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(57) **Abrégé/Abstract:**

A method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the nucleotide of the subject at one or more polymorphic positions selected from: (i) position 101 of SEQ ID NO:1 (rs953211) (ii) position 101 of SEQ ID NO:2 (rs1509290) (iii) position 101 of SEQ ID NO:3 (rs1509289) and/or detecting one or more biomarkers genetically linked to said polymorphic positions.



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(54) Title: BIOMARKERS FOR PREDICTING WEIGHT LOSS AND WEIGHT MAINTENANCE

(57) Abstract: A method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the nucleotide of the subject at one or more polymorphic positions selected from: (i) position 101 of SEQ ID NO:1 (rs953211) (ii) position 101 of SEQ ID NO:2 (rs1509290) (iii) position 101 of SEQ ID NO:3 (rs1509289) and/or detecting one or more biomarkers genetically linked to said polymorphic positions.

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Biomarkers for predicting weight loss and weight maintenance

FIELD OF INVENTION

The present invention provides biomarkers and biomarker combinations that can be used to predict the degree of weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions.

BACKGROUND

Obesity is a chronic metabolic disorder that has reached epidemic proportions in many areas of the world and is the major risk factor for serious co-morbidities such as type 2 diabetes mellitus, cardiovascular disease, dyslipidaemia and certain types of cancer (World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253).

It has long been recognized that low calorie dietary interventions can be very efficient in reducing weight and that this weight loss is generally accompanied by an improvement for the risk of obesity related co-morbidities, in particular type 2 diabetes mellitus. Empirical data suggests that a weight loss of at least 10% of the initial weight results in a considerable decrease in risk for obesity related co-morbidities (World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253). However, the capacity to lose weight shows large inter-subject variability.

It has been shown that a percentage of the population do not successfully lose weight on a low calorie diet (Ghosh, S. et al., Obesity (Silver Spring), (2011) 19(2):457-463). This leads to an unrealistic expectation of weight loss, which in turn causes non-compliance, drop-outs and generally unsuccessful dietary intervention.

Some studies also demonstrate that there are methods in the art for monitoring weight loss which include monitoring levels of particular biomarkers in plasma (e.g. Lijnen et al., Thromb Res. 2012 Jan, 129(1): 74-9; Cugno et al., Intern Emerg Med. 2012 Jun, 7(3): 237-42; and Bladbjerg et al., Br J Nutr. 2010 Dec, 104(12): 1824-30). However,

these methods do not provide a prediction or indication of the degree of weight loss attainable by a particular subject. There is no predictive value in looking at the correlation of biomarker levels with weight loss.

Keeping weight lost stable also presents a major challenge to the patient. It is known that only one year after a weight loss intervention, about one-third of the lost weight is regained (Hensrud, *Obes. Res.* 9 Suppl 4, 348S–353S, 2001). Moreover, diet-induced weight loss induces several physiological changes that facilitate weight regain (Sumithran and Proietto., *Clin. Sci. Lond. Engl.* 1979 124, 231–241 (2013)). These changes include alterations in energy expenditure, substrate metabolism and hormone pathways involved in appetite regulation. Our understanding of these physiological and molecular changes remains so far limited.

The solution for successful planning and design of dietary interventions, for example low calorie diets, lies in the availability of a method which predicts a weight loss trajectory. In addition, successful planning and design of weight management interventions would be aided by the availability of a test to predict the success (or failure) of a patient at keeping his/her weight loss stable during a weight maintenance program.

United States Patent Application US 2011/0124121 discloses a method for predicting weight loss success. The methods disclosed comprises selecting a patient who is undergoing or considering undergoing a weight loss therapy such as gastric banding, measuring one or more hormone responses of the patient to caloric intake and predicting success of a weight loss therapy based on the hormone response. The hormones measured are gastrointestinal hormones such as a pancreatic hormone.

European Patent Application EP 2 420 843 discloses a method for determining the probability that a person will maintain weight loss after an intentional weight loss by determining the level of angiotensin I converting enzyme (ACE) before and after the dietary period.

