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(54) Title: TARGETING AMP DEAMINASE 2 FOR AMELIORATING CRAVING FOR SUGAR AND OTHER SUBSTANCES

(57) Abstract: The invention relates to the use of adenosine monophosphate deaminase 2 (AMPD2) inhibitors alone or in combination with various agents to treat a wide variety of diseases including, but not limited to, sugar craving, salt craving, umami craving, and addictions including drug, tobacco, nicotine and alcohol addictions. Embodiments of the invention may also relate to stimulation of AMPD2 activity to treat anorexia nervosa or stimulate food intake in a subject.



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## TARGETING AMP DEAMINASE 2 FOR AMELIORATING CRAVING FOR SUGAR AND OTHER SUBSTANCES

### FIELD

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The present inventors have identified adenosine monophosphate deaminase isoform 2 (AMPD2) as a key enzyme that contributes to metabolic conditions characterized by craving for sugar, salt, and umami foods and drinks, as well as other cravings such as nicotine, alcohol, opiates, cocaine and other drug cravings familiar to drug addictions. In particular, isoform specific inhibitors targeting AMPD 2, or pan AMPD inhibitors will be effective for the prevention and/or treatment of these conditions. On the other hand, potentiators of AMPD2 activity and/or expression will be an effective therapeutic approach for anorexia nervosa as it would stimulate appetite.

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### BACKGROUND

Fructokinase (ketohexokinase, KHK) is a key enzyme in fructose metabolism, and phosphorylates fructose to fructose-1-phosphate. In turn, fructose 1-phosphate (F-1-P) is metabolized by aldolase B and triokinase to dihydroxyacetone phosphate (DHAP) and glyceraldehyde that continue to be metabolized, eventually generating glucose, glycogen and triglycerides. While this latter pathway is the classical caloric pathway by which fructose is metabolized, there is also a noncaloric sidechain reaction triggered when fructose is phosphorylated to F-1-P by fructokinase C (one of the isoforms). Specifically, the phosphorylation of fructose occurs very rapidly due to an inefficient negative regulation of fructokinase by downstream products –unlike glucokinase and most sugar kinases- thus causing adenosine triphosphate (ATP) and intracellular phosphate to become depleted. Adenosine monophosphate (AMP) then accumulates from ATP depletion (FIG.1) and is metabolized by the enzyme, adenosine monophosphate deaminase 2 (AMPD2) to generate inosine monophosphate (IMP) which is progressively broken down, eventually to uric acid by xanthine oxidoreductase (XO).

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Humans are known to like multiple types of sugars (table sugar also known as sucrose but also monosaccharides alone –glucose, fructose- or in mixtures –high fructose corn syrup-), as the stimulation of sweet taste causes a rapid feeling of pleasantness due in part to the stimulation of dopamine in the brain(1, 2). and/or by inefficiently stimulating the activity of the sugar satiety hormone fgf21 produced in the liver Other sweet substances, such as high fructose corn syrup, glucose, fructose, and even artificial sugars (sucralose) can also

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stimulate dopamine responses. However, it has been shown that the repeated ingestion of sugar in mice can lead to a craving or addiction syndrome characterized by altered levels of dopamine and dopamine receptors in dopaminergic neurons, thus leading to the necessity of more sugar to induce similar dopamine responses in the brain. Animals addicted to sugar develop features similar to that observed with drug addiction, and show signs of anxiety or withdrawal following elimination of sugar from the diet or the administration of naloxone (3, 4). The mechanism relates in part to a reduction in dopamine receptors (especially D2 receptors) in the nucleus accumbens from chronic dopamine stimulation, leading to a loss of control mechanisms in the frontal and prefrontal cortex.(5) The importance of this pathway is being increasingly recognized as a mechanism that results in lack of normal control, and may have a role in the pathogenesis of obesity, attention deficit hyperactivity disorder, drug addictions, sex addictions, and even aggressive behavior and dementia (2, 6-11). Thus, identifying a way to modulate metabolic pathways which cause or result in these cravings, including sugar, and other food cravings as well as cravings in general, such as nicotine, alcohol, opiates, cocaine and related agents, and including sexual cravings, among others could be beneficial in reducing diseases that result from these cravings, e.g., metabolic syndrome, and diabetes, as well as drug addiction and dependence.

Our published data demonstrate that the sidechain reaction involving ATP depletion, activation of AMPD isoform 2, and the generation of uric acid plays a role in driving the metabolic syndrome in response to fructose<sup>27-29</sup>. However, while blocking xanthine oxidase to reduce uric acid levels can improve fructose-induced metabolic syndrome, it does not have any effect on the craving for fructose or sucrose. Likewise, while blocking fructokinase C does block the craving of fructose (US Pat. Pub. No. 2013/0224218)., it was not known, prior to this disclosure, if this is due to blocking the side chain reaction initiated by AMPD, or whether it is from blocking the ATP consumption and intracellular phosphate consumption, or from blocking intracellular F-1-P formation and its further metabolism via the caloric pathway (Figure 1). Thus, these observations indicate that to date, there was no *a priori* reason to believe that blocking AMPD2 could treat fructose or sugar craving (see data below).

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention is explained in the following description in view of the drawings that show

(note that the word “figure” in the drawings has been abbreviated to “FIG.”):

**FIG. 1** shows the classic caloric pathway of fructose metabolism and the side chain reaction in which AMPD2 is activated.

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**FIG. 2** shows that AMPD2 deficiency is associated with a decreased or an absence of craving to sugars, including fructose (F), sucrose (S), high fructose corn syrup (HFCS) (mixture containing a 2 to 1 ratio of fructose and glucose monomers), glucose water (G), and sweetener – sucralose-water (SW) at 8 weeks. Concentrations were 15% in the water except for sucralose which was 0.04%. AMPD2 deficiency is also associated with reduced drinking of glucose compared to wildtype (WT) mice and no increased consumption of artificial sweeteners, such as sucralose-containing water.

**FIG. 3** illustrates that AMPD2 deficient mice (KO) (knockout) do not show an increase in fructose-water intake as compared to wild type (WT) mice after 10 days of exposure to drinking fructose-containing (15%) water. The left panel shows results of overall water (W) and fructose-water (F) intake in wild type (WT) and AMPD2 deficient (KO) mice at 10 days after the start of the experiment.  $**P<0.01$  between mice of the same group (WT or KO),  $##P<0.01$ . The right panel shows the overall water (W) and fructose-water (F) intake in AMPD2 deficient (D1KO), AMPD2 deficient (D2KO) and AMPD3 deficient, (D3KO) mice at 8 weeks ( $**P<0.01$  between mice of the same group (D1KO, D2KO or D3KO),  $##P<0.01$ ). The right panel shows that the observed effects are specific to isoform 2 of AMPD as deficiency of AMPD1 or AMPD3 in mice do not eliminate the craving for sugar or fructose.

**FIG. 4** shows a dose-dependent effect in AMPD2 activity in the liver between wild type (W) (i.e, containing the AMPD2 gene in both alleles of their genome), heterozygous (Het) mice (i.e, containing only one allele copy of AMPD2 in their genome) and AMPD2 deficient mice (K) (containing no AMPD2 alleles in their genome).  $***P<0.001$  versus WT. The right panel shows daily fructose-water intake in wild type (WT), AMPD2 heterozygous (Het), and AMPD2 deficient (KO) mice.  $*P<0.05$  and  $**P<0.01$  versus WT.

**FIG. 5** provides a representative western blot from nucleus accumbens (NAcc) extracts for AMPD2 and deltaFosB expression from wild type (Wt) and AMPD2 deficient (K) mice exposed to water (wat), fructose (Fru), glucose (glu) or sucralose (S) for 24 hours. Left: The western blot demonstrates that AMPD2 protein is expressed in nucleus accumbens of wild

type (Wt) but not AMPD2 deficient mice (K). It also shows that upon exposure of glucose, fructose and sucralose but not regular tap water there is an elevation in the expression of the transcription factor, deltaFosB ( $\Delta$ FosB), in the nucleus accumbens in wild type (Wt) but not AMPD2 deficient mice (K) (right panel). Drugs and compounds that cause addiction are known to increase  $\Delta$ FosB in the nucleus accumbens and this change is thought to be critical for addiction to occur (see Li et al, and Ruffle cited supra). The results of the experiments performed by the inventors herein show that AMPD2 blockade results in decreased activation of  $\Delta$ FosB induced by sugar, including some not regulated by fructokinase (sucralose) suggest AMPD2 blockade may be blocking addiction pathways directly at the nucleus accumbens.

**FIG. 6** shows the role of AMPD2 in the craving of alcohol. On the left panel is shown a two bottle preference study in which wild type mice can choose between regular drinking water and water that contains alcohol, with the alcohol concentration being increased from 3% (days 0-3), 6% (days 3-8) and 10% (day 8 onward). Wild type mice will rapidly prefer to drink alcohol containing water, and this increases significantly with increasing alcohol concentrations. In contrast, AMPD2 KO mice (right panel) markedly prefer regular water over alcohol-containing water, and show no increase in alcohol intake with time. This shows evidence AMPD2 KO mice not only do not crave alcohol, but have no inclination for addiction-like behavior in which alcohol intake increases with time.

**FIG.7** shows the role of AMPD2 in nicotine craving. Again, this is a two bottle preference study in which mice choose between regular drinking water and water containing nicotine. Wild type mice drinking nicotine-containing water about 30 percent of the time, while the percent of nicotine water drunk by AMPD2 KO mice is significantly less (left panel). This results in significantly less nicotine exposure over time in the AMPD2 KO mouse (right panel). Thus, blocking AMPD2 should lead to less smoking in individuals with nicotine dependence.

**FIG. 8** shows the effect of an orally administered pan AMPD inhibitor (see Admyre et al., *Chem Biol*, 2014, 21(11):1486-96) on fructose craving in wild type mice. Mice were administered the racemate of the active compound 1 (Figure 2 in the paper by Admyre et al *Chem Biol* 2014; 21:1488-1496) by gavage at a dose of 25 mg/kg twice daily beginning two days before administering fructose (15%) in the drinking water. As shown in the left panel,

this dose could significantly block AMPD2 activity in the liver. As shown in the right panel, this dose could also significantly reduce the intake of fructose-containing water. Indeed, mice given the inhibitor drank the same amount of fructose-water as mice given regular water, documenting that the inhibitor completely blocked the craving for fructose.

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**FIG. 9** shows graphical data illustrating how inositol monophosphate (IMP) is a stimulant for food intake. The left panel shows that IMP (300 $\mu$ M) stimulates weight gain in animals given fructose (5%) in the drinking water. The center panel shows that IMP stimulates weight gain in mice on regular chow, and also potentiates the effect of monosodium glutamate (MSG, 5%). The right panel shows energy intake on mice receiving regular chow with regular drinking water, water containing MSG, or MSG plus IMP.

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#### DETAILED DESCRIPTION

The specifics of the discovery include the use of an agent that can specifically inhibit AMPD2 to treat specific conditions as outlined below.

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To date, studies inhibiting AMPD are limited. It has been reported that inhibition of AMPD does not improve glucose control of insulin resistance or diabetes (16). However, it has been identified for the first time herein, novel roles for AMPD, which constitutes the basis for this patent application.

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Our recent studies have identified roles for AMPD and its blockade in reducing or eliminating sugar (for example fructose, sucrose, high fructose corn syrup) cravings as well as potentially other cravings, including salt cravings and/or umami cravings that were not previously noted in the literature. Furthermore, our recent studies have identified roles for AMPD inhibition in ameliorating drug and other addictions.

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In one non-limiting embodiment, a primary discovery relates to the finding that AMPD2 has an important role in driving a craving for sugar, e.g., sucrose, as well as glucose and artificial sugars (FIG. 2). Sugar craving is a distinct process and consists of a specific desire for sugar. It has been shown to be mediated by dopaminergic signaling in the brain and is similar to the addictive response one can observe with narcotics<sup>30, (12)</sup> It had been thought to be mediated by taste receptors, but when the taste receptor signaling is blocked, craving for sugar still occurs.<sup>31</sup> Thus, the specific mechanism responsible for sugar craving had been

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unknown.

We have previously found that AMPD2 has a role in driving obesity and metabolic syndrome (US Patent 8,697,628B, April 15 2014). However, prior to the discovery provided herein, AMPD2 had not been identified as a target for inhibiting or reducing sugar craving or for the craving for other foods or drug addiction. The discovery that blocking AMPD2 blocks sugar craving was unexpected. Indeed, one might *a priori* think that blocking AMPD2 should be similar to blocking uric acid production (which is a downstream product of AMPD), in which lowering uric acid improves fructose induced metabolic syndrome but does not block sugar craving<sup>29</sup> (13).

