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ORIGINAL RESEARCH

LG839: Anti-Obesity Effects and Polymorphic Gene Correlates of Reward Deficiency Syndrome

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ABSTRACT

Introduction: This study systematically assessed the weight management effects of a novel experimental DNA-customized nutraceutical, LG839 (LifeGen®, Inc., La Jolla, CA, USA).
Methods: A total of 1058 subjects who participated in the overall D.I.E.T. study were genotyped and administered an LG839 variant based on polymorphic outcomes. A subset of 27 self-identified obese subjects of Dutch descent, having the same DNA pattern of four out of the five candidate genes tested (chi-square analysis) as the entire data set, was subsequently evaluated. Simple *t* tests comparing a number of weight management parameters before and after 80 days of treatment with LG839 were performed.
Results: Significant results were observed for weight loss, sugar craving reduction, appetite suppression, snack reduction, reduction of late night eating (all $P<0.01$), increased perception of overeating, enhanced quality of sleep, increased happiness (all $P<0.05$), and increased energy

(P<0.001). Polymorphic correlates were obtained for a number of genes (*LEP*, *PPAR-γ2*, *MTHFR*, *5-HT2A*, and *DRD2* genes) with positive clinical parameters tested in this study. Of all the outcomes and gene polymorphisms, only the *DRD2* gene polymorphism (A1 allele) had a significant Pearson correlation with days on treatment (r=0.42, P=0.045).
Conclusion: If these results are confirmed in additional rigorous, controlled studies, we carefully suggest that DNA-directed targeting of certain regulator genes, along with customized nutraceutical intervention, provides a unique framework and strategic modality to combat obesity.

Keywords: LG839; neurotransmitters; obesity; reward deficiency syndrome; sugar craving behavior; weight loss

INTRODUCTION

Obesity was formerly characterized as purely a behavioral disorder involving poor dietary habits and lack of physical exercise, due in large part to a lack of willpower or self-restraint.[1] Over the past decade, however, the consensus of the medical community has evolved and the Institute of Medicine at the National Academy of Sciences, the National Institute of Health (NIH), and the International Classification of Diseases, among many others, now recognize obesity as a chronic, multifactorial medical disease of energy metabolism and appetite regulation involving genetics, environment, physiology, and biochemistry.[1-3] However, in persons not classified as obese but rather as overweight, the US Food and Drug Administration (FDA) recently noted in its final rules under the Dietary Supplements Health and Education Act of 1994, that despite obesity being a disease, a person being overweight but less than obese refers not to a disease but to the structure and function of the body.[4]

Obesity-related medical conditions contribute to 300,000 deaths in the United States each year, second only to smoking as a cause of preventable death; and obesity has been established as a major risk factor for hypertension, cardiovascular disease, type 2 diabetes, and some cancers in both men and women.[5]

Obesity affects an estimated 58 million people in the United States and its prevalence is increasing.[6] Approximately one-third of American adults are estimated to be obese, and 60% are overweight.[7] In response to this rising epidemic, the medical, food, and fitness communities have consistently advised the population to make behavioral modifications, such as improving diet and exercising. However, as people have changed their eating and exercise habits, the rates of obesity have continued to rise. The combined prevalence of overweight and obese persons in the United States has increased from 46% of the adult populations (National Health and Nutrition Examination Survey [NHANES] II, 1976-1980) to over

60% of the adult population in NHANES III (1988-1994).[7] A 2006 survey suggested that people who are obese have similar eating and exercise habits, as well as similar behaviors in snacking, reading nutritional labels, and eating out, when compared with normal weight people.[7]