There is, however, still a need for a method of accurately predicting the degree of weight loss and weight maintenance in a subject. Consequently, it was the objective of the present invention to provide biomarkers that can be detected easily and that can

facilitate the prediction of weight loss and weight maintenance in a subject. Such biomarkers may be used to predict weight trajectories during weight loss and during weight maintenance and may help for stratification of patients into adapted treatment groups according to their biological weight loss and weight maintenance capacities.

Protocadherin Fat 4, also known as cadherin family member 14 (CDHF14) or FAT tumor suppressor homolog 4 (FAT4), is a protein that in humans is encoded by the *FAT4* gene. FAT4 is associated with the Hippo signaling pathway. The Hippo pathway has emerged as a conserved signaling pathway that is essential for the proper regulation of organ growth in *Drosophila* and vertebrates (Halder, 2011, Development, Jan;138(1):9-22).

It has recently been shown that *Drosophila* Fat (Ft) cadherin has a direct role in regulating mitochondrial morphology and metabolism (Sing et al., 2014, Cell, 158, 1293-1308). It was shown that proteolytic cleavage of Ft releases a soluble fragment (Ft^{mito}) that is imported into the mitochondria and that such cleavage functions as a switch mechanism to coordinate cell cycle and metabolism. It was suggested that altering the levels of Ft^{mito} may allow an organism to directly adjust metabolic rates in accordance with changing energy requirements.

SUMMARY OF THE INVENTION

The present invention identifies biomarkers useful in predicting the predisposition of a subject to change in weight-related phenotypes by applying one or more dietary interventions to a subject. The present invention also identifies biomarkers useful in predicting the predisposition of a subject to maintenance of weight-related phenotypes following one or more dietary interventions.

In particular, the invention discloses specific polymorphisms/alleles of the *FAT4* gene that are related to change in weight-related phenotypes as well as diagnostic tools and kits based on these susceptibility alterations. Thus, the invention can be used in predicting the outcome to a weight management program (including weight loss program or weight maintenance program).

Accordingly the present invention provides in one aspect a method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary

interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the presence of one or more polymorphic markers in the *FAT4* gene or a regulatory element thereof.

The present invention provides in one aspect a method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the nucleotide of the subject at one or more polymorphic positions selected from:

- (i) position 101 of SEQ ID NO:1 (rs953211)
- (ii) position 101 of SEQ ID NO:2 (rs1509290)
- (iii) position 101 of SEQ ID NO:3 (rs1509289)

and/or detecting one or more biomarkers genetically linked to said polymorphic positions.

In one embodiment, the one or more biomarkers (e.g., SNPs) are within the *FAT4* gene or a regulatory element thereof. In one embodiment, the biomarker is less than 20, 15, 10, 5, 4, 3, 2, 1 kilobases (kb) from said polymorphic positions defined above.

Preferably the method comprises determining the nucleotide of the subject at both position 101 of SEQ ID NO:1 and position 101 of SEQ ID NO:2. In one embodiment, the method comprises determining the nucleotide of the subject at position 101 of SEQ ID NO:1, position 101 of SEQ ID NO:2 and position 101 of SEQ ID NO:3.

In one embodiment the method comprises determining the presence of A or G at position 101 of SEQ ID NO:1, and/or A or G at position 101 of SEQ ID NO:2, and/or A or G at position 101 of SEQ ID NO:3.

In one embodiment the method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:1 wherein the

weight loss is represented by a change in body mass index (BMI), fat mass, hip circumference or waist circumference.

In another embodiment the method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:2 wherein the weight loss is represented by a change in BMI, fat free mass, hip circumference or waist circumference.

In another embodiment the method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:3 wherein the weight loss is represented by a change in BMI, fat mass, fat free mass, hip circumference or waist circumference.

In another embodiment the method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:1 and the presence of A or G at position 101 of SEQ ID NO:2 wherein the weight loss is represented by BMI, fat mass, fat free mass, hip circumference or waist circumference.

In a particularly preferred embodiment subjects having a genotype comprising a G at position 101 of SEQ ID NO:1 and an A at position 101 of SEQ ID NO:2 are identified as having a predisposition maintain weight loss following a dietary intervention.