Consequently, the novel discovery of the inventors provided for the first time herein, that blocking or inhibiting AMPD2 decreases sugar craving is not intuitive. Furthermore, our data also demonstrate that the blockade of other isoforms of AMPD, namely AMPD1 and AMPD3, does not prevent sugar craving thus indicating that this is a specific effect related to AMPD2 inhibition, discovered by the inventors herein (FIG. 3).

Our recent studies show that mice lacking AMPD2 show no craving for fructose and that this effect is dose-dependent as heterozygous mice, i.e. mice that have approximately 50 % expression and activity of AMPD2, have an intermediate effect in their liking of sugar (FIG. 4). As mentioned, Interestingly, AMPD2 deficient mice also show significantly less craving than wild type mice for other sugars including glucose, sucrose and high fructose corn syrup and artificial sweeteners like sucralose (FIG. 2). Some of these features, such as blocking the craving for artificial sugars or for glucose, are not involved in fructose metabolism and clearly demonstrate that some of the actions of AMPD2 on craving are outside the fructose metabolic pathway. Indeed, the finding that AMPD2 is highly expressed in dopaminergic neurons of the nucleus accumbens (where dopamine signaling in response to food or drugs occurs) suggests that AMPD2 has effects on craving at the central level independently of fructose metabolism, especially since to date fructokinase C has not been detected in this part of the brain.

We also have found that mice lacking AMPD1 or AMPD3 (the two other isoforms of AMPD) do not show craving for fructose, showing that the craving effects of sugar and fructose are specific for the AMPD2 isoform (FIG. 3). Thus, to block the craving to sugar, sucralose or other dopamine stimulating agents, one must either use a specific AMPD2 inhibitor or a pan

AMPD inhibitor with activity against the AMPD2 isoform.

One of the major pathways involved in addiction is the activation of the transcription factor, delta Fos B ( $\Delta$ FosB) in the nucleus accumbens. This activation is common to multiple  
5 addictive drugs, including alcohol, cocaine, and narcotics (Chao & Nestler, *Annu Rev Med*, 2004, 55:113-32). As shown in FIG. 5, delta FosB is also activated in the nucleus accumbens in response to sugar (sucrose), fructose, glucose and other sweeteners. However, mice lacking AMPD2 show no induction of  $\Delta$ FosB.

10 Consistent with direct activity on the nucleus accumbens, we have also found that AMPD2 KO mice are protected from the craving of alcohol (FIG. 6). Specifically, when Wild type C57Bl6 mice are provided a choice between drinking water containing alcohol versus regular water (two bottle preference testing), the mice preferentially drink alcohol, and this increases over time as the alcohol content is progressively increased from 3 to 10 percent. In  
15 contrast, AMPD2 KO mice prefer water over alcohol, and drink less alcohol containing water as the alcohol concentration is increased.

Nicotine is another substance known to be addicting in humans. AMPD2 also has a role in the craving for nicotine (FIG. 7). When wild type mice are offered water containing either  
20 nicotine or regular water, they will drink nicotine-containing water approximately 30 percent of the time, but AMPD2 KO mice only drink nicotine-containing water about 10 percent of the time, and over time this results in a significant decreased nicotine intake in mice lacking AMPD2.

25 The benefit from blocking AMPD2 on craving is not limited to mice genetically lacking AMPD2. Recently an inhibitor for AMPD2 was published, and we made a racemate of the active compound 1 (Figure 2 in the paper by Admyre et al *Chem Biol* 2014; 21:1488-1496). As shown in FIG. 8, the administration of the AMPD inhibitor orally by gavage (50 mg/kg/d) resulted in both an inhibition of AMPD2 activity in the liver and also blocked the craving for  
30 fructose.

The use of AMPD inhibitors may thus provide a variety of benefits. First, they will have significant benefit in blocking the craving of sugar or other sweeteners. This indirectly may lead to a reduction in sugar intake, that could have downstream benefits for those trying to



diet or lose weight, or who are trying to block the metabolic effects of fructose. Second, blocking AMPD2 will also have other benefits by blocking the craving for other addictive or 'habit-forming' substances, including, for example, nicotine, cocaine, opioids, alcohol, cannabinoids, methylphenidate, phencyclidine, and substituted amphetamines. This benefit is expected due to the ability of AMPD2 blockade to prevent the upregulation of  $\Delta$ FosB in the nucleus accumbens, which is a critical regulator of craving and addiction to cocaine, narcotics, alcohol, opiates, amphetamines, marijuana, and other addicting drugs (see Li et al. *Synapse* 2008, 62(5):358-69, and Ruffle, *Am J Drug Alcohol Abuse*, 2014 40(6):428-37).

In another embodiment, AMPD2 could be activated in order to treat or subjects suffering from anorexia nervosa or to stimulate food intake, such as in a person with cachexia from cancer or other illnesses. AMPD2 can also generate IMP (see FIG. 1), which is taste enhancer. We found, however, that IMP also enhanced the intake of food (which was not anticipated based on the literature), including in response to fructose or regular chow. FIG. 9 shows the effect of adding IMP to the water of rats receiving fructose (5%) in their water. Within 3 weeks the addition of IMP was associated with increased weight gain. Likewise, IMP was also found to stimulate weight gain and food intake in animals in normal chow (right panel) and that this potentiated the effects of MSG (5% in the water). Hence, IMP, a product of AMPD2, when given in the diet can enhance food intake and weight gain, and hence may be useful as a means for increasing food intake in subjects with low body weight, anorexia nervosa, or cachexia of any cause.

Exemplary disorders to be treated, and exemplary risk factors to be ameliorated by the compositions and methods described herein include, but are not limited to, sugar craving, salt craving, umami craving, alcohol or drug addiction, sex addiction, addiction to nicotine, and/or other craving or addiction-related behavior of a mammal. Other disorders include obesity, fatty liver, or diabetes where craving for sugar and sweets may be problematic. In further embodiments, the compositions and methods described herein may be or may also be administered to a subject to provide a diminished craving in the subject for fructose and/or fructose-containing sugars or other sugars such as sucrose, glucose or sucralose, in non-limiting examples, to reduce body mass index or to improve kidney function. As set forth herein, the methods and compositions described herein may be administered to a subject as the single therapy or along with a conjunctive therapeutic agent, which is administered to a subject to treat or prevent the same or distinct disorder or physical condition as the AMPD2 inhibitor.

## DEFINITIONS

5 As used herein, the terms "administering" or "administration" of an agent, drug, or peptide to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. The administering or administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, or topically. Administering or administration  
10 includes self-administration and the administration by another.

As used herein, the terms "co-administered," "co-administering," or "concurrent administration", when used, for example with respect to administration of a conjunctive agent along with administration of an AMPD or AMPD2 inhibitor refers to administration of the  
15 AMPD or AMPD2 inhibitor and the conjunctive agent such that both can simultaneously achieve a physiological effect. The two agents, however, need not be administered together. In certain embodiments, administration of one agent can precede administration of the other, however, such co-administering typically results in both agents being simultaneously present in the body (e.g. in the plasma) of the subject.

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As used herein, the terms "diabetic" or "diabetes" refers to Type 1 diabetes, wherein the pancreas produces little or no insulin; Type 2 diabetes, wherein the body becomes resistant to the effects of insulin or produces little or no insulin.; or disease state occurring as sequelae of other primary diseases that include the symptoms of either or both of elevated  
25 blood sugar (hyperglycemia) and the excretion of sugar in the urine (glycosuria).

As used herein, the terms "disease," "disorder," or "complication" refers to any deviation from a normal state in a subject. In exemplified embodiments, the disease, disorder or complication pertains to a craving or addiction. The disease, disorder or complication may  
30 be characterized where the expression and/or activity of an AMPD2 protein differs between subjects with disease and subjects not having disease or where a change in  $\Delta$ FosB expression in the nucleus accumbens differs between subjects with disease and subjects not having disease. Diseases may include psychological diseases, such as drug or alcohol addictions, and also cravings for sugar, salt, umami, and other cravings known to those  
35 skilled in the art. Diseases may include diabetic-related diseases.

As used herein, by the term "effective amount," "amount effective," "therapeutically effective amount," or the like, it is meant an amount effective at dosages and for periods of time necessary to achieve the desired result. In the case of the co-administration of an AMPD2 inhibitor with a conjunctive agent as described herein, the conjunctive agent, the AMPD2 inhibitor, or the combination of the AMPD2 inhibitor and the conjunctive agent may supply the effective amount.

As used herein, the term "expression" in the context of a gene or polynucleotide involves the transcription of the gene or polynucleotide into RNA. The term may also, but not necessarily, involve the subsequent translation of the RNA into polypeptide chains and their assembly into proteins.

As used herein, the terms "interfering molecule" refer to all molecules, e.g., RNA or RNA-like molecules, which have a direct or indirect influence on gene expression, such as the silencing of a target gene sequence. Examples of other interfering RNA molecules include siRNAs, short hairpin RNAs (shRNAs), single-stranded siRNAs, microRNAs (miRNAs), methylated siRNAs or other siRNAs treated to protect the siRNA from degradation by circulating RNases, and dicer-substrate 27-mer duplexes. Examples of "RNA-like" molecules include, but are not limited to, siRNA, single-stranded siRNA, microRNA, and shRNA molecules that contain one or more chemically modified nucleotides, one or more non-nucleotides, one or more deoxyribonucleotides, and/or one or more non-phosphodiester linkages. Thus, siRNAs, single-stranded siRNAs, shRNAs, miRNAs, and dicer-substrate 27-mer duplexes are subsets of "interfering molecules." "Interfering molecules" also may include PMOs.

As used herein, the terms "phosphothioate morpholino oligomer(s)," "a PMO" "PMOs" refer to molecules having the same nucleic acid bases naturally found in RNA or DNA (i.e. adenine, cytosine, guanine, uracil or thymine), however, they are bound to morpholine rings instead of the ribose rings used by RNA. They may also linked through phosphorodiamidate rather than phosphodiester or phosphorothioate groups. This linkage modification eliminates ionization in the usual physiological pH range, so PMOs in organisms or cells are uncharged molecules. The entire backbone of a PMO is made from these modified subunits.

As used herein, the term "antisense sequence" refers to an oligomeric compound that is at

least partially complementary to a target nucleic acid molecule to which it hybridizes. In certain embodiments, an antisense compound modulates (increases or decreases) expression of a target nucleic acid. Antisense compounds include, but are not limited to, compounds that are oligonucleotides, oligonucleosides, oligonucleotide analogs,  
5 oligonucleotide mimetics, and chimeric combinations of these.

As used herein, the term "RNA interference" (RNAi) refers to a post-transcriptional gene silencing (PGSR) process whereby one or more exogenous small interfering RNA (siRNA) molecules are used to silence expression of a target gene. RNAi includes double-stranded  
10 RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in humans are known in the art (Fire A, et al., 1998 Nature 391: 806-811; Fire, A. Trends Genet. 15,358-363 (1999); Sharp, P. A. RNA interference 2001.  
15 Genes Dev. 15,485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2,110-1119 (2001); Tuschl, T. Chem. Biochem. 2,239-245 (2001); Hamilton, A. et al., Science 286,950-952 (1999); Hammond, S. M., et al., Nature 404,293-296 (2000); Zamore, P. D., et al., Cell 101,25-33 (2000); Bernstein, E., et al., Nature 409,363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15,188-200 (2001); W00129058 ; W09932619 ; Elbashir SM, et al., 2001 Nature  
20 411: 494-498). RNAi may be used to knock down expression of AMPD Gl#s 4557310,14043442, and 4502078 in various cell lines using small interfering RNAs (siRNA, Elbashir et al, supra). Examples of RNAi agents include siRNA and shRNA.

As used herein, "siRNAs" (short interfering RNAs) refer to double-stranded RNA molecules, generally around 15-30 nucleotides in length, that are complementary to the sequence of the mRNA molecule transcribed from a target gene.  
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As used herein, "shRNAs" (small hairpin RNAs) are short "hairpin-turned" RNA sequences that may be used to inhibit or suppress gene expression.  
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As used herein, a "composition," "pharmaceutical composition" or "therapeutic agent" all include a composition comprising at least an AMPD2 inhibitor. Optionally, the "composition," "pharmaceutical composition" or "therapeutic agent" further comprises pharmaceutically acceptable diluents or carriers. In the case of an interfering molecule, for example, the  
35 interfering molecule may be combined with one or more pharmaceutically acceptable

diluents, such as phosphate-buffered saline, for example. As used herein, a pharmaceutical composition particularly refers to a composition comprising at least an AMPD2 inhibitor that is intended to be administered to a subject as described herein.