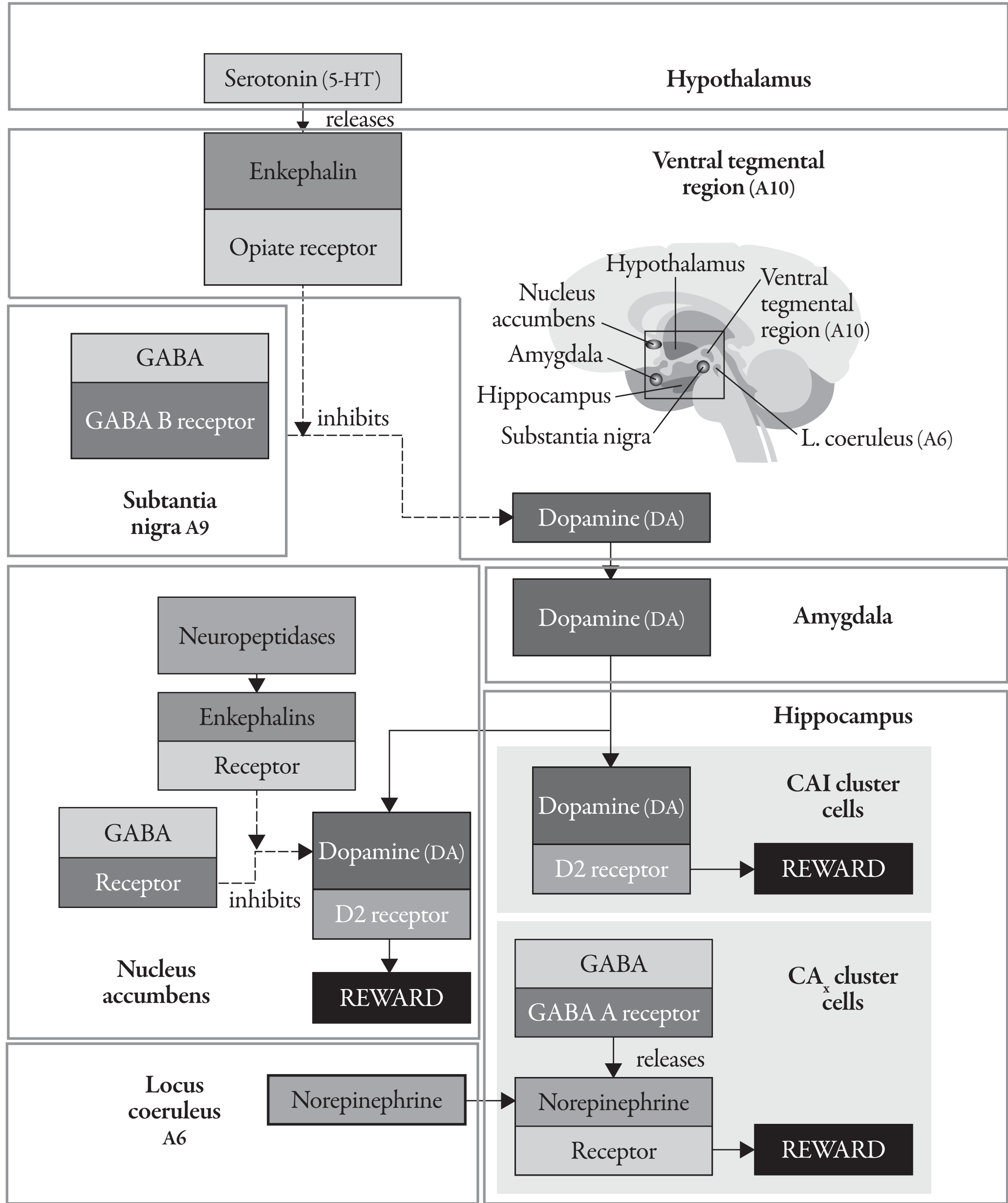
Despite NIH, World Health Organization, and National Academy of Sciences statements that genetic factors play a role in obesity, a genetic test has not yet been developed to diagnose or stratify this condition, nor have there been treatments to target genetic associations. To address this unmet clinical need, our laboratory has developed a patent-protected genetic test and companion customized nutritional product called LG839 (LifeGen®, Inc., La Jolla, CA, USA).[8] The basic components consist of Dl-phenylalanine, L-tyrosine, 5-hydroxytryptophane, L-glutamine, chromium salts, vitamin B6, rhodiola rosea, and passion flower.

Our previous research into alcoholism, another prevalent healthcare condition that has also been traditionally characterized in behavioral terms, suggested that the condition had a genetic component.[9] This research, and work by other laboratories, continued to explain how analyzing certain genotypes could guide dietary intake and environments to overcome this genetic predisposition.[9] Similar to alcoholism, obesity may be due in part to certain genetic predispositions, and by the body engaging in behavior to overcome these deficiencies, the host may engage in behaviors that are individually unhealthy or detrimental. In fact, it has been shown that the prevalence of polymorphisms of the *DRD2* receptor gene significantly increases in obese subjects with comorbid substance use disorder.[10]

This initial research began to elucidate a concept that has been defined as reward deficiency syndrome (RDS). RDS results from a dysfunction in the brain reward cascade (Figure 1), which directly links abnormal craving behavior with a defect in the *DRD2* dopamine receptor gene as well as in other dopaminergic genes (*D1*, *D3*, *D4*, *D5*).[12-14] A number of other candidate genes are also associated with reward dependence in the neurogenetics of RDS.[15] However, dopamine is a very powerful neurotransmitter in the brain that controls feelings of well-being produced through the interaction of dopamine and neurotransmitters such as serotonin and the opioids.[15] The complex interactions of these neurotransmitters ultimately regulate the dopaminergic activity in the mesolimbic system of the brain, which has been termed the brain reward cascade.[11]