By “G at position 101” referred to above it is meant that the subject has at least one copy of G at said site, i.e., the subject may have the genotype G/G (homozygous) or G/A (heterozygous).

By “A at position 101” referred to above it is meant that the subject has at least one copy of A at said site, i.e., the subject may have the genotype A/A (homozygous) or G/A (heterozygous).

Preferably the method for assessing the predisposition of a subject to maintenance of weight loss comprises determining the presence of a genotype selected from:

- (i) G/G (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;
- (ii) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2;
- (iii) G/A (heterozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2; and
- (iv) G/A (heterozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

and wherein the presence of said genotype indicates that a subject has a predisposition to maintain weight loss following a dietary intervention.

Preferably the method for assessing the predisposition of a subject to maintenance of weight loss comprises determining the presence of a genotype selected from:

- (i) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;
- (ii) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/G (heterozygous) at position 101 of SEQ ID NO:2;
- (iii) A/A (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;
- (iv) A/G (heterozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2; and
- (v) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

and wherein the presence of said genotype indicates that a subject has a predisposition not to maintain weight loss following a dietary intervention.

Preferably the maintenance of weight loss is assessed by measuring a change in BMI.

In another embodiment the method for assessing the predisposition of a subject to weight loss attainable by applying on or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:1 and wherein the weight loss is represented by a change in hip circumference.

In another embodiment the method for assessing the predisposition of a subject to weight loss attainable by applying on or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:2 and wherein the weight loss is represented by a change in BMI or hip circumference.

In another embodiment the method for assessing the predisposition of a subject to weight loss attainable by applying on or more dietary interventions comprises determining the presence of A or G at position 101 of SEQ ID NO:3 and wherein the weight loss is represented by a change in BMI or hip circumference.

In another embodiment the method for assessing the predisposition of a subject to weight loss attainable by applying on or more dietary interventions comprises detecting the presence of A or G at position 101 of SEQ ID NO:1 and the presence of A or G at position 101 of SEQ ID NO:2 and wherein the weight loss is represented by a change in BMI or a change in hip circumference.

In a particularly preferred embodiment subjects having a genotype comprising a G at position 101 of SEQ ID NO:1 and an A at position 101 of SEQ ID NO:2 are identified as having a predisposition to lose weight.

By “G at position 101” referred to above it is meant that the subject has at least one copy of G at said site, i.e., the subject may have the genotype G/G (homozygous) or G/A (heterozygous).

By “A at position 101” referred to above it is meant that the subject has at least one copy of A at said site, i.e., the subject may have the genotype A/A (homozygous) or G/A (heterozygous).

Preferably the method for assessing the predisposition of a subject to weight loss attainable by applying on or more dietary interventions comprises determining the presence of a genotype selected from:

(i) G/G (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

(ii) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2;

(iii) G/A (heterozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2; and

(iv) G/A (heterozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

and wherein the presence of said genotype indicates that a subject has a predisposition to lose weight following a dietary intervention.

Preferably the method for assessing the predisposition of a subject to weight loss attainable by applying on or more dietary interventions comprises determining the presence of a genotype selected from:

(i) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

(ii) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/G (heterozygous) at position 101 of SEQ ID NO:2;

(iii) A/A (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

(iv) A/G (heterozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2; and

(v) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

and wherein the presence of said genotype indicates that a subject has a predisposition not to lose weight following a dietary intervention.

In another aspect of the present invention there is provided a method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the nucleotide of the subject at one or more polymorphic positions shown in Figure 7 (SEQ ID NOs 10 to 101).

The present invention provides in another aspect a method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary

interventions and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the sequence of *FAT4* gene or a regulatory element thereof.

The dietary intervention referred to herein is preferably a low calorie diet.

The dietary intervention may comprise administering at least one diet product to the subject.

A low calorie diet may comprise a decreased consumption of fat and/or an increase in consumption of low fat foods. By way of example only, low fat foods may include wholemeal flour and bread, porridge oats, high-fibre breakfast cereals, wholegrain rice and pasta, vegetables and fruit, dried beans and lentils, baked potatoes, dried fruit, walnuts, white fish, herring, mackerel, sardines, kippers, pilchards, salmon and lean white meat.