5 As used herein, the term “AMPD2 inhibitor” is an agent that inhibits AMPD2. It also includes a PAN AMPD inhibitor that has AMPD2 inhibitory activity. AMPD inhibitors are known<sup>12-14</sup> and include molecules and compounds such as those described herein, and those described in Admyre and Kasibhalta references incorporated by reference herein<sup>12-14</sup>. For example, AMPD inhibitors include: N3-Substituted coformycin aglycon analogues with improved AMP  
10 deaminase (AMPDA) inhibitory potency are described. Replacement of the 5-carboxypentyl substituent in the lead AMPDA inhibitor 3-(5-carboxypentyl)-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol (2) described in the previous article with various carboxyarylalkyl groups resulted in compounds with 10-100-fold improved AMPDA inhibitory potencies. The optimal N3 substituent had m-carboxyphenyl with a two-carbon alkyl tether. For example, 3-[2-(3-  
15 carboxy-5-ethylphenyl)ethyl]-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol (43g) inhibited human AMPDA with a  $K(i) = 0.06$  microM. The compounds within the series also exhibited >1000-fold specificity for AMPDA relative to adenosine deaminase<sup>13-14</sup>. 3-[2-(3-carboxy-4-bromo-5,6,7,8-tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol (24b), represents a 10- to 250-fold enhancement in AMPDA inhibitory  
20 potency without loss in the enzyme specificity. The potency of the inhibitor 24b (AMPDA  $K(i) = 0.002$  microM) is 10(5)-fold lower than the  $K_m$  for the substrate AMP. It represents the most potent nonnucleotide AMPDA inhibitor known<sup>13-14</sup>. Additional compounds include those disclosed in Admyre et al. (supra): Compound 1, Compound II and Compound III 6-(4-((1-(Isoquinolin-8-yl)ethylamino)methyl)phenyl)nicotinic acid (including enantiomers), Compound  
25 IV 4'-((1-(Isoquinolin-8-yl)ethylamino)methyl)-3-methoxybiphenyl-4-carboxylic acid, and others: N-(Isoquinolin-8-ylmethylene)-2-methylpropane-2-sulfinamide; 4'-Formyl-3-methoxybiphenyl-4-carboxylic acid<sup>12</sup>.

As used herein, the term “AMPD” refers to AMPD2 and other isoforms of AMPD, including  
30 AMPD2, unless otherwise specified.

As used herein, the term “subject” refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment.

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As used herein, the terms "treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen), reduce symptoms, or delay progression the targeted disease, disorder or complication.

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As used herein, the terms "craving" and "addiction" may be used interchangeably in certain embodiments. Typically "craving" is used in reference to sugar, salt and umami, and "addiction" is used in reference to addictive substances that, for example, stimulate dopamine, including alcohol, sex and drugs such as nicotine, cocaine, opioids, cannabinoids, methylphenidate, phencyclidine, and substituted amphetamines,. See Nieh et al., 2015, Cell 160, 528–541; Shulte et al., PLoSone, 10(2): e0117959. Doi:10.1371/journal. Pone.0177959, and Avena et al., *Neurosci Biobehav Rev.* 2008; 32(1): 20–39 for sugar addiction. In a specific embodiment, an AMPD2 related addiction refers to an addiction associated with elevated amounts of  $\Delta$ FosB in the nucleus accumbens of a subject.

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#### *Description of exemplary embodiments*

The mechanism by which sugar and other cravings occur has remained a mystery. It was originally thought that there might be a major role for taste receptors, but when taste receptor signaling is blocked, sugar (sucrose) still induces a robust dopamine response in the brain (14). The present inventors have shown that the craving for fructose and sugar (sucrose) is dependent on AMPD2. Specifically, AMPD2 deficient, also known as, knockout mice (KO mice) show a decrease in preference to no preference of water containing fructose (fructose-water), or fructose-containing sugar (sucrose, high fructose corn syrup) over drinking water alone. Also, AMPD2 deficiency in these mice is associated with reduced intake of glucose. In contrast, mice expressing AMPD2 (wild type, WT mice) exposed to both water and fructose-water show an increase in consumption of and an increase in preference to drinking fructose-water as compared to non-fructose water. Similarly, wild type mice have increased preference for sucrose, high fructose corn syrup and glucose than AMPD2 deficient mice.

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These studies show that blocking AMPD2 can decrease or inhibit the craving of fructose and other sugars including fructose- and non-fructose containing sugars, and also reduce the craving for fructose-containing sugars, sucralose, and glucose, for example.

In accordance with one aspect of the present invention, there are provided methods and compositions comprising AMPD2 inhibitors for blocking AMPD2 to correspondingly reduce the craving of sugars, including fructose, sucralose, glucose, sucrose, and other monosaccharides. The craving for sugars that can be converted to fructose in the body, such as sorbitol, will also be reduced.

In accordance with another aspect of the present invention, since repeated sugar intake from craving can induce obesity, there are provided methods and compositions comprising AMPD2 inhibitors that are able to block sugar craving syndromes and hence be an adjunctive treatment for obesity. Accordingly, one embodiment provided is treatment of obesity by administering a therapeutically effective amount of an AMPD2 inhibitor.

In accordance with another aspect of the present invention, there is provided a method for reducing a craving for fructose and/or fructose-containing sugars or other sugars in a subject, the method comprising inhibiting AMPD2 activity in the subject. In one embodiment, the method comprises administering an AMPD2 inhibitor to the subject.

In accordance with another aspect of the present invention, there is provided a composition useful for decreasing a craving for sugar, sugar-containing compounds, other sweeteners and monosaccharides, the composition comprising an AMPD2 inhibitor and optionally, a conjunctive therapeutic agent.

In accordance with another aspect of the present invention, there is provided a method for treating a sugar craving, a salt-craving, and/or an umami craving. The method comprises administering to the subject a therapeutically effective amount of an AMPD2 inhibitor. A composition for treating a sugar craving, a salt-craving, an umami craving, and methods or compositions for treating other addictions, including narcotic or other drug (cocaine) addictions, alcohol addictions, smoking (nicotine) addiction, sex addiction among other addictions is also provided, as well as a method of treating such cravings or addiction by administering a therapeutically effective amount of an AMPD2 inhibitor containing composition to a subject are also provided herein.

In accordance with another aspect of the present invention, there is provided a method for diminishing, inhibiting or eliminating addiction-related behavior of a subject, wherein said method comprises administering a composition comprising an AMPD2 inhibitor (to inhibit

AMPD2) to the subject, and wherein the addiction-related behavior is associated with a craving for sugar, salt, umami, or addiction to drug or alcohol intake or a sex addiction. A composition for diminishing, inhibiting or eliminating addiction-related behavior of a mammal comprising an AMPD2 inhibitor may likewise be provided.

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The present inventors have made novel discoveries that were not predicted based on the currently published literature. First, the inventors have discovered that a limitation or elimination of craving for sugar and or fructose can be accomplished with the inhibition or blocking of AMPD2 enzyme or a pan-AMPD inhibitor in a subject. Furthermore, the  
10 inventors have discovered that inhibition of the AMPD2 isoform or a general AMPD inhibitor that has activity against AMPD2 may also be able to block other types of craving unrelated to fructose metabolism (such as for glucose, sucralose, or other) is particularly relevant in eliminating or limiting cravings as described herein.

15 Further, the present inventors have also shown a dose-dependent effect of AMPD2 inhibition. The inventors have demonstrated that heterozygous mice have half the AMPD2 expression, and, half the response to fructose-water as compared to wild type mice and knockout mice. Heterozygous mice have less preference to fructose-water than wild type mice, but show an increased preference to fructose-water than knockout mice.

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Moreover, the present inventors have found that AMPD2 inhibitors are also useful in decreasing a craving or decreasing a preference for sweeteners and other monosaccharides (i.e, fructose, glucose) as well as artificial sweeteners (sucralose) compared to wild type animals. In one example, a 24 hour study was conducted in which mice were exposed to  
25 water, sucralose (0.04%), glucose (10%), and fructose (15%), and the water consumption was higher in the wild type mice than in AMPD2 deficient mice (KO) exposed to these sweeteners and monosaccharides.

In accordance with one aspect of the present invention, the AMPD2 inhibitors described  
30 herein are useful as an adjunct treatment for obesity in subjects by helping in reducing sugar craving and consumption.

In accordance with another aspect of the present invention, there is provided a composition for treating or preventing a complication characterized by an increased presence or activity  
35 of AMPD2 by administering an AMPD2 inhibitor.



The AMPD2 inhibitor may include one or more of a ribozyme, an interfering molecule, a peptide, a small molecule, or an antibody targeted to AMPD2. In a particular embodiment, the AMPD2 inhibitor comprises an interfering molecule that can participate in changes in  
5 gene expression of AMPD2, such as the silencing of expression of the AMPD2. Without limitation, the interfering molecule may comprise a phosphothioate morpholino oligomer (PMO), miRNA, siRNA, shRNA, any other antisense sequence, or any combination thereof.

AMPD2 can be inhibited by a number of means as set forth further below, including silencing  
10 via miRNA, shRNA, siRNA, or a PMO directed to a portion of the sequence described at the genbank accession numbers provided below. See U.S Patent Publication 20060110440 for background on siRNA silencing, the entirety of which is hereby incorporated by reference. As discussed above, agents can be developed to inhibit AMPD2 to achieve a beneficial effect on obesity, metabolic syndrome and related diseases and complications, sugar and  
15 other cravings discussed herein and other addictions including drug and alcohol addictions.

It is noted that the compositions and methods disclosed herein may be administered to any subject as defined herein. In one embodiment, the subject is human. In another embodiment, the subject is a pet, e.g., cat, dog, or the like, and in a particular embodiment,  
20 is an overweight pet or animal. It is further noted that a corresponding composition, e.g., a pharmaceutical composition, may be provided for use in any method described

### *Gene Expression*

25 In another embodiment, test compounds which increase or decrease AMPD2 gene expression are identified. An AMPD polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the AMPD polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of  
30 the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in

its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of AMPD2 mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of an AMPD2 polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined in vivo, in a cell culture, or in an in vitro translation system by detecting incorporation of labeled amino acids into an AMPD2 polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses an AMPD2 polynucleotide can be used in a cell-based assay system.

The AMPD2 polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

#### *Pharmaceutical Compositions*

The invention also pertains to compositions, e.g., pharmaceutical compositions, comprising one or more therapeutic agents that inhibit AMPD2. In one embodiment, the therapeutic agents inhibit AMPD2. Therapeutic agents include those that are identified by screening methods that utilize AMPD2 polypeptides and/or polynucleotides. Therapeutic agent(s) can be administered to a patient to achieve a therapeutic effect, i.e. useful in modulating AMPD2 activity and in turn, treating and/or preventing obesity, sugar cravings, and/or other cravings described herein. Compositions of the invention can comprise, for example, therapeutic agents identified by a screening method embodiment described herein, which are identified by their ability to bind to or affect activity of AMPD2 polypeptides, or bind to and/or affect expression of AMPD2 polynucleotides. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Compositions of the invention can be administered by any number of routes including, but  
5 not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as  
10 tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton,  
15 Pa., which is incorporated herein by reference). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Accordingly, some examples of an agent having therapeutic activity as described herein, include but are not limited to a modulating agent, an antisense nucleic acid molecule, small molecule AMPD2 inhibitors, peptide inhibitors, a specific antibody, ribozyme, interfering molecules, or an AMPD2 polypeptide binding molecule targeted to AMPD2. In one embodiment, the agent comprises an interfering molecule and the interfering molecule  
25 comprises a phosphothioate morpholino oligomer (PMO), miRNA, methylated miRNA, treated-miRNA, siRNA, shRNA, antisense RNA, and any combination thereof.

Each of the compositions and methods described herein may include an effective amount of the AMPD2 inhibitor. In one embodiment, the AMPD2 inhibitor is combined with one or  
30 more conjunctive therapeutic agents to bring about a desired effect in the subject. This effect may be realized by an effective amount of the AMPD2 inhibitor, an effective amount of the conjunctive agent, or an effective amount of the combination of the AMPD2 and the one or more conjunctive therapeutic agents. It is understood that the administration of the AMPD2 inhibitor with one or more conjunctive therapeutic agents may advantageously  
35 increase an efficacy of the AMPD2 inhibitor, the conjunctive therapeutic agent, or both.

In certain embodiments, inhibiting AMPD2 involves downregulation of gene expression, translation or activity of AMPD genes.

- 5 The methods and compositions described herein may be directed at inhibiting expression of inhibiting the gene expression, translation or activity of genes. In a particular embodiment, the methods and compositions described herein are directed at inhibiting the gene expression, translation or activity of AMPD2.
- 10 Agents can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. In addition, compositions may include a conjunctive agent in addition to the therapeutic agents of the present invention. A comprehensive discussion of many different agents that can be used in combination with a therapeutic agent comprising an AMPD2 inhibitor is described in U.S. Patent Pub. 20080255101, the entirety of which is
- 15 incorporated by reference herein. According to specific embodiments, small molecule AMPD2 inhibitors include, but are not limited to, 3-[2-(3-carboxy-4-bromo-5,6,7,8-tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo[4,5-d] [1,3] diazepin-8-ol<sup>13</sup>.