RDS involves a form of sensory deprivation of the brain's reward or pleasure mechanisms and is manifested as a consequence of an individual's biochemical inability to derive reward from ordinary, everyday activities. The concept of RDS can explain how simple genetic anomalies give rise to complex aberrant behavior.[16,17] In this regard, other genetic polymorphisms have been associated with obesity, including the dopamine D2 receptor (*DRD2*), methylenetetrahydrofolate reductase (*MTHFR*), leptin (*LEP*), serotonergic receptor (*5-HT2A*), and peroxisome proliferator-activated receptor gamma 2 (*PPAR-γ2*) genes.[18-23]

Figure 1. Brain reward cascade. In this cascade stimulation of the serotonergic system in the hypothalamus leads to the stimulation of delta/mu receptors by serotonin to cause a release of enkephalins. Activation of the enkephalinergic system induces an inhibition of GABA transmission at the substantia nigra by enkephalin stimulation of mu receptors at GABA neurons. This inhibitory effect allows for the fine-tuning of GABA activity. This provides the normal release of dopamine at the projected area of the nucleus accumbens (reward site of the brain).[11]
Figure reproduced with permission from Blum K, Kozlowski GP. Ethanol and neuromodulator interactions: a cascade model of reward. In: Ollat H, Parvez S, Parvez H, eds. Alcohol and Behavior. *Utrecht: VSP Press; 1990:131-149.*



Preliminary, hypothesis-generating studies with an LG839 variant called PCAL-103 in obese and overweight subjects indicated that PCAL-103 facilitated withdrawal from carbohydrate bingeing, reduced relapse rates, and increased weight loss compared with controls.[24] Subsequent prospective studies have demonstrated that PCAL-103 significantly increased weight loss, decreased food cravings and binge eating, prevented weight regain after a period of fasting, and also reduced stress and enhanced general well-being.[25,26]

In light of these early hypothesis-generating studies, and the paucity of research involving neurotransmitter manipulation of the brain reward system coupled with genetic polymorphic identification, we designed a pilot study to evaluate the process of DNA-customization of a nutritional solution for weight management. We carried out this experiment cognizant that larger randomized, placebo-controlled studies are warranted before any solid conclusions can be drawn from the present evaluation.

MATERIALS AND METHODS

Subjects

The first stage of the study involved a broad sample of 1058 subjects who participated in the overall D.I.E.T. study and who had self-identified themselves as obese or overweight. All subjects were recruited from customers who had purchased LG839 as part of a commercial pilot in the Netherlands from January 2006 to February 2007, and study participants were approached without a particular schema. The study subjects were primarily an ethnically homogenous group of Dutch descent. The second stage of the study involved a narrow sample of 27 subjects who had self-identified themselves as obese or overweight. These subjects participated in the LG839 pilot in the Netherlands, were willing to complete a retrospective survey about their results, and were willing to participate without compensation.

Study Design

This cross-sectional, observational study included a genetic analysis involving a panel of genes, and an evaluation questionnaire providing two key self-reported retrospective data sets on the subjects' health before taking LG839 and then after taking LG839. Self-reported evaluations were collected through an online questionnaire disseminated over email to study participants.

Study Outcomes

The primary objective was to evaluate the safety and efficacy of LG839 in obese and overweight subjects, and specifically to look at differences in weight, mood, sleep, digestion, and other health issues before and after taking LG839. All participants provided written consent, and the study protocol was approved by the institutional review board of the nonprofit research organization, PATH Medical & Research Foundation, located in New York, USA. Data were collected online independently by LooseFoot Computing Ltd. of Regina, Saskatchewan, Canada.

Laboratory Measurements and Genotyping

Laboratory testing was performed in a high-complexity, CLIA-certified laboratory under contract with the company based in San Diego, California, USA. All subjects were genotyped based on a neutral identification number and read without knowledge of the individual being typed. Total genomic DNA was extracted from each coded blood sample, and aliquots were used for polymerase chain reaction (PCR) analysis.

DRD2

The *DRA1* and *DRA2* genotyping was performed using PCR.[9,27] The oligonucleotide primers 5'-CCGTCGACCCTTCCTGAGTGTCATCA-3' and 5'CCGTCGACGGCTGGCCAAGTTGTCTA-3' were used to amplify a 310-base pair (bp) fragment spanning the polymorphic Taq1A1 site of the *DRD2* gene. PCR was performed in 30 mL reaction mixtures containing 1.5 mM MgCl2, 2 mM 2'-deoxynucleotide 5'-triphosphates, 0.5 μM primers, 1 μg of template DNA, 1.5 U Taq polymerase (Boehringer Mannheim Corp., IN, USA), and PCR buffer (20 mM Tris-HCL [pH 8.4] and 50 mM KCl). After an initial denaturation at 94°C for 4 min, DNA was amplified with 35 cycles of 30 sec at 94°C, 30 sec at 58°C, and 30 sec at 72°C, followed by a final extension step of 5 min at 72°C. The PCR product was digested with 5 U of Taq1 for 22 hours at 65°C for the Taq1A polymorphism. Digestion products were then resolved on a 3% agarose gel (5 V/cm) containing 0.65 μg/mL ethidium bromide. There were three *DRD2* Taq1A genotypes: 1) the predominant homozygote A2/A2, which exhibits two restriction fragments of 180 and 130 bp; 2) the heterozygote A1/A2, which exhibits three restriction fragments of 310, 180, and 130 bp; and 3) the rare homozygote A1/A1, which produces only the uncleaved 310-bp fragment.