In one embodiment the low calorie diet may comprises a calorie intake of about 600 to about 1200 kcal/day and/or may comprise administration of at least one diet product.

The low calorie diet may also comprise administration of up to, for example, about 400 g vegetables/day.

Preferably the diet product is Optifast® or Modifast®.

Thus, the diet may comprise a product such as Optifast® or Modifast®. This may be supplemented with three portions of non-starchy vegetables such that the total energy intake is about 2.5 MJ (600 kcal/day). This may be further supplemented with at least 2 L of water or other energy free beverages per day.

In another embodiment, the diet may comprise, for example, a composition which is 46.4% carbohydrate, 32.5% protein and 20.1% with fat, vitamins, minerals and trace elements; 2.1MJ per day (510 kcal/day); This may be supplemented with three portions of non-starchy vegetables such that the total energy intake is about 2.5 MJ (600 kcal/day). This may be further supplemented with at least 2 L of water or other energy free beverages per day.

In one embodiment, the low calorie diet has a duration of up to 12 weeks, e.g. 6 to 12 weeks.

The methods referred to herein may further comprise determining one or more anthropometric measures and/or lifestyle characteristics of the subject.

The anthropometric measure may be selected from, for example, the group consisting of gender, weight, height, age and body mass index, and the lifestyle characteristic may be, for example, whether the subject is a smoker or a non-smoker.

According to another aspect of the present invention there is provided a method for optimizing one or more dietary interventions for a subject comprising:

assessing the predisposition of a subject to weight loss attainable by one or more dietary interventions and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions according to the method defined herein; and
applying one or more dietary interventions to the subject.

According to another aspect of the present invention there is provided a method for selecting a modification of lifestyle of a subject, the method comprising:

- (a) performing a method of assessing the predisposition of a subject to weight loss attainable by one or more dietary interventions and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions according to the method defined herein; and
- (b) selecting a suitable modification in lifestyle based upon the predicted weight loss attainable and/or predicted maintenance of weight loss from (a).

In one embodiment, the modification of lifestyle comprises a dietary intervention, preferably a dietary intervention defined herein.

According to another aspect of the present invention there is provided a low calorie diet for weight loss, wherein the diet product is administered to a subject that is predicted to attain weight loss by the method defined herein.

According to another aspect of the present invention there is provided a diet product for use as part of a low calorie diet for weight loss, wherein the diet product is administered to a subject that is predicted to attain weight loss or maintenance by the method defined herein.

According to another aspect of the present invention there is provided a diet product for use as part of a low calorie diet for weight loss, wherein the diet product is administered to a subject that is predicted to attain both weight maintenance and weight loss by the method defined herein.

According to another aspect of the present invention there is provided a diet product for use in treating obesity or an obesity-related disorder, wherein the diet product is administered to a subject that is predicted to attain weight maintenance and/or weight loss by the method defined herein.

In one embodiment, the diet product may comprise a product such as Optifast® or Modifast®.

In another embodiment, the diet product may comprise, for example, a composition which is 46.4% carbohydrate, 32.5% protein and 20.1% with fat, vitamins, minerals and trace elements; 2.1MJ per day (510 kcal/day); This may be supplemented with three portions of non-starchy vegetables such that the total energy intake is about 2.5 MJ (600 kcal/day). This may be further supplemented with at least 2 L of water or other energy free beverages per day.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a polymorphic position within the *FAT4* gene or a regulatory element thereof.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide primer capable of detecting a polymorphic position within the *FAT4* gene or a regulatory element thereof.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a polymorphic position selected from:

- (i) position 101 of SEQ ID NO:1;
- (ii) position 101 of SEQ ID NO:2; or
- (iii) position 101 of SEQ ID NO:3.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide primer capable of detecting a polymorphic position selected from:

- (i) position 101 of SEQ ID NO:1;
- (ii) position 101 of SEQ ID NO:2; or
- (iii) position 101 of SEQ ID NO:3.