- Exemplary compounds include for use in combination or co-administration with the
- 20 therapeutic agent(s) or composition comprising an AMPD2inhibitor as described herein may be selected from the group consisting of angiotensin-converting enzyme (ACE) inhibitors, aldosterone antagonists, amphetamines, amphetamine-like agents, Angiotensin II receptor antagonists, anti-oxidants, aldose reductase inhibitors, biguanides, sorbitol dehydrogenase inhibitors, thiazolidinediones (glitazones), thiazide and thiazide-like diuretics, triglyceride
- 25 synthesis inhibitors, uric acid lowering drugs, e.g., xanthine oxidase inhibitors, ketohexokinase (fructokinase) inhibitors and combinations thereof.

- Those skilled in the art will appreciate that numerous delivery mechanisms are available for delivering a therapeutic agent to an area of need. By way of example, the agent may be
- 30 delivered using a liposome as the delivery vehicle. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 µg of DNA per 16 nmole of liposome delivered to about 10<sup>6</sup> cells, more preferably about 1.0 µg of DNA per 16 nmole of liposome delivered to about 10<sup>6</sup> cells, and even more preferably about 2.0 µg of DNA per 16 nmol of liposome delivered to about 10<sup>6</sup> cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes conventionally used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Pat. No. 5,705,151). Preferably, from about 0.1 µg to about 10 µg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 µg to about 5 µg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 µg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues in vivo using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis et al. Trends in Biotechnol. 11, 202-05 (1993); Chiou et al., GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J. A. Wolff, ed.) (1994); Wu & Wu, J. Biol. Chem. 263, 621-24 (1988); Wu et al., J. Biol. Chem. 269, 542-46 (1994); Zenke et al., Proc. Natl. Acad. Sci. U.S.A. 87, 3655-59 (1990); Wu et al., J. Biol. Chem. 266, 338-42 (1991).

## 2.1 Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose of therapeutic agents identified by a screening method herein is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which modulates AMPD2 activity compared to that which occurs in the absence of the therapeutically effective dose.

Therapeutic efficacy and toxicity, e.g., ED<sub>50</sub> (the dose therapeutically effective in 50% of the population) and LD<sub>50</sub> (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD<sub>50</sub> /ED<sub>50</sub>.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

Preferably, a therapeutic agent reduces expression of an AMPD gene or the activity of an AMPD polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of an AMPD gene or the activity of an AMPD polypeptide can be assessed such as by hybridization of nucleotide probes to AMPD-specific mRNA, quantitative RT-PCR, immunologic detection of an AMPD polypeptide, or measurement of AMPD activity.

## 2.2 *Conjunctive Therapeutic Agents*

In any of the embodiments described above, any of the compositions of the invention can be co-administered with other appropriate therapeutic agents (conjunctive agent or conjunctive therapeutic agent) for the treatment or prevention of a target disease. Selection of the appropriate conjunctive agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Exemplary conjunctive agents that may be formulated and/or administered with any form of an AMPD2 inhibitor as described herein include, but are not limited to, angiotensin-converting enzyme (ACE) inhibitors, aldosterone antagonists, amphetamines, amphetamine-like agents, Angiotensin II receptor antagonists, anti-oxidants, aldose reductase inhibitors, biguanides, sorbitol dehydrogenase inhibitors, thiazolidinediones (glitazones), thiazide and thiazide-like diuretics, triglyceride synthesis inhibitors, uric acid lowering agents, e.g., xanthine oxidase inhibitors, fructokinase inhibitors, and combinations thereof.

Exemplary ACE inhibitors include, but are not limited to, Benazepril (Lotensin), Captopril, Enalapril (Vasotec), Fosinopril, Lisinopril (Prinivil, Zestril), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), Trandolapril (Mavik), and combinations thereof.

Exemplary aldosterone antagonists include, but are not limited to, Spironolactone, Eplerenone, Canrenone (canrenoate potassium), Prorenone (prorenoate potassium), Mexrenone (mexrenoate potassium), and combinations thereof.

Exemplary amphetamines include, but are not limited to, amphetamine, methamphetamine, methylphenidate, p-methoxyamphetamine, methylenedioxymethamphetamine, 2,5-dimethoxy-4-methylamphetamine, 2,4,5-trimethoxyamphetamine, and 3,4-methylenedioxymethamphetamine, N-ethylamphetamine, fenethylline, benzphetamine, and chlorphentermine as well as the amphetamine compounds of Adderall.RTM.; actedron;

actemin; adipan; akedron; allodene; alpha-methyl-(.-)-benzeneethanamine; alpha-methylbenzeneethanamine; alpha-methylphenethylamine; amfetamine; amphate; anorexine; benzebar; benzedrine; benzyl methyl carbinamine; benzolone; beta-amino propylbenzene; beta-phenylisopropylamine; biphetamine; desoxynorephedrine; dietamine; DL-amphetamine; 5 elastonon; fenopromin; finam; isoamyne; isomyn; mecodrin; monophos; mydrial; norephedrane; novydrine; obesin; obesine; obetrol; octedrine; oktedrin; phenamine; phenedrine; phenethylamine, alpha-methyl-; percomon; profamina; profetamine; propisamine; racephen; raphetamine; rhinalator, sympamine; simpatedrin; simpatina; sympatedrine; and weckamine. Exemplary amphetamine-like agents include but are not 10 limited to methylphenidate. Exemplary compounds for the treatment of ADD include, but are not limited to, methylphenidate, dextroamphetamine/ amphetamine, dextroamphetamine, and atomoxetine (non-stimulant).

Exemplary Angiotensin II receptor antagonists or angiotensin receptor blockers (ARBs)

15 include, but are not limited to losartan, irbesartan, olmesartan, candesartan, valsartan, and combinations thereof.

Exemplary anti-oxidant compounds include but are not limited to L-ascorbic acid or L-ascorbate (vitamin C), menaquinone (vitamin K 2 ), plastoquinone, phyloquinone (vitamin K 20 1 ), retinol (vitamin A), tocopherols (e.g.,  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocotrienols, ubiquinol, and ubiquione (Coenzyme Q10)); and cyclic or polycyclic compounds including acetophenones, anthroquinones, benzoquinones, biflavonoids, catechol melanins, chromones, condensed tannins, coumarins, flavonoids (catechins and epicatechins), hydrolyzable tannins, hydroxycinnamic acids, hydroxybenzyl compounds, isoflavonoids, lignans, naphthoquinones, 25 neolignans, phenolic acids, phenols (including bisphenols and other sterically hindered phenols, aminophenols and thiobisphenols), phenylacetic acids, phenylpropenes, stilbenes and xanthenes. Additional cyclic or polycyclic antioxidant compounds include apigenin, auresin, aureusidin, Biochanin A, capsaicin, catechin, coniferyl alcohol, coniferyl aldehyde, cyanidin, daidzein, daphnetin, deiphinidin, emodin, epicatechin, eriodictyol, esculetin, ferulic acid, formononetin, gernistein, gingerol, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3- 30 hydroxycoumarin, juglone, kaemferol, lunularic acid, luteolin, malvidin, mangiferin, 4-methylumbelliferone, mycertin, naringenin, pelargonidin, peonidin, petunidin, phloretin, p-hydroxyacetophenone, (+)-pinosresinol, procyanidin B-2, quercetin, resveratol, resorcinol, rosmarinic acid, salicylic acid, scopolein, sinapic acid, sinapoyl-(S)-maleate, sinapyl aldehyde, 35 syrginyl alcohol, telligrandin umbelliferone and vanillin. Antioxidants may also be obtained



from plant extracts, e.g., from blackberries, blueberries, black carrots, chokecherries, cranberries, black currants, elderberries, red grapes and their juice, hibiscus, oregano, purple sweet potato, red wine, rosemary, strawberries, tea (e.g., black, green or white tea), and from various plant ingredients as ellagic acid.

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Exemplary aldose reductase inhibitors include, but are not limited to, epalrestat, ranirestat, fidarestat, sorbinil, and combinations thereof.

Exemplary biguanides include, but are not limited to, metformin, and less rarely used

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phenformin and buformin, proguanil, and combinations thereof.

Exemplary thiazolidinediones include, but are not limited to, troglitazone, pioglitazone, ciglitazone, rosiglitazone, englitazone, and combinations thereof.

15 

Exemplary sorbitol dehydrogenase inhibitors are disclosed in US Patent Nos. 6,894,047, 6,570,013, 6,294,538, and US Published Patent Application No. 20050020578, the entirety of which are incorporated by reference herein.

Exemplary thiazide and thiazide-like diuretics include, but are not limited to,

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benzothiadiazine derivatives, chlortalidone, metolazone, and combinations thereof.

Exemplary triglyceride synthesis inhibitors include, but are not limited to, diglyceride acyltransferase 1 (DGAT-1) inhibitors.

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Exemplary uric acid lowering agents include, but are not limited to, xanthine oxidase inhibitors, such as allopurinol, oxypurinol, tisopurine, febuxostat, inositols (e.g., phytic acid and myo-inositol), fructokinase inhibitors, and combinations thereof.

Exemplary fructokinase inhibitors include, but are not limited to, osthol, alpha mangosteen,

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luteolin, osthonol, or indazole derivatives (see US Pub No. 2011/0263559) or

Pyrimidinopyrimidine derivatives (US2011/0263559).

It is appreciated that suitable conjugant therapeutic agents for use in the present invention may also comprise any combinations, prodrugs, pharmaceutically acceptable salts, analogs, and derivatives of the above compounds.

5 In one embodiment, the AMPD inhibitor may be administered to the subject along with one or more other therapeutic agents that are active in acute and chronic kidney disease. Exemplary conjugant therapeutic agents for this use include but are not limited to angiotensin-converting enzyme (ACE) inhibitors, aldosterone antagonists, Angiotensin II  
10 dehydrogenase inhibitors, thiazolidinediones (glitazones), xanthine oxidase inhibitors, and/or any other agent used to treat acute or chronic kidney disease.

It is appreciated by one skilled in the art that when any one or more the AMPD inhibitors described herein are combined with another therapeutic agent, the AMPD inhibitor(s) may  
15 critically allow for increased efficacy of the therapeutic agent or allow for reduction of the dose of the other therapeutic agent that may have a dose-related toxicity associated therewith.

The mode of administration for a conjunctive formulation in accordance with the present  
20 invention is not particularly limited, provided that the AMPD2 inhibitor and the conjunctive agent are combined upon administration. Such an administration mode may, for example, be (1) an administration of a single formulation obtained by formulating an AMPD2 inhibitor and the conjunctive agent simultaneously; (2) a simultaneous administration via an identical route of the two agents obtained by formulating an AMPD2 inhibitor and a conjunctive agent  
25 separately; (3) a sequential and intermittent administration via an identical route of the two agents obtained by formulating an AMPD2 inhibitor and a conjunctive agent separately; (4) a simultaneous administration via different routes of two formulations obtained by formulating an AMPD2 inhibitor and a conjunctive agent separately; and/or (5) a sequential and intermittent administration via different routes of two formulations obtained by formulating an  
30 AMPD2 inhibitor and a conjunctive agent separately (for example, an AMPD2 or its composition followed by a conjunctive agent or its composition, or inverse order) and the like.

The dose of a conjunctive formulation may vary depending on the formulation of the AMPD2  
35 inhibitor and/or the conjunctive agent, the subject's age, body weight, condition, and the

dosage form as well as administration mode and duration. One skilled in the art would readily appreciate that the dose may vary depending on various factors as described above, and a less amount may sometimes be sufficient and an excessive amount should sometimes be required.

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The conjunctive agent may be employed in any amount within the range causing no problematic side effects. The daily dose of a conjunctive agent is not limited particularly and may vary depending on the severity of the disease, the subject's age, sex, body weight and susceptibility as well as time and interval of the administration and the characteristics, preparation, type and active ingredient of the pharmaceutical formulation. An exemplary

10 daily oral dose per kg body weight in a subject, e.g., a mammal, is about 0.001 to 2000 mg, preferably about 0.01 to 500 mg, more preferably about 0.1 to about 100 mg as medicaments, which is given usually in 1 to 4 portions.

15 When an AMPD2 inhibitor and a conjunctive agent are administered to a subject, the agents may be administered at the same time, but it is also possible that the conjunctive agent is first administered and then the AMPD2 inhibitor is administered, or that the AMPD2 is first administered and then the conjunctive agent is administered. When such an intermittent administration is employed, the time interval may vary depending on the active ingredient

20 administered, the dosage form and the administration mode, and for example, when the conjunctive agent is first administered, the AMPD2-inhibitor may be administered within 1 minute to 3 days, preferably 10 minutes to 1 day, more preferably 15 minutes to 1 hour after the administration of the conjunctive agent. When the AMPD2 inhibitor is first administered, for example, then the conjunctive agent may be administered within 1 minute to 1 day,

25 preferably 10 minutes to 6 hours, more preferably 15 minutes to 1 hour after the administration of the AMPD2 inhibitor.