MTHFR

Genotyping for the *MTHFR* C677T polymorphism was performed using PCR and restriction fragment length polymorphism analysis. We designed PCR primers 5'-CCCAGCCACTCACTGTTTTAG-3' and 5'-TGGGAAGAACTCAGCGAACT-3' with DNASIS Pro Ver.2.0 (Hitachi Software Engineering Co. Ltd., Tokyo, Japan). Since the C to T transition at nucleotide 677 produces a Hinf I digestion site, the amplified 469-bp product derived from the mutant gene was cleaved into 393-bp and 76-bp fragments by Hinf I (TaKaRa Bio Inc., Shiga, Japan), which leaves the wild type gene unaffected. After electrophoresis through a 6% polyacrylamide gel, the digestion products were visualized by staining with ethidium bromide.

5-HT2A

Genotyping of the −1438G/A polymorphism of the *5-HT2A* gene was carried out by PCR and restriction digestion as described previously.[20] Genomic leukocyte DNA (100 ng in a final volume of 10 μL)

was amplified by PCR using the following primers: 5′-AAGCTGCAAGGTAGCAACAGC-3′ and 5′-AACCAACTTATTTCCTACCAC-3′. The primers amplified a product of 468 bp. The PCR conditions were as follows: an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 15 sec, and extension at 72°C for 30 sec, with a final extension of 10 min at 72°C. The PCR reaction product was digested at 37°C overnight with 5 U of the restriction enzyme MspI (New England Biolabs, MA, USA). The −1438G allele was cut into 244-bp and 224-bp fragments, whereas the −1438A allele remained undigested. The fragments were separated on a 2% agarose gel and visualized by staining with ethidium bromide.

PPAR-γ2

Two hundred base pairs of sequence surrounding *PPAR-γ2* Pro12Ala were provided to Applied Biosystems (Foster City, CA, USA) to develop TaqMan allelic discrimination assays using their assay by design platform. Genotyping of the Pro12Ala AD was performed using the following primers (0.9 mol/L each): forward 5′-TTATGGGTGAAACTCTGGGAGATT-3′ and reverse 5′-TGCAGACAGTGTATCAGTGAAGGA-3′ and the Taqman MGB probes Fam-TTCTGGGTCAATAGG and Vic-CTTTCTGCGTCAATAG (0.1 mol/L each; Applied Biosystems). Four nanoliters of a 10 ng/L stock of DNA was dispensed into 384-well PCR plates using a Biomek FX robot (Beckman Coulter, Fullerton, CA, USA), to which 6 μL of a mix containing primers, TaqMan MGB probes, and TaqMan Universal PCR Master Mix (Applied Biosystems) were added. These were sealed with optical seals (Applied Biosystems) and incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, and 60°C for 1 min before analysis on a 7900HT plate reader (Applied Biosystems). Individual genotypes were determined using SDSv2.1 software (Applied Biosystems).

LEP

We used the following dinucleotide repeats (D7S1873, D7S1875, D7S514, and D7S680) present on the YAC contig containing the human *LEP* gene as described by Green.[28] The primers used for PCR were the same as in their report. Of these, D7S1875 was closest to the *LEP* gene. We refer to this as LEP_{1875}. The frequency of the different alleles of the LEP_{1875} polymorphism has been reported previously.[27] The alleles ranged in size from 199 to 225 bp in length. Our a priori approach to the examination of dinucleotide repeats in behavioral disorders is based on other research.[29,30]

Comings et al.[27] was first to divide the alleles into approximately equal groups, since there was a natural tendency for a bimodal distribution, which was the case for the LEP_{1875} polymorphism and the cut was made at 208 bp. The resulting genotypes were <208 bp/<208 bp, <208 bp/≥208 bp, and ≥208 bp/≥208 bp. In this experiment we used

Coming's cut-off point in our analysis providing for only positive and negative allelic associations.

Nutraceutical Customization

Based upon the genetic profiles derived, certain ingredients in LG839 were customized according to subjects' genotypes (Table 1).

Measurements of Efficacy and Safety

Separate from the laboratory measurements, all measurements of efficacy and safety were self-reported by the study subjects using a one-time retrospective online questionnaire. The primary endpoint was weight loss in kilograms. Secondary endpoints included changes in appetite, sugar cravings, waist circumference, mood, sleep, and digestion. The changes in these factors were measured generally using a scale of 1 to 5 where 5 was the least healthy rating and 1 was the most healthy rating to provide consistency across the multiple measures. Tertiary endpoints included changes in incidences of depression and cravings for alcohol/tobacco/recreational drugs. To monitor persistency, we asked study subjects to self-report on their frequency of compliance to the product's serving instructions.