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe as defined in.

According to another aspect of the present invention there is provided a diagnostic kit comprising (i) an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of G at position 101 of SEQ ID NO:1 and (ii) an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of A at position 101 of SEQ ID NO:2.

In one embodiment the diagnostic kit further comprises an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of A at position 101 of SEQ ID NO:3.

In a further aspect of the present invention, there is provided a computer program product comprising computer implementable instructions for causing a programmable computer to predict the degree of weight loss attainable by a subject according to the methods described herein.

Description of the figures

Figure 1: Association between SNPs located nearby the *FAT4* gene and change in BMI during weight maintenance. The X axis shows the SNP's coordinate on chromosome 4 and the Y axis shows the $-\log_{10}$ p-value from an allelic test. This figure displays results from SNPs assayed on the genotyping array.

Figure 2: Association between SNPs located nearby the *FAT4* gene and change in BMI during weight maintenance. The X axis shows the SNP's coordinate on chromosome 4 and the Y axis shows the $-\log_{10}$ p-value from an allele dosage test. This figure displays results from SNPs either assayed on the genotyping array or whose genotype was obtained from imputation.

Figure 3: Sequence of rs953211 (SEQ ID NO:1), rs1509290 (SEQ ID NO:2) and rs1509289 (SEQ ID NO: 3). The polymorphic position is shown in square brackets, i.e., [A/G].

Figure 4: Genomic sequence of *FAT4* gene (SEQ ID NO: 4). The polymorphic positions rs953211, rs1509290 and rs1509289 are identified as bold bases within the underlined sequences.

Figures 5A, 5B and 5C: Sequences of three *FAT4* transcripts (SEQ ID NOs 5, 6 and 7)

Figure 6 A and 6B: Sequences of two *FAT4* protein sequences (SEQ ID NOs: 8 and 9).

Figure 7: SNPs located nearby the *FAT4* gene (± 10 Kb). Shown are ninety-two SNPs have a p-value smaller than 5%.

DETAILED DESCRIPTION OF THE INVENTION

Predicting weight loss and weight maintenance

The present invention provides a method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions. The invention allows accurate prediction of weight trajectory of a patient, prior to a weight loss intervention and/or prior to a weight maintenance intervention.

The method may be used to make an informed prediction of the subject's capacity to lose weight and/or maintain weight loss, and select or adjust one or more dietary intervention accordingly.

For example, the present invention can be used for predicting the outcome of a weight management program, for adapting the weight management program and for stratifying patients following such a program into groups of successful or less successful profiles. For example, the method can be used to identify subjects with either a "high" or "low" likelihood of achieving weight loss by applying a dietary intervention. Similarly, the method can be used to identify subjects with either a "high" or "low" likelihood of maintaining weight loss following a dietary intervention.

Where the dietary intervention is a low calorie diet, the method could be used to aid in the appropriate diet for the subject or to adjust the daily calorie intake or duration of a particular diet and aid in setting realistic expectations for the subject.

In particular, the method provides a skilled person with a useful tool for assessing which subjects will most likely benefit from a particular dietary intervention, e.g. a low calorie diet. The present method therefore enables dietary interventions such as a low calorie diet and modifications in lifestyle to be optimised.

Weight loss as defined herein may refer to a reduction in parameters such as weight (e.g. in kilograms), body mass index (kgm^{-2}), waist-hip ratio (e.g. in centimetres), fat mass (e.g. in kilograms), hip circumference (e.g. in centimetres) or waist circumference (e.g. in centimetres).

Weight loss may be calculated by subtracting the value of one or more of the aforementioned parameters at the end of the dietary intervention from the value of said parameter at the onset of the dietary intervention.

The degree of weight loss may be expressed as a percentage change of one of the aforementioned weight phenotype parameters (e.g., a percentage change in a subject's body weight (e.g. in kilograms) or body mass index (kgm^{-2})). For example, a subject predisposed to lose weight following a dietary intervention may be likely to lose at least 10% of their initial body weight, at least 8% of their initial body weight, or at least 5% of their initial body weight. By way of example only, a subject may be likely to lose between 5 and 10 % of their initial body weight.