It is understood that when referring to an AMPD2 inhibitor and a conjunctive agent, it is meant an AMPD2 inhibitor alone, a conjunctive agent alone, as a part of a composition, e.g.,

30 composition, which optionally includes one or more pharmaceutical carriers. It is also contemplated that more than one conjunctive agent may be administered to the subject if desired.

35

### *Polypeptides*

AMPD polypeptides according to an aspect of the present invention comprise at least 12, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 or 265 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NOs: 7, 8, 9, 10, 11, and 12 or a biologically active variant thereof, as defined below. An AMPD polypeptide of the invention therefore can be a portion of an AMPD protein, a full-length AMPD protein, or a fusion protein comprising all or a portion of AMPD protein.

### *Biologically Active Variants*

AMPD polypeptide variants which are biologically active, i.e., confer an ability to deaminate adenosine monophosphate (AMP), also are considered AMPD polypeptides for purposes of this application. Preferably, naturally or non-naturally occurring AMPD polypeptide variants have amino acid sequences which are at least about 55, 60, 65, or 70, preferably about 75, 80, 85, 90, 96, 96, or 98% identical to the amino acid sequence shown in SEQ. ID NOS.: 7, 8, 9, 10, 11, 12 for all the AMPD2 protein isoforms derived from each transcript variant identified or a fragment thereof. Percent identity between a putative AMPD polypeptide variant and an amino acid sequence of SEQ. ID NOS: 7, 8, 9, 10, 11, 12 determined using the Blast2 alignment program (Blosun62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a AMPD polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active AMPD polypeptide can readily be determined by assaying for AMPD activity, as described herein, for example. Mass.), and Quantum Biotechnologies (Montreal,

Canada; 1-888-DNA-KITS).

### *Polynucleotides*

- 5 An AMPD polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for an AMPD polypeptide. A coding sequence for AMPD polypeptide of SEQ ID NOS: 1, 2, 3, 4, 5, or 6 for each transcript variant identified to date.
- 10 Degenerate nucleotide sequences encoding AMPD polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 60, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NOS: 1, 2, 3, 4, 5, or 6 for each transcript variant identified to date also are AMPD-like enzyme polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined
- 15 using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of AMPD polynucleotides which encode biologically active AMPD polypeptides also are AMPD polynucleotides.

20

#### *4.1 Identification of Polynucleotide Variants and Homologs*

- Variants and homologs of the AMPD polynucleotides described above also are AMPD polynucleotides. Typically, homologous AMPD polynucleotide sequences can be identified
- 25 by hybridization of candidate polynucleotides to known AMPD polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions: 2 X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50° C. once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each homologous sequences can be
- 30 identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

- Species homologs of the AMPD polynucleotides disclosed herein also can be identified by
- 35 making suitable probes or primers and screening cDNA expression libraries. It is well known

that the  $T_m$  of a double-stranded DNA decreases by 1-1.5° C with every 1% decrease in homology (Bonner et al., J. Mol. Biol. 81, 123 (1973)). Variants of AMPD polynucleotides or polynucleotides of other species can therefore be identified by hybridizing a putative homologous AMPD polynucleotide with a polynucleotide having a nucleotide sequence of  
5 SEQ ID NO.: 1, 2, 3, 4, 5, or 6 for each transcript variant identified to date or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

10

Nucleotide sequences which hybridize to AMPD polynucleotides or their complements following stringent hybridization and/or wash conditions also are AMPD polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2<sup>nd</sup> ed.,  
15 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20° C below the calculated  $T_m$  of the hybrid under study. The  $T_m$  of a hybrid between an AMPD polynucleotide having a  
20 nucleotide sequence shown in SEQ ID NO.: 1, 2, 3, 4, 5, or 6 for each transcript variant identified to date or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, Proc. Natl. Acad. Sci. U.S.A. 48, 1390 (1962):

25

$T_m = 81.5^\circ \text{C} - 16.6(\log_{10} [\text{Na}^+]) + 0.41(\% \text{ G+C}) - 0.63(\% \text{ formamide}) - 600/l$ , where  $l$  = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4 X SSC at 65° C, or 50% formamide, 4 X SSC at 42° C, or 0.5 X SSC, 0.1% SDS at 65° C. Highly stringent  
30 wash conditions include, for example, 0.2 X SSC at 65° C.

### *Preparation of Polynucleotides*

A naturally occurring AMPD polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated AMPD polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises AMPD nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

AMPD DNA molecules can be made with standard molecular biology techniques, using AMPD mRNA as a template. AMPD DNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook et al. (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention. The inventors have successfully demonstrated this approach.

Alternatively, synthetic chemistry techniques can be used to synthesize AMPD polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a AMPD polypeptide having, for example, an amino acid sequence shown in SEQ ID NOs: 7, 8, 9, 10, 11, 12 for all the AMPD2 protein isoforms derived from each transcript variant identified or a biologically active variant thereof.

### *Expression of Polynucleotides*

To express an AMPD polynucleotide, the polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding AMPD polypeptides and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described, for example, in Sambrook et al. (1989) and in Ausubel et al.,

30

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding an AMPD enzyme polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (e.g., baculovirus), plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those nontranslated regions of the vector enhancers, promoters, 5' and 3' untranslated regions which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding an AMPD polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

#### *Host Cells*

According to certain embodiments of the subject invention, an AMPD polynucleotide will need to be inserted into a host cell, for expression, processing and/or screening. A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed AMPD polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation,



phosphorylation, lipidation, and acylation. Posttranslational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and W138),  
5 are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, Va. 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high yield production of recombinant proteins.  
10 For example, cell lines which stably express AMPD polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 12 days in an enriched medium before they are switched to a selective medium. The purpose of the  
15 selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced AMPD sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R. I. Freshney, ed., 1986.

#### *Detecting Expression*

A variety of protocols for detecting and measuring the expression of an AMPD polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in  
25 the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a KHK polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton et al., SEROLOGICAL METHODS: A  
30 LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox et al., J. Exp. Med. 158, 12111216, 1983).

#### *Expression and Purification of Polypeptides*

35 Host cells transformed with nucleotide sequences encoding AMPD polypeptide can be

cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode KHK polypeptides can be designed to contain signal sequences which direct secretion of soluble AMPD polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound AMPD polypeptide.

### *Antibodies*

Antibodies are referenced herein and various aspects of the subject invention utilize antibodies specific to AMPD polypeptide(s). As described above, one example of a therapeutic agent may pertain to an antibody. Any type of antibody known in the art can be generated to bind specifically to an epitope of an AMPD polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, which are capable of binding an epitope of an AMPD polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, e.g., at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of an AMPD polypeptide can be used therapeutically, as mentioned, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen. Antibodies useful for embodiments of the subject invention may be polyclonal, but are preferably monoclonal antibodies.

### *Ribozymes*

Ribozymes may be one category of compounds useful as therapeutic agents for modulating AMPD activity. Ribozymes are RNA molecules with catalytic activity. See, e.g., Cech, Science 236, 15321539; 1987; Cech, Ann. Rev. Biochem. 59, 543568; 1990, Cech, Curr.

Opin. Struct. Biol. 2, 605609; 1992, Couture & Stinchcomb, Trends Genet. 12, 510515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff et al., U.S. Pat. No. 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

Accordingly, another aspect of the invention pertains to using the coding sequence of an AMPD polynucleotide to generate ribozymes which will specifically bind to mRNA transcribed from the AMPD polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (see Haseloff et al. Nature 334, 585591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach et al., EP 321,201).

Specific ribozyme cleavage sites within an AMPD RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate AMPD RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease AMPD expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and

maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

5

As taught in Haseloff et al., U.S. Pat. No. 5,641,673, the entirety of which is incorporated by reference, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both

10

Reference is made to standard textbooks of molecular biology that contain definitions and methods and means for carrying out basic techniques, encompassed by the present invention. See, for example, Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1982) and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989); *Methods in Plant Molecular Biology*, Maliga et al, Eds., Cold Spring Harbor Laboratory Press, New York (1995); *Arabidopsis*, Meyerowitz et al, Eds., Cold Spring Harbor Laboratory Press, New York (1994) and the various references cited therein.

20

#### *Interfering molecules*

AMPD can be inhibited by a number of means including silencing via miRNA, shRNA, or siRNA, for example, directed to a portion of the sequence described at the genbank accession numbers provided above. siRNA molecules can be prepared against a portion of SEQ ID NOs: 1, 2, 3, 4, 5, 6 according to the techniques provided in U.S Patent Publication 20060110440 and used as therapeutic compounds. RNAi against AMPD is commercially available from, for example, Santa Cruz Biotechnology, including AMPD2 siRNA (catalog # sc-78844), AMPD2 shRNA plasmid (catalog #sc-78844-SH), AMPD2 shRNA lentiviral particles (catalog # sc-78844-V), in non limiting examples. shRNA constructs are typically made from one of three possible methods; (i) annealed complementary oligonucleotides, (ii) promoter based PCR or (iii) primer extension. See Design and cloning strategies for constructing shRNA expression vectors, Glen J McIntyre, Gregory C Fanning BMC Biotechnology 2006, 6:1 (5 January 2006).

35

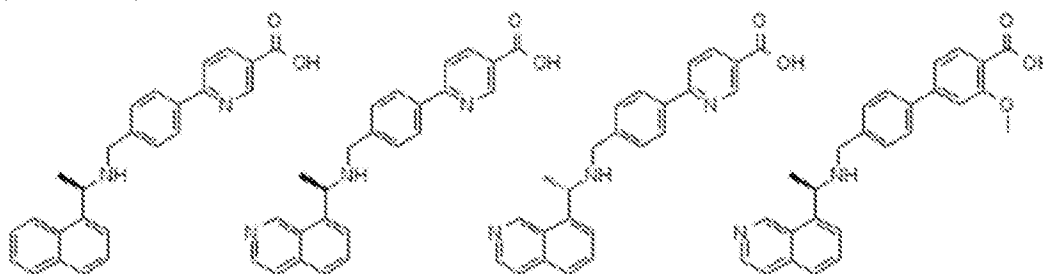
For background information on the preparation of miRNA molecules, see e.g., U.S. patent applications 20110020816, 2007/0099196; 2007/0099193; 2007/0009915; 2006/0130176; 2005/0277139; 2005/0075492; and 2004/0053411, the disclosures of which are hereby incorporated by reference herein. See also, U.S. Pat. Nos. 7,056,704 and 7,078,196 (preparation of miRNA molecules), incorporated by reference herein. Synthetic miRNAs are described in Vatolin, et al 2006 J Mol Biol 358, 983-6 and Tsuda, et al 2005 Int J Oncol 27, 1299-306, incorporated by reference herein.

It is within the scope of aspects of the present invention to provide agents to silence AMPD2 genes to achieve a therapeutic effect using interfering molecules.