Table 1. Customization of the nutraceutical LG839 according to genotype.

Genotype	Ingredient	Serving
MTHFR (C677T) Homozygous or normal/wild type (CC)[31,32]	Vitamin B9 (folic acid)	800 µg
MTHFR (C677T) Heterozygous mutant (CT) or homozygous mutant (TT)[33]	Vitamin B9 (folic acid)	5 mg
5-HT2A (1438A) Heterozygous or normal[34]	SuperCitrimax (–)hydroxycitric acid (extract of *Garcinia cambogia*)	2500 mg
5-HT2A (1438A) Heterozygous or homozygous mutant or leptin LEP_{1875}[35]	SuperCitrimax (–)hydroxycitric acid (extract of *Garcinia cambogia*)	4667 mg
DRD2 Wild type A2/A2[35-37]	Synaptamine™ complex (DL-phenylalanine, chromium, L-glutamine, L-tyrosine, L-5-HTP, vitamin B6 [pyridoxal-5-phosphate])	1725 mg
DRD2 Heterozygous A1/A2 or homozygous A1/A1[38]	Synaptamine™ complex (DL-phenylalanine, chromium, L-glutamine, L-tyrosine, L-5-HTP, vitamin B6 [pyridoxal-5-phosphate])	2750 mg

5-HT2A=serotonergic receptor; *DRD2*=dopamine D2 receptor; *LEP*=leptin; *MTHFR*=methylenetetrahydrofolate reductase.

Statistical Analysis

The means and standard deviations were calculated before and during LG839 treatment for each item in the questionnaire. To test for a change, the paired *t* test was used on the change score, with change calculated as response during treatment minus the response before treatment for each item. Significance was denoted at each level of 0.05, 0.01, and 0.001 in the results.

Two-sample *t* tests were used with Satterthwaite's approximate *t* test when the *F* test for equal variances rejected for comparing changes in each item for those with each genetic polymorphism compared to those without the polymorphism.

Genotypic and allelic distributions were compared with the Pearson chi-square test for homogeneous variances. Three groups were compared: historical literature controls, the sample of 1058, and the subset for this analysis (n=27). The effect of each polymorphism on quantitative variables was tested using multiple linear regression. Pearson's correlation was used to determine which responses and which genetic polymorphisms were related to days on treatment. Stepwise multiple regression was used to determine the model for each outcome considering the five genetic polymorphisms, days on LG839 treatment, and extent to which subjects followed instructions. R^2 correlations were given for the final model's ability to explain variance in the outcome measurement. These were contribution to the overall variance of a preassigned total sum outcome index (weight loss in kilograms, reduction in waist circumference in inches, sugar cravings, snack intake, late night bingeing behavior, exercise, appetite, and energy level). In addition, Cronbach's alpha was used to determine that the index sum indeed was reliable. It was reliable, as Cronbach's alpha was 0.73 for the total sum of change scores. This total provides an overall response to use for the multiple regression, in addition to the individual item outcomes measured in the questionnaire.

Percentages of each gene polymorphism for the self-identified sample of obese (n=1058) and for the variable sample size of normal controls from the literature were each compared to the sample (n=27) in this study for each gene polymorphism individually by Fisher's exact test.[39] We also pooled the genotypes from each group (1058 obese subjects; 27 obese subjects [subset]; literature controls) and statistically compared genotypic patterns for significant differences.

The Statistical Analysis System (SAS Institute, 2006, version 9.1 for the PC, Cary, North Carolina, USA) was used for these calculations.

RESULTS

Significant improvements were observed for weight loss, sugar craving reduction, appetite suppression, snack reduction, reduction of late night eating (all P<0.01), increased perception of overeating, enhanced quality of sleep, increased happiness (all P<0.05), and increased energy (P<0.001) (Table 2).

Table 2. Overall changes in obesity-associated health measures.

Assessment area and question	*n*	Mean change*	SD	*P*
Anxiety	25	–0.32	0.85	0.073
Appetite	25	–0.76	1.20	0.004
Drug craving	25	–0.20	0.71	0.170
Tobacco craving	25	–0.00	0.00	NA
Depression	25	–0.20	0.58	0.096
Ease of sleep	25	–0.28	1.02	0.183
Energy	25	–0.84	1.03	0.0004
Exercise	25	+0.56	2.02	0.179
Get along with others	25	+0.08	0.49	0.425
Happiness	25	–0.36	0.70	0.017
Interrupted sleep	25	–0.48	1.61	0.149
Late night eating	25	–0.84	1.43	0.007
Perception of overeating	25	–0.72	1.49	0.023
Quality of sleep	25	–0.52	1.00	0.016
Regularity of digestion	25	–0.40	1.04	0.067
Snacking	25	–0.80	1.29	0.005
Sugar craving	25	–0.88	1.51	0.008
Waist, cm	16	–0.75	1.73	0.104
Weight, kg	26	–2.62	4.55	0.006

*Responses during LG839 administration minus baseline responses.
NA=not applicable.