In one embodiment, a degree of weight loss of at least 10% of initial body weight results in a considerable decrease in risk for obesity related co-morbidities.

Weight maintenance as defined herein may refer to the maintenance in parameters such as weight (e.g. in kilograms), body mass index (kgm^{-2}), waist-hip ratio (e.g. in centimetres) fat mass (e.g. in kilograms), hip circumference (e.g. in centimetres) or waist circumference (e.g. in centimetres) following a dietary intervention

The degree of weight maintenance may be calculated by determining the change in one or more of the aforementioned parameters during a period of time following the end of the dietary intervention. The period of time may be for example at least 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 or 52 weeks following the end of the dietary intervention.

The degree of weight maintenance may be expressed as the percentage of weight change during a period following the end of the dietary intervention. For example, a subject predisposed to weight maintenance may be likely to regain less than 50, 40, 30, 20, 10, 5% or 1% of the weight lost during the dietary intervention.

Biomarkers

The term "polymorphism" refers to two or more alternate forms (alleles) in a population of a genetic locus that differ in nucleotide sequence or have variable

numbers of repeated nucleotide units. Polymorphisms occur in coding regions (exons), non-coding regions of genes or outside of genes (intergenic regions).

An “allele” is a particular form of a gene, genetic marker or other genetic locus, that is distinguishable from other forms of the gene, genetic marker or other genetic locus.. The term allele includes, for example and without limitation, one form of a single nucleotide polymorphism (SNP). An individual can be homozygous for a certain allele in diploid cells; i.e. the allele on both paired chromosomes is identical; or heterozygous for said allele; i.e. the alleles on both paired chromosomes are not identical.

The term “gene” refers to a unit of DNA which performs one function. Usually, this is equated with the production of one RNA or one protein. A gene may contain coding regions, introns, untranslated regions and control regions.

As used herein, the phrase "genetic marker" refers to a feature of an individual's genome (e.g., a nucleotide or a polynucleotide sequence that is present in an individual's genome) that is associated with one or more loci of interest. Typically, a genetic marker is polymorphic and the variant forms of genetic markers include, for example, single nucleotide polymorphisms (SNPs), indels (i.e., insertions/deletions), simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), cleaved amplified polymorphic sequence (CAPS) markers, Diversity Arrays Technology (DArT) markers, and amplified fragment length polymorphisms (AFLPs), Microsatellites or Simple sequence repeat (SSRs) among many other examples.

A “single nucleotide polymorphism (SNP)” is a DNA sequence variation occurring when a single nucleotide - A (for Adenine), T (for Thymine), C (for Cytosine), or G (for Guanine)- in the genome (or other sequence shared between individuals of a species) differs between individuals of a species (or between paired chromosomes in an individual).

A “genotype” as used herein refers to the combination of both alleles of a genetic marker, e.g. without limitation of a SNP, on a single genetic locus on paired (homologous) chromosomes in an individual.

The term “haplotype” refers to variants or alleles from distinct markers (e.g. SNPs) that are co-located on the same chromosome.

"linkage disequilibrium" (also called "allelic association") refers to a phenomenon wherein particular alleles at two or more loci tend to remain together in linkage groups when segregating from parents to offspring with a greater frequency than expected from their individual frequencies in a given population.

In one aspect the present invention provides a method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the nucleotide of the subject at one or more polymorphic positions selected from:

- (i) position 101 of SEQ ID NO:1 (rs953211)
- (ii) position 101 of SEQ ID NO:2 (rs1509290)
- (iii) position 101 of SEQ ID NO:3 (rs1509289)

and/or detecting one or more biomarkers genetically linked to said polymorphic positions.

Preferably the method comprises determining the presence of A or G at position 101 of SEQ ID NO:1, and/or A or G at position 101 of SEQ ID NO:2, and/or A or G at position 101 of SEQ ID NO:3.

