone and 3,7-dihydroxyflavone.

17. The method according to claim **15** wherein the compound is suitable for modulating prostaglandins and comprises at least one of the compounds selected from acteoside, amentoflavone, chlorella, green tea, hinokiflavone, quercetin-3-O-rutinoside, ricinoleic acid, sennosides, viprostol, latanoprost, isopropyl unoprostone, bimatoprost.

18. The method according to claim **15** wherein the step of administering or applying to a subject in need thereof a therapeutically effective amount of a compound of natural or synthetic origin is preceded by a step for the diagnosis of lipedema that confirmed the tested person is affected by lipedema, said step for the diagnosis of lipedema comprising at least one of the following steps:

(i) detecting step to identify rare and polymorphic variants in the sequence of AKR1C1 gene, copy number variants (CNV), complex rearrangements and epigenetic modifications;

(ii) detecting step to quantify mRNA encoding an AKR1C1 isoform or to verify the presence of mRNA encoding an AKR1C polypeptide or fragment thereof;

(iii) detecting an increment or reduction of AKR1C1 enzymatic substrate or product or metabolites, in a biological sample of a lipedema patient compared to controls;

(iv) identifying natural and synthetic molecules capable of modulating AKR1C1 with possible therapeutic effect on lipedema.

19. The method according to claim **18** wherein the confirmation of the fact that the tested person is affected by lipedema is obtained by the detection of a biomarker in a body fluid in a concentration exceeding a determined limit value.

20. A composition for the treatment of human lipedema, in particular in the form of a food supplement, cream or ointment, comprising an inhibitor of AKR1C1 or a compound that modulates the catalytic activity of the AKR1C1 enzyme or of prostaglandins, in particular at least one of the components indicated in tables 6-8, in particular benzodiazepines, such as medazepam, derivatives of pyrimidine, phthalimide and anthranilic acid, competitive inhibitors with a core structure of steroid carboxylate and flavones, and liquiritin, flavanone, flavone, 3-hydroxyflavone, 5-hydroxyflavone, equilin, diazepam, 20α-hydroxydydrogesterone, coumarin, glycyrrhetinic acid, 7-hydroxyflavone and 3,7-dihydroxyflavone, acteoside, amentoflavone, chlorella, green tea, hinokiflavone, quercetin-3-O-rutinoside, ricinoleic acid, sennosides, viprostol, latanoprost, isopropyl unoprostone, bimatoprost.

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