It is to be noted that because of missing values for the genotyping statistics (*n*=23), even with this small sample size, we obtained significant polymorphic correlates with regard to a number of well-known genes (*LEP*, *PPAR-γ2*, *MTHFR*, *5-HT2A*, and *DRD2*). In this regard, carrying certain known polymorphisms (*MTHFR* [C677T allele]; *LEP* [D7S1875 allele]; *5-HT2A* [1438G/A allele]; *DRD2* [A1 allele]; *PPAR-γ2* [Pro12Ala allele]) correlated negatively with positive clinical parameters tested in this study. The percentage prevalence for each gene polymorphism is presented in Table 3.

A chi-square test was used to determine whether a genetic difference occurred between the 1058 subjects participating in the overall D.I.E.T. study, the historical literature controls, and the subset of participants (*n*=27) utilized in the present study. The test revealed no difference

Table 3. Genotypic comparisons between the 1058 obesity sample, the subset of 27 obese subjects, and historical literature controls*.

Name and description of gene studied	Prevalence (%), mutant (combined heterozygous/homozygous) Subset of self-selected obese subjects	Obesity sample	Literature control sample	Frequency for wild type	Frequency for mutant type
PPAR-γ2	4/23 (17.4)	265/1058 (25.0)	314/2244 (14.0)	0.90	0.10
5-HT2A	16/25 (64.0)	679/1058 (64.2)	173/283 (61.1)	0.56	0.44
MTHFR	16/25 (64.0)	739/1058 (69.9)	54/100 (54.0)	0.60	0.40
DRD2	9/23 (39.1)	403/1058 (38.1)	945/3258 (29.0)	0.76	0.24
LEP‡	14/25 (56.0)	800/1058 (75.6)†	94/206 (45.6)	0.60	0.40

*Due to missing data the mean was n=23.
†Significantly different from n=23 sample by Fisher's exact test.
‡We are cognizant that many studies on the LEP gene argued its association with obesity.
5-HT2A=serotonergic receptor; *DRD2*=dopamine D2 receptor; *LEP*=leptin; *MTHFR*=methylenetetrahydrofolate reductase; *PPAR-γ2*=peroxisome proliferator-activated receptor gamma 2.

between any gene polymorphism evaluated for those present in each individual group compared to the average for all three groups. Some gene polymorphisms differed among the three groups, but the subset of n=23 (reflecting missing data) always had a percentage near the average for all three groups and thus did not differ from the overall gene polymorphism distribution. These percentages were 31.3% overall for the *DRD2* gene and 39.1% for the subset. The *5-HT2A* receptor gene was present in 68.4% overall and 64.0% in the subset. The *PPAR-γ2* gene was present in 17.5% overall and 17.4% for the subset. The *MTHFR* gene was present in 69.9% overall and 64.0% for the subset. The *LEP* gene was present in 70.4% overall, but in only 56.0% of the subset. This was the largest difference, but the subset is intermediate to the high sample of 1058 (75.6%) and low sample of literature controls (n=206, 45.6%) and its deviation of 56.0% positive compared to the average of 70.4% positive does not contribute significantly to the overall chi-square test. However, when we compared each group separately against each other and controls we found similar results, except for the *LEP* gene (Table 3).

The significant results are as follows: *PPAR-γ2* was significantly associated with increased interruptions of sleep (P<0.04) as well as reduced hours of exercise (P<0.02); *MTHFR* was significantly associated with weight loss resistance (P<0.05); *5-HT2A* was significantly associated with loss of waist circumference resistance (P<0.008); and *DRD2* was significantly associated with reduced hours of exercise (P<0.04). We found no association with the *LEP* gene polymorphism for any parameter tested.

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The multiple regression analysis identified several outcomes that could be jointly predicted by multiple variables. Both days on treatment and compliance with instructions jointly predicted exercise change (r^2=0.58, *P*=0.0004). Both the *PPAR-γ2* gene polymorphism and compliance with instructions jointly predicted change in getting along with others (r^2=0.42, *P*=0.007). Both the *5-HT2A* gene and *LEP* gene jointly predicted waist size change (r^2=0.56, *P*=0.024). All other outcomes related to this study were only significant for the univariate results given earlier. Of all the outcomes and gene polymorphisms, only the *DRD2* gene polymorphism had a significant Pearson correlation with days on treatment (*r*=0.42, *P*=0.045).