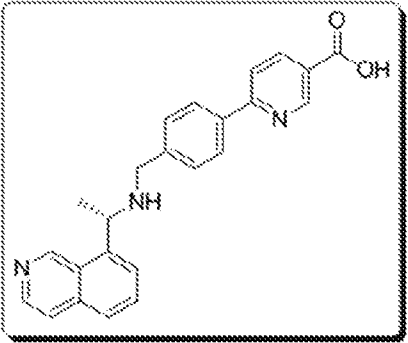
#### *Exemplary AMPD2 inhibitor compounds*

To document that small molecule compounds are available to inhibit AMPD2 specifically, the present inventors proposed the following compounds and the following scheme to arrive at the compound below:

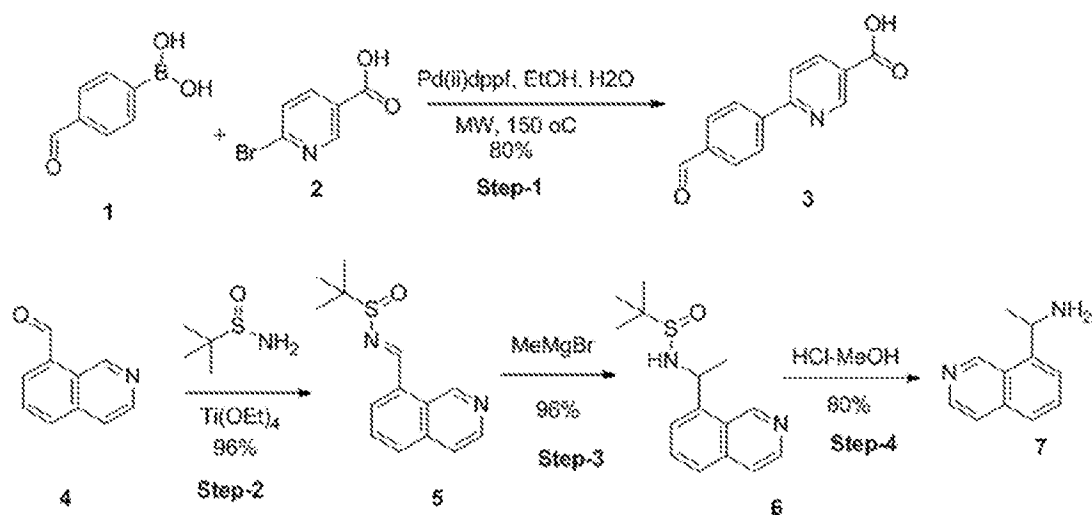
AMPD2 inhibitors provided <sup>3</sup>:  
in Admyre et al.(supra)

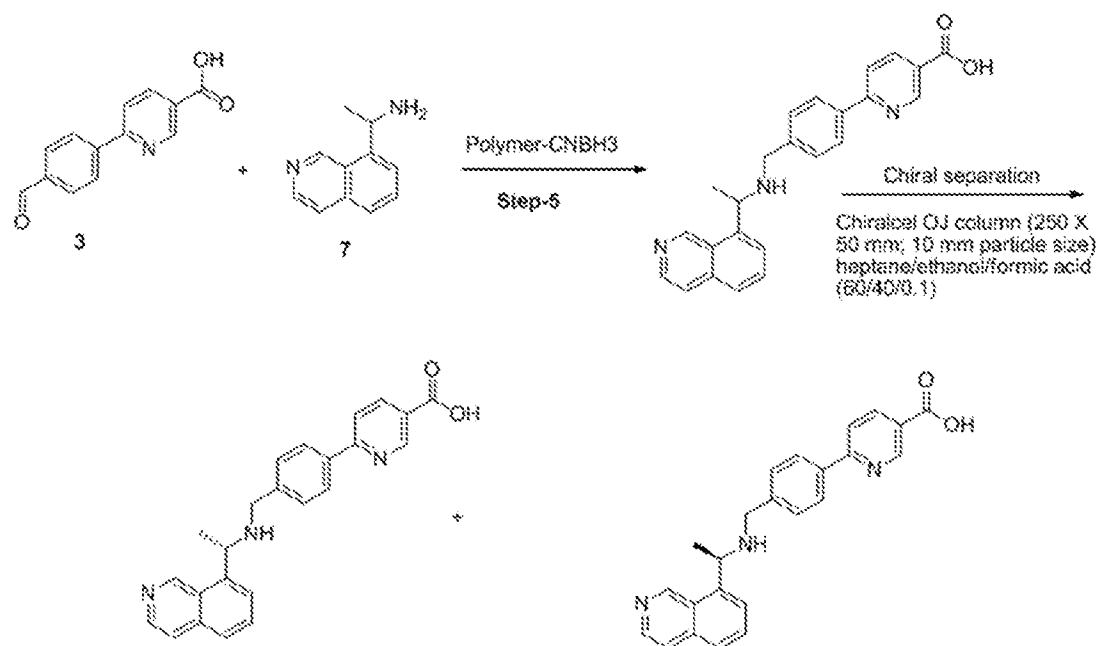


In a particular embodiment, the AMPD inhibitor is as follows:

|           |                                                                                    |
|-----------|------------------------------------------------------------------------------------|
| Proposal  | (S)-6-(4-((1-(isoquinolin-8-yl)ethylamino)methyl)phenyl)nicotinic acid             |
| Structure |  |
| Quantity  | ~50 mg                                                                             |

## Proposed Scheme:





## Chemicals

Required:

| S. No. | Chemical Name                                                                                       | CAS No.     | Vendor        | Quantity | Cost (\$) |
|--------|-----------------------------------------------------------------------------------------------------|-------------|---------------|----------|-----------|
| 1      | 4-formylphenylboronic acid                                                                          | 87199-17-5  | Sigma-Aldrich | 10 g     | 205       |
| 2      | 6-bromonicotinic acid                                                                               | 6311-35-9   | Sigma-Aldrich | 10 g     | 225       |
| 3      | 1,1'-bis(diphenylphosphino)-ferrocenedichloropalladium(II)                                          | 72287-26-4  | Sigma-Aldrich | 5 g      | 255       |
| 4      | isoquinoline-8-carbaldehyde                                                                         |             | Sigma-Aldrich |          |           |
| 5      | Titanium ethoxide                                                                                   | 3087-36-3   | Sigma-Aldrich | 50g      | 55        |
| 6      | 2-methylpropane-2-sulfinamide (racemic)                                                             | 146374-27-8 | Sigma-Aldrich | 5 g      | 193       |
| 7      | Methylmagnesium bromide 3M                                                                          | 75-16-1     | Sigma-        | 100 ml   | 48        |
|        |                                                                                                     |             | Aldrich       |          |           |
| 8      | Polymer supported cyanoborohydride                                                                  |             | Sigma-Aldrich | 25 g     | 268       |
|        |                                                                                                     |             |               |          |           |
|        | Chiralcel OJ column (250 X 50 mm; 10 $\mu$ m particle size) heptane/ethanol/formic acid (60/40/0.1) |             |               |          |           |

*Derivatives of AMPD2 inhibitors*

- 5 According to certain embodiments, the term AMPD2 inhibitors may include derivatives, salts, esters, enol ethers, enol esters, acetals, ketals, orthoesters, hemiacetals, hemiketals, solvates, hydrates, metabolites or prodrugs thereof. Derivatives may be readily prepared by those of skill in this art using known methods for such derivatization. (see also, Admyre et al cited supra, which describes different derivatives of the compounds described above,
- 10 particularly those shown in FIG. 2A). The compounds produced may be administered to



animals or humans without substantial toxic effects and either are pharmaceutically active or are prodrugs. Pharmaceutically acceptable salts include, but are not limited to, amine salts, such as but not limited to N,N'-dibenzylethylenediamine, chlorprocaine, choline, ammonia, diethanolamine and other hydroxyalkylamines, ethylenediamine, N-methylglucamine, procaine, N-benzylphenethylamine, 1-para-chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole, diethylamine and other alkylamines, piperazine and tris(hydroxymethyl)aminomethane; alkali metal salts, such as but not limited to lithium, potassium and sodium; alkali earth metal salts, such as but not limited to barium, calcium and magnesium; transition metal salts, such as but not limited to zinc; and other metal salts, such as but not limited to sodium hydrogen phosphate and disodium phosphate; and also including, but not limited to, salts of mineral acids, such as but not limited to hydrochlorides and sulfates; and salts of organic acids, such as but not limited to acetates, lactates, malates, tartrates, citrates, ascorbates, succinates, butyrates, valerates and fumarates. Pharmaceutically acceptable esters include, but are not limited to, alkyl, alkenyl, alkynyl, alk(en)(yn)yl, aryl, aralkyl, and cycloalkyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids and boronic acids. Pharmaceutically acceptable enol ethers include, but are not limited to, derivatives of formula  $C=C(OR)$  where R is hydrogen, alkyl, alkenyl, alkynyl, alk(en)(yn)yl, aryl, aralkyl, or cycloalkyl. Pharmaceutically acceptable enol esters include, but are not limited to, derivatives of formula  $C=C(OC(O)R)$  where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, aralkyl, or cycloalkyl. Pharmaceutically acceptable solvates and hydrates are complexes of a compound with one or more solvent or water molecules, or 1 to about 100, or 1 to about 10, or one to about 2, 3 or 4, solvent or water molecules.

According to further embodiments, derivatives may include, but are not limited to, specific substitutions of reactive constituents on or emanating from the AMPD2 inhibitor, and may include, but are not limited to, one or more of the following: a hydrogen, hydroxy, halo, haloalkyl, thiocarbonyl, alkoxy, alkenoxy, alkylaryloxy, aryloxy, arylalkyloxy, cyano, nitro, imino, alkylamino, aminoalkyl, thio, sulfhydryl, thioalkyl, alkylthio, sulfonyl, C1-C6 straight or branched chain alkyl, C2-C6 straight or branched chain alkenyl or alkynyl, aryl, aralkyl, heteroaryl, carbocycle, or heterocycle group or moiety, or CO<sub>2</sub> R<sub>7</sub> where R<sub>7</sub> is hydrogen or C1-C9 straight or branched chain alkyl or C2-C9 straight or branched chain alkenyl group or moiety.

It is to be understood that the compounds provided herein may contain chiral centers. Such chiral centers may be of either the (R) or (S) configuration, or may be a mixture thereof.

Thus, the compounds provided herein may be enantiomerically pure, or be stereoisomeric or

diastereomeric mixtures. It is to be understood that the chiral centers of the compounds provided herein may undergo epimerization in vivo. As such, one of skill in the art will recognize that administration of a compound in its (R) form is equivalent, for compounds that undergo epimerization in vivo, to administration of the compound in its (S) form.

5 As used herein, alkyl refers to an unbranched or branched hydrocarbon chain. An alkyl group may be unsubstituted or substituted with one or more heteroatoms.

As used herein, alkenyl refers to an unbranched or branched hydrocarbon chain comprising one or more double bonds. The double bond of an alkenyl group may be unconjugated or conjugated to another unsaturated group. An alkenyl group may be unsubstituted or  
10 substituted with one or more heteroatoms.

As used herein, alkynyl refers to an unbranched or branched hydrocarbon chain comprising one or more triple bonds therein. The triple bond of an alkynyl group may be unconjugated or conjugated to another unsaturated group. An alkynyl group may be unsubstituted or substituted with one or more heteroatoms.

15 As used herein, alk(en)(yn)yl refers to an unbranched or branched hydrocarbon group comprising at least one double bond and at least one triple bond. The double bond or triple bond of an alk(en)(yn)yl group may be unconjugated or conjugated to another unsaturated group. An alk(en)(yn)yl group may be unsubstituted or substituted with one or more heteroatoms. Exemplary alkyl, alkenyl, alkynyl, and alk(en)(yn)yl groups herein include, but  
20 are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl, n-butyl, sec-butyl, tert-butyl, isopentyl, neopentyl, tert-pentyl, isohexyl, allyl (propenyl) and propargyl (propynyl).

As used herein, "aryl" refers to aromatic monocyclic or multicyclic groups containing from 6 to 19 carbon atoms. Aryl groups include, but are not limited to groups such as unsubstituted or substituted fluorenyl, unsubstituted or substituted phenyl, and unsubstituted or substituted  
25 naphthyl.

As used herein, "heteroaryl" refers to a monocyclic or multicyclic aromatic ring system, in certain embodiments, of about 5 to about 15 members where one or more, in one embodiment 1 to 3, of the atoms in the ring system is a heteroatom, that is, an element other than carbon, including but not limited to, nitrogen, oxygen or sulfur. The heteroaryl group  
30 may be optionally fused to a benzene ring. Heteroaryl groups include, but are not limited to, furyl, imidazolyl, pyrimidinyl, tetrazolyl, thienyl, pyridyl, pyrrolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, triazolyl, quinolinyl or isoquinolinyl.

As used herein, "halo," "halogen," or "halide" refers to F, Cl, Br or I.

While various embodiments of the present invention have been shown and described herein, it will be obvious that such embodiments are provided by way of example only. Numerous variations, changes and substitutions may be made without departing from the invention herein. Accordingly, it is intended that the invention be limited only by the spirit and scope of the appended claims.

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The teachings of the references, including patents and patent related documents, cited herein are incorporated herein in their entirety to the extent not inconsistent with the teachings herein.

## SEQUENCE INFORMATION:

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SEQ. ID NO. 5 (NM\_001257361):

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 35 421 actcggacct acagctctac aaggaacagg gtgaggggca gggtgaccgg agcctgcggg

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## SEQ. ID NO 6 (NM\_001308170):

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10 SEQ ID NO. 7 (NP\_004028):

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## CLAIMS

What is claimed is:

5

1. A composition useful for decreasing a craving for fructose, sucrose, and/or a simple sugar-containing compound, said composition comprising any compound with AMPD2 inhibitory activity at a therapeutically effective amount to inhibit activity against AMPD2.

10

2. The composition of claim 1, wherein the AMPD inhibitor comprises a member from the group consisting of a ribozyme, an interfering molecule (RNAi), peptide nucleic acid, peptide, small molecule, an antibody targeted to AMPD, and combinations thereof.