DISCUSSION

In the present study, albeit with a small sample size, we showed significant effects on multiple obesity-related parameters using the experimental DNA-customized nutraceutical LG839. The most robust findings were for weight loss, sugar craving reduction, appetite suppression, snack reduction, and reduction of late night bingeing. Other significant findings included increased perception of overeating, increased energy, increased quality of sleep, and overall happiness. Of even greater significance was the finding that certain gene polymorphisms correlated with resistance to a number of outcome measures including weight loss, loss of waist circumference, exercise, and sleep interruptions. It appears that in the presence of these polymorphic correlations, LG839 induced significant positive changes suggesting anti-obesity activity.

These results are consistent with earlier reports involving LG839 variants.[24-26] Furthermore, a loss of 2.5 kg in only 80 days is quite statistically robust especially when considering guidelines from the US FDA regarding meaningful weight loss of any medically approved anti-obesity pharmaceutical.

The NIH 1998 *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults* defines normal weight as a body mass index (BMI) of 18.5-24.9 kg/m^2, overweight as a BMI of 25-29.9 kg/m^2, and obese as a BMI of ≥30 kg/m^2. The *Clinical Guidelines* recommend weight loss through a combination of diet modification, increased physical activity, and behavior therapy for obese patients, and for patients who are overweight or have a high-risk waist circumference, when accompanied by two or more risk factors. In the event that lifestyle changes do not promote weight loss after 6 months, the *Clinical Guidelines* recommend that drugs should be considered as adjunctive therapy for select patients who have a BMI of ≥30 kg/m^2, or a BMI of ≥27 kg/m^2 if concomitant obesity-related risk factors or disease exist. The FDA's approach to the approval of prescription weight-loss drugs mirrors the recommendations provided in the NIH's *Clinical Guidelines*. For example, the prescription of orlistat was studied and approved for long-term weight loss in patients moderately-to-severely overweight (BMI 27-29.9 kg/m^2) with comorbid conditions such as hypertension, type 2 diabetes, or dyslipidemia, and in obese subjects (BMI

≥30 kg/m^2) regardless of the presence of comorbidities. Limiting approval of prescription weight-loss drugs to moderately-to-severely overweight individuals with comorbidities and to obese patients maximizes the therapeutic risk-benefit profile by targeting drug therapy to individuals whose risk for weight-related disease is high and likely to outweigh the risks associated with any given pharmacological agent. In clinical studies, orlistat demonstrated weight loss of 5% or more at 6 and 12 months.[40] In light of the current results, which suggest a potential weight loss of 2.5% in 80 days, or less than 3 months, the LG839 product warrants further investigation to evaluate whether these pilot study results are replicable in a randomized, placebo-controlled study of a longer duration.

Gene Polymorphic Correlates

Obesity is a biogenetic condition involving both environmental and genetic antecedents. Similar to diseases such as alcoholism that were once classified as purely behavioral abnormalities, obesity is now accurately characterized as a disease with biological and genetic factors. It has been reported that in both Caucasian women and men, heritability for BMI was 0.58 and 0.63; for body fat percentage, 0.59 and 0.63; and for lean body mass 0.61 and 0.56, respectively.[41] Because of the complexity of obesity and related conditions involving reward deficiencies, it is likely that more than one defective gene is involved. However, work from Volkow's laboratory using PET scanning showed a *DRD2* paucity in subjects with RDS versus non-RDS subjects.[42] It is of note that in this pilot study we found significant results for a number of gene-outcome parameters.

PPAR

In terms of gene associations, it is noteworthy that the development and metabolism of adipocytes are regulated in a complex manner and the important role of the transcription factor *PPAR-γ* has been recently recognized.[22,23] *PPAR-γ* is a member of the nuclear hormone receptor family of transcription factors, which are known to be involved in transcription of target genes.[22,23] *PPAR-γ2* is specific for adipose tissue; this characteristic makes it a candidate gene for the regulation of insulin and glucose metabolisms.[43] Among genetic variants identified in the *PPAR-γ2* gene, the Pro12Ala variant is highly prevalent in Caucasians.[43] Conflicting results regarding the association between this mutation and complex traits, such as obesity, insulin sensitivity, and type 2 diabetes have been reported. Some reports suggest that the X12Ala (either Pro12Ala or Ala12Ala) genotype is related to improved insulin sensitivity, particularly in subgroups with obesity, although this conclusion is still controversial.[22-24,44-47] Interestingly, we found a negative association between the Pro12Ala or Ala12Ala polymorphism of the *PPAR-γ2* gene and both quality of sleep and exercise. In fact, there are a number of studies relating the *PPAR-γ2* gene and exercise.[43,48] However, this is the first report linking sleep interruption and the Pro12Ala polymorphism of the *PPAR-γ2* gene.