15

3. The composition of claims 1 and 2, wherein the composition further comprises a conjunctive therapeutic compound either as a pharmacologically active compound or mixture of compounds, comprising:

one or more of fructokinase inhibitors, xanthine oxidase inhibitors including febuxostat, allopurinol and oxypurinol, angiotensin converting enzyme inhibitors, or angiotensin receptor blockers.

20

4. The composition of claims 1-3, wherein the AMPD inhibitor comprises a small molecule, and wherein the small molecule is selected from the group consisting of: 3-[2-(3-carboxy-4-bromo-5,6,7,8-tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo[4,5-d] [1,3] diazepin-8-ol; 6-(4-((1-(Isoquinolin-8-yl)ethylamino)methyl)phenyl)nicotinic acid (including enantiomers); 4'-((1-(Isoquinolin-8-yl)ethylamino)methyl)-3-methoxybiphenyl-4-carboxylic acid; and N3-Substituted coformycin aglycon analogues.

25

5. A method for treating an AMPD2 related craving or addiction in a subject comprising administering a therapeutically effective amount of an AMPD2 inhibitor and/or a pan AMPD inhibitor, to the subject.

30

6. The method of claim 5, wherein the AMPD2 inhibitor comprises at least one member of the group consisting of a ribozyme, an interfering molecule, a peptide, a small molecule, an antibody targeted to at least AMPD2, and combinations thereof.

7. The method of claim 6, wherein the AMPD2 inhibitor comprises an interfering molecule, and wherein the interfering molecule comprises a member from the group consisting of a phosphothioate morpholino oligomer (PMO), miRNA, siRNA, methylated siRNA, treated-siRNA, shRNA, antisense RNA, a dicer-substrate 27-mer duplex, and any combination thereof.

8. The method of claim 5, wherein the administering is done to decrease or treat a sugar, salt, or umami craving, or a combination thereof.

9. The method of claim 5, wherein the administering is done to treat an addiction to alcohol, tobacco, nicotine, cocaine, methamphetamines, amphetamines and marijuana.

10. The method of claim 5, wherein the administering is done to treat an addiction to opioids, cannabinoids, methylphenidate, phencyclidine, and substituted amphetamines, or sex.

11. The method of claim 5, wherein the administering is done to provide a diminished craving in the subject from at least one member selected from the group consisting of fructose, fructose-containing sugars, glucose, sucralose, and combinations thereof.

12. The method of claim 5, wherein the subject is diabetic.

13. A pharmaceutical formulation for treating a sugar craving characterized by a presence or activity of AMPD2 by decreasing AMPD2, comprising a therapeutically effective amount of an AMPD2 inhibitor, and optionally a pharmaceutically acceptable carrier.

14. The formulation of claim 13, wherein the AMPD2 inhibitor comprises at least one member of the group consisting of a ribozyme, an interfering molecule, a peptide, a small molecule, or an antibody targeted to AMPD2.

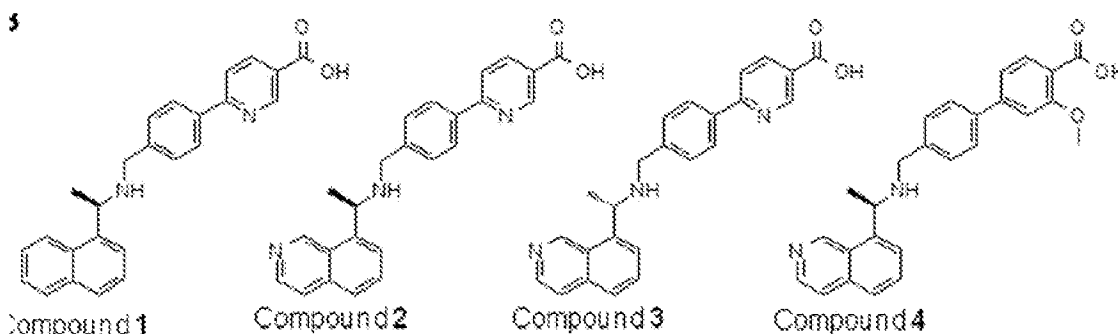
15. The formulation of claim 14, wherein the AMPD2 inhibitor comprises an interfering molecule, and wherein the interfering molecule comprises a member from the group consisting of a phosphothioate morpholino oligomer (PMO), miRNA, siRNA,

methyated siRNA, treated siRNA, shRNA, antisense RNA, a dicer-substrate 27-mer duplex, and any combination thereof.

16. The formulation of claim 14, wherein the AMPD2 inhibitor comprises a small molecule, and wherein the small molecule comprises:

(S)-6-(4-((1-(isoquinolin-8-yl) ethylamino) methyl) phenyl) nicotinic acid.

17. The formulation of claim 14, wherein the AMPD2 inhibitor comprises one of the following compounds:



18. A method for treating anorexia nervosa and/or to increase food intake in a subject, comprising administering a therapeutically effective amount of IMP to the subject.

19. A method for treating anorexia nervosa and/or to increase food intake in a subject comprising inducing expression of AMPD2, or stimulating AMPD2 activity in the subject.

20. The method of claims 5-12, wherein the AMPD2 related addiction is an addiction associated with reduction in dopamine receptors in the nucleus accumbens.

21. The method of claims 5-12 and 20, wherein the therapeutically effective amount reduces the upregulation of  $\Delta$ FosB or amount of  $\Delta$ FosB in the nucleus accumbens in the subject.

22. The method of claim 21, wherein the upregulation of  $\Delta$ FosB or amount of  $\Delta$ FosB in the nucleus accumbens in the subject has been elevated in response to alcohol, tobacco, nicotine, cocaine, methamphetamine, amphetamine, marijuana, opioid, cannabinoid,

methylphenidate, phencyclidine, substituted amphetamines, or sex administration to the subject.

23. The method of claims 5-12, or 20-22, wherein the AMPD2 inhibitor comprises  
5 one of the following compounds:

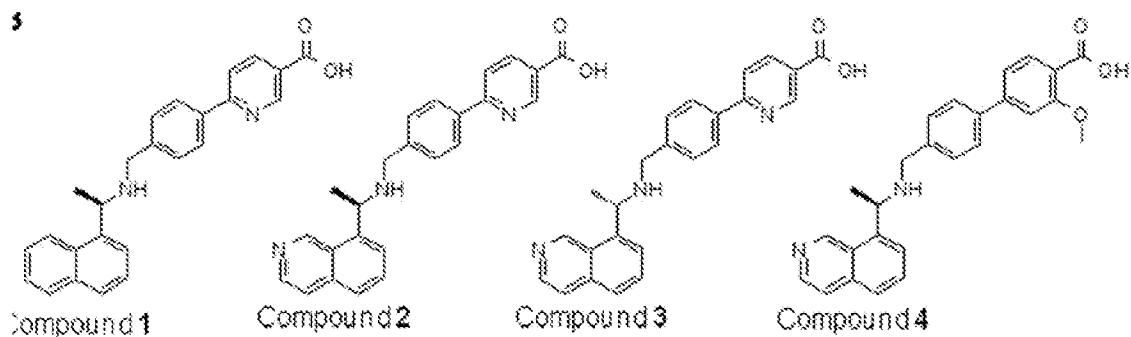


Figure 1 Fructose Metabolism Involves Activation of AMP Deaminase 2

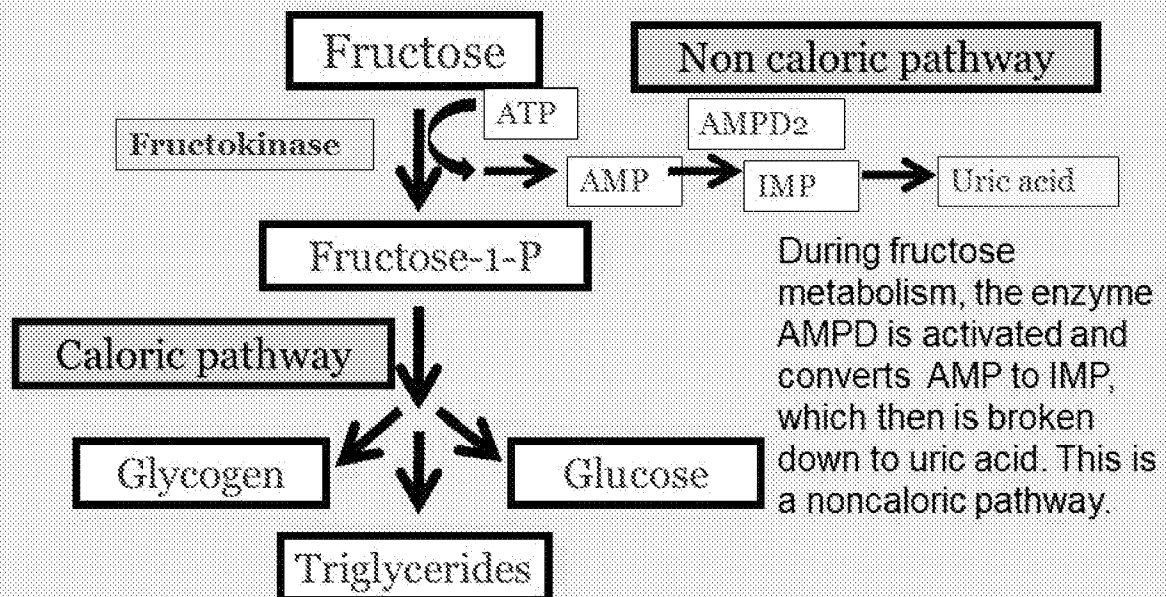
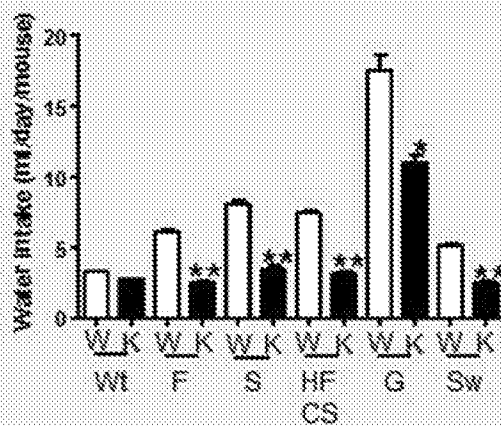


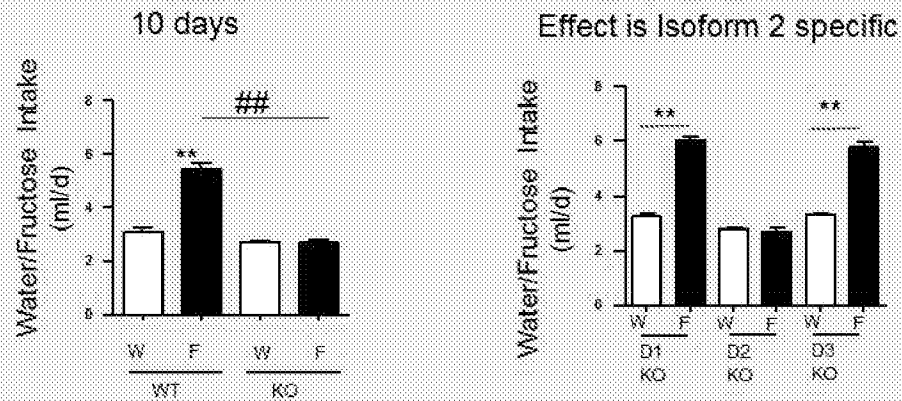


Figure 2 AMPD2 KO Mice Do not Like Simple Sugars or Artificial Sugar



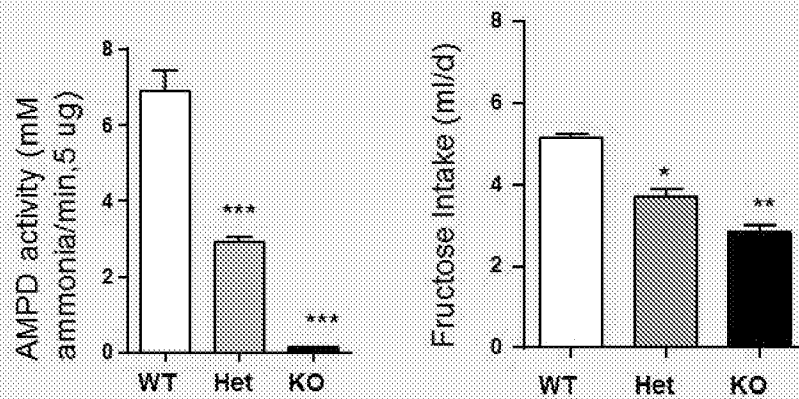
Mice were offered water (Wt) or water with 15% fructose (F), sucrose (S), High fructose corn syrup (HFCS), glucose (G) or 0.04% sucralose (SW). While Wild type mice (W) preferred sweetened fluids over water, AMPD2 Knockout mice (K) showed much less liking of sweetened fluids.