MTHFR

Di Renzo and associates have identified a subset of metabolically obese, but normal weight individuals, with potentially increased risks of developing the metabolic syndrome, despite their normal BMI.[18] They determined the relationship between body fat distribution, resting metabolic rate, total body water amount, and selected gene polymorphisms on interleukin-15 receptor-alpha (IL-15Ralpha) *MTHFR* C677T, to distinguish normal weight obese (NWO) from non-obese subjects with a normal metabolic profile and obese individuals. The distribution of *MTHFR* C677T and IL-15Ralpha genotypes was significantly different between classes. The data suggested that NWO individuals demonstrated a significant relationship between the decrease in the basal metabolism, increased body fat mass, and total body water amount. Possession of wild type homozygote genotypes regarding IL-15Ralpha cytokine and C677T *MTHFR* enzyme characterized NWO individuals.[18]

Our results showing a negative correlation between the *MTHFR* gene and LG839 induction of weight loss is in agreement with the Di Renzo study.

5-HT2A

While the serotonergic system and receptors have been significantly associated with a number of eating disorders,[49] to our knowledge this is the first report to observe a negative association between the −1438G/A allele of the *5-HT2A* receptor gene and resistance to a reduction in waist circumference by administration of LG839.

DRD2

A Ser311Cys mutation of the human *DRD2* gene produces a marked functional impairment of the receptor and is associated with higher BMI in some populations. Tataranni hypothesized that the Ser311Cys mutation of *DRD2* may inhibit energy expenditure.[50] In a population of nondiabetic Pima Indians, total energy expenditure was lower in homozygotes for the Cys311-encoding allele when compared with those heterozygous and homozygous for the Ser311-encoding allele (P=0.056). The 24-hour resting energy expenditure was also lower in homozygotes for the Cys311-encoding allele when compared with those heterozygous and homozygous for the Ser311-encoding allele (P=0.026). These findings were the first evidence that a genetic mutation is associated with reduced energy expenditure in humans. The findings of the present study, whereby the TaqA1 allele was significantly associated with a reduced number of exercise hours, provides additional evidence that *DRD2* gene polymorphisms may influence both energy expenditure and also actual exercise activity in a predisposed individual. It is very interesting that of all the genes tested, a positive correlation was obtained with the *DRD2* A1 allele and number of days in treatment. This is significant because it would appear that those individuals carrying the *DRD2* A1 allelic polymorphism continued treatment due to potential benefits of LG839 targeted at a deficiency of dopamine receptor (D2) density.

LEP

It is not surprising that no correlation between weight loss parameters and the *LEP* gene was found. In a previous study, there was no correlation of the *LEP* gene with weight in older women, although a correlation was observed in young women.[27] This may be expected since weight gain is more likely to have a genetic basis in the young while environmental factors are likely to have more influence in older women. In this regard, the age range of the 27 participants in the present study was between 30 and 65 years, with most being older than 40 years of age. Furthermore, we found that the only difference of either the self-selected obesity sample or the normal control sample was for the *LEP* polymorphism where there was a significant increase in the obesity sample (P=0.034) supporting many earlier studies.

Several potential limitations of the study should be noted. The sample size was small, and replication in larger samples will be important. The duration of treatment with regard to LG839 administration was not as long as other typical anti-obesity studies. The study was not placebo-controlled or randomized. It is to be noted that placebo effects are strong when studies are performed with regard to weight management and as such these results are considered preliminary and hypothesis-generating until these controlled studies are successfully executed.[1,2,40] There could also be bias based on the small selection of individuals who agreed to participate in the study compared with the 1058 potential subjects. However, in an attempt to offset this bias we did find similar genotypes for all participants in the study, as presented in the results section. Additionally, due to the high rate of respondents (23/27 subjects) who complained about the local call center working for the sponsoring company, there may have been a negative bias in survey respondents. The questionnaire utilized was not a standardized assessment and may have contributed to bias. Finally, because the subjects received a customized formula with different dosages of certain ingredients based on polymorphic genetic profiles, rather than a common formula for all subjects, this in itself complicates assessment and could result in uncontrolled bias.

CONCLUSION

Based on this preliminary investigation, we suggest that DNA-directed targeting of certain regulator genes along with customized nutraceutical intervention may provide a unique framework and strategic modality to combat obesity, weight problems, and their associated comorbidities. In light of many dietary supplement ingredients and other tactics used empirically for weight loss without preliminary analysis to deem their merit, this study maintains previous foundational research involving the LG839 technology that a scientific and rationale approach of laboratory testing and laboratory-directed nutritional supplementation may present beneficial health outcomes in the treatment of obesity, along with healthy eating and exercise.

This hypothesis-generating observational pilot study (albeit with small

sample size) has validated previous clinical study work that LG839 provides significant reductions in weight, appetite, snacking between meals, late night eating, and sugar cravings, as well as increases in energy, sleep quality, and overall well-being. However, as an observational study, it has generated information that must be utilized in the protocol design of larger, randomized, placebo-controlled studies. Interpretation of these pilot study results, which could represent spurious statistical outcomes based on sample size,[51] must await future confirmation.

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