Figure 3 AMPD2 Deficient Mice Do not Crave Fructose



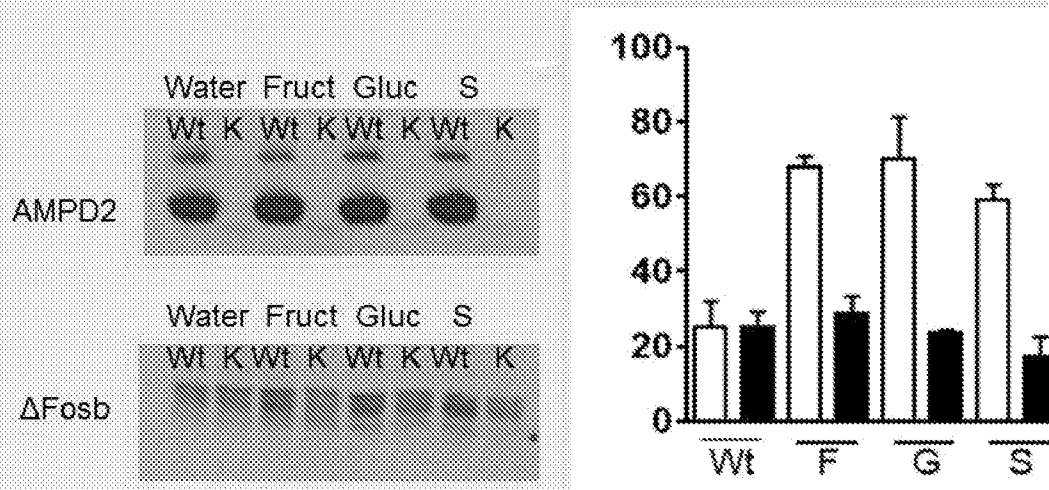
Left: Wild type (WT) mice drink more 15% fructose water (F) than regular water whereas AMPD2 KO mice (KO) do not crave fructose. Right: AMPD1 KO (D1) and AMPD3 (D3) KO mice prefer fructose (F) to water but not the AMPD2 (D2) KO mouse.

**Figure 4 AMPD2 Heterozygotes show half the Effect as the AMPD2 KO (Dose Dependency)**



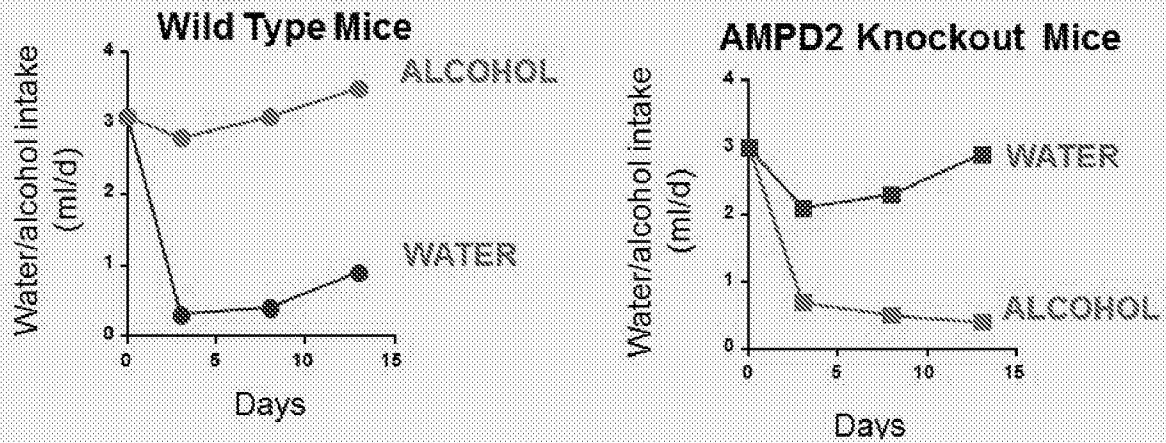
Left: Wild type (WT) mice have significant AMPD activity in their liver while AMPD KO mice (K) have none and AMPD heterozygote (Hets) have intermediate activity. Right: While WT mice like to drink fructose (15%) drinking water, Hets drink significantly less and KO drink even less.

Figure 5 AMPD2 KO Maintain Low Delta FosB



AMPD2 protein is present by western blot in the nucleus accumbens of wild type (Wt) mice administered water, fructose (15%, fruct or F), glucose (gluc or G), or sucralose (S) but not in AMPD2 KO (K) mice. Delta Fosb is also induced in wild type mice given fructose, glucose or sucralose. However, induction of delta FosB is not observed in AMPD2 KO mice administered sweeteners.

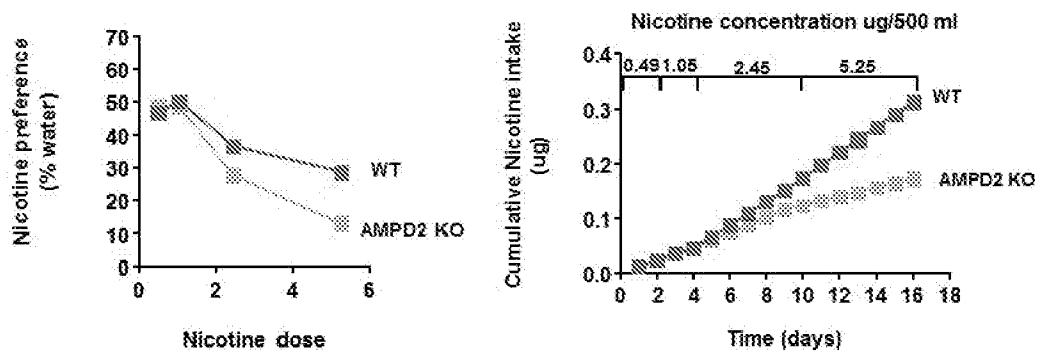
**Figure 6 AMPD2 KO Mice Do not Crave Alcohol:**  
Two bottle preference with water or increasing alcohol (3 days 3%, 5 days 6%, 7 days 10%)



Left: Wild type mice will prefer alcohol containing water (red) over regular water. Right: AMPD2 KO mice prefer water over alcohol..

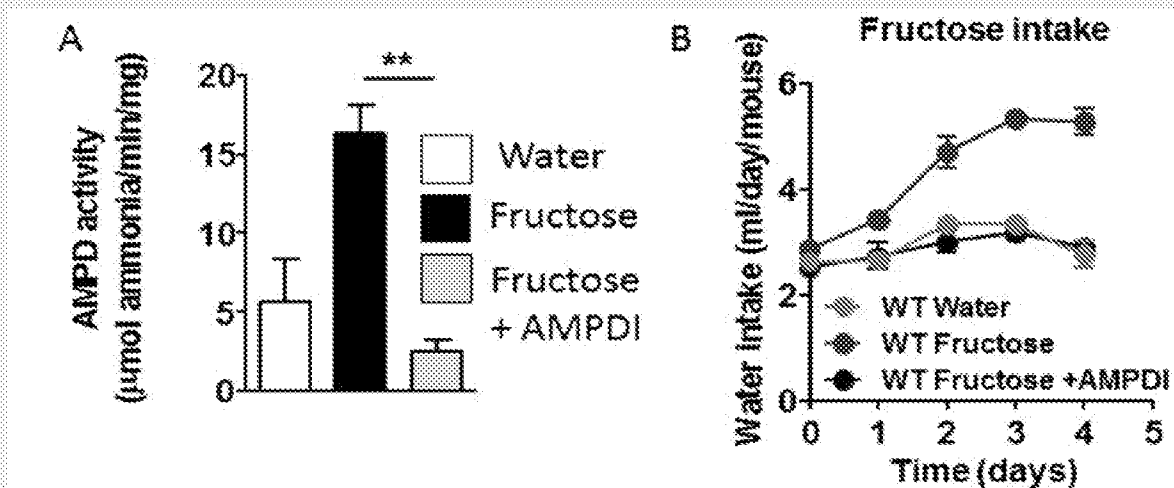
**Figure 7 AMPD2 Knockout Mice Show Reduced Preference for Nicotine and Decreased Total Intake**

2 bottle choice experiment: WATER versus NICOTINE



Using a 2-bottle choice preference (water versus increasing levels of nicotine) in wild type WT (red) and AMPD2 Knockout mice (blue). WT mice demonstrated preference for nicotine over 30% of the time compared to water while AMPD2 KO mice preferred nicotine less than 10% of the time. Right: Cumulative nicotine intake over 16 days in the 2-bottle choice preference. Differences between nicotine intake between WT and KO are highly statistically significant.

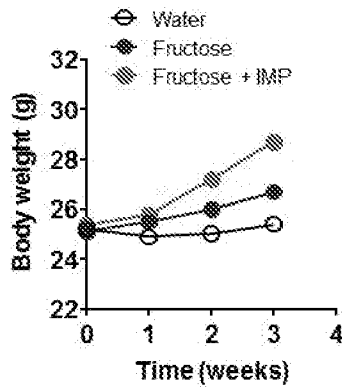
Figure 8 Pharmacologic Inhibition of AMPD Blocks Craving to Fructose



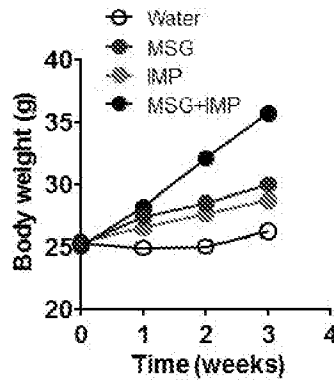
Left: Wild type mice given fructose (15% in drinking water) show increased AMPD2 activity in their liver that is blocked using the AMPD inhibitor (AMPDI). Right: While mice like to drink fructose (15%) drinking water (blue), this is prevented in the mice given the inhibitor by oral gavage.

## Figure 9 IMP is a stimulant for Food intake

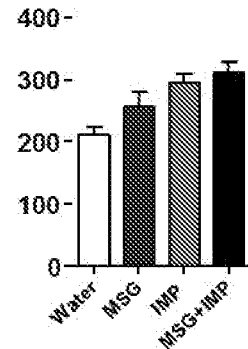
Wild type animals gain weight with IMP added to fructose



Wild type animals gain weight with IMP added to fructose



Cumulative caloric intake (3 weeks)



Left: IMP (300  $\mu$ M), the product of AMPD, stimulates weight gain in animals given fructose (5%) in the drinking water. Center: IMP also stimulates weight gain in mice on regular chow, and also potentiates the effect of monosodium glutamate (MSG, 5%). Right panel: Energy intake on mice receiving regular chow with regular drinking water, water containing MSG, or MSG plus IMP.

## INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/US2017/027207****A. CLASSIFICATION OF SUBJECT MATTER****A61K 31/713(2006.01)i, A61K 38/00(2006.01)i, A61K 31/4406(2006.01)i, A61K 31/47(2006.01)i, A61K 31/4725(2006.01)i, C12N 15/113(2010.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K 31/713; A61K 39/395; G01N 33/52; C07D 231/44; A01N 43/56; A01N 61/00; A61K 38/00; C12Q 1/28; A61K 31/4406; A61K 31/47; A61K 31/4725; C12N 15/113

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) &amp; Keywords: AMPD2 inhibitory, craving for fructose, addiction, fructokinase, BDBM154581

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                  | Relevant to claim No. |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | US 8697628 B2 (GARCIA, G. et al.) 15 April 2014<br>See columns 3, 10-11; and figures 7A, 7B.                                                                                                                        | 1-2, 13-17            |
| Y         | US 2013-0224218 A1 (JOHNSON, R. J. et al.) 29 August 2013<br>See abstract; paragraph [0021]; and figure 1.                                                                                                          | 1-2, 13-17            |
| Y         | ADMYRE, T. et al., 'Inhibition of AMP deaminase activity does not improve glucose control in rodent models of insulin resistance or diabetes'<br>Chemistry & Biology, 2014, Vol. 21, pp. 1486-1496<br>See figure 2. | 16-17                 |
| A         | WO 01-07413 A1 (3-DIMENSIONAL PHARMACEUTICALS, INC.) 01 February 2001<br>See the whole document.                                                                                                                    | 1-2, 13-17            |
| A         | EP 0745684 A1 (KYOWA MEDEX CO., LTD.) 04 December 1996<br>See the whole document.                                                                                                                                   | 1-2, 13-17            |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

08 August 2017 (08.08.2017)

Date of mailing of the international search report

**08 August 2017 (08.08.2017)**

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 5-12,18-23  
because they relate to subject matter not required to be searched by this Authority, namely:  
Claims 5-12 and 18-23 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.
2. ☒ Claims Nos.: 22  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Claim 22 refers to one of claims which are not drafted in accordance with PCT Rule 6.4(a).
3. ☒ Claims Nos.: 3-4,20-21,23  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/US2017/027207**

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Publication<br>date                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
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