It should be noted that in this application, SNPs are referred to by, for example, reference to a position in SEQ ID NO:1 (e.g. position 101), SEQ ID NO:2 (e.g. position 101) or SEQ ID NO:3 (e.g. position 101). However, when such references are made, it will be understood that the invention is not to be limited to the exact sequence as set out in the SEQ ID NO but includes variants and derivatives thereof. Instead, identification of SNP locations in similar sequences are contemplated (i.e. SNPs at positions which the skilled person would consider correspond to the positions identified in the SEQ ID numbers). The person skilled in the art can readily align similar sequences and locate the same SNP locations.

It should further be noted that detection of the nucleotide in the complement strand to SEQ ID NO:1, 2 or 3 that base-pairs with the nucleotide at position 101 of SEQ ID NO:1, 2 or 3 is of course within the scope of the claimed invention.

In the context of the present invention, detecting the presence of a biomarker in a *FAT4* gene or regulatory element thereof comprises determining the identity of one or more nucleotides at a polymorphic site in a *FAT4* encoding gene or regulatory element thereof of the individual. Similarly, detecting the presence of a biomarker genetically linked to the polymorphic positions 101 of SEQ ID NO:1, position 101 of SEQ ID NO:2 and position 101 of SEQ ID NO:3 comprises determining the identity of one or more nucleotides at a polymorphic site genetically linked to said positions.

The polymorphic site will be one which has an association with weight loss attainable by applying one or more dietary interventions and/or maintenance of weight loss following one or more dietary interventions in a subject population. By this is meant that a particular nucleotide or nucleotide sequence at the polymorphic site is correlated with said weight loss and/or maintenance.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system.

Detection of alleles

The nucleic acids obtained from the sample can be genotyped to identify the particular allele present for a marker locus. A sample of sufficient quantity to permit direct detection of marker alleles from the sample may be obtained.

Alternatively, a smaller sample is obtained from the subject and the nucleic acids are amplified prior to detection. Optionally, the nucleic acid sample is purified (or partially purified) prior to detection of the marker alleles.

Examples of allele detection methods are given below:

Allele Specific PCR

Allele-specific PCR differentiates between target regions differing in the presence of absence of a variation or polymorphism. PCR amplification primers are chosen based upon their complementarity to the target sequence, such as a sequence disclosed herein. The primers bind only to certain alleles of the target sequence.

Allele Specific Oligonucleotide Screening Methods

Further screening methods employ the allele-specific oligonucleotide (ASO) screening methods (e.g. see Saiki et al., Nature 324:163-166, 1986).

Oligonucleotides with one or more base pair mismatches are generated for any particular allele. ASO screening methods detect mismatches between one allele in the target genomic or PCR amplified DNA and the other allele, showing decreased binding of the oligonucleotide relative to the second allele (i.e. the other allele) oligonucleotide. Oligonucleotide probes can be designed that under low stringency will bind to both polymorphic forms of the allele, but which at high stringency, bind to the allele to which they correspond. Alternatively, stringency conditions can be devised in which an essentially binary response is obtained, i.e., an ASO corresponding to a variant form of the target gene will hybridize to that allele, and not to the wildtype allele.

Ligase Mediated Allele Detection Method

Ligase can also be used to detect point mutations, such as the SNPs in a ligation amplification reaction (e.g. as described in Wu et al., Genomics 4:560-569, 1989). The ligation amplification reaction (LAR) utilizes amplification of specific DNA sequence using sequential rounds of template dependent ligation (e.g. as described in Wu, supra, and Barany, Proc. Nat. Acad. Sci. 88:189-193, 1990).

Denaturing Gradient Gel Electrophoresis

Amplification products generated using the polymerase chain reaction can be analyzed by the use of denaturing gradient gel electrophoresis. Different alleles can be identified based on the different sequence-dependent melting properties and electrophoretic migration of DNA in solution. DNA molecules melt in segments, termed melting domains, under conditions of increased temperature or denaturation.

Each melting domain melts cooperatively at a distinct, base-specific melting temperature (T_m). Melting domains are at least 20 base pairs in length, and can be up to several hundred base pairs in length.

Differentiation between alleles based on sequence specific melting domain differences can be assessed using polyacrylamide gel electrophoresis, as described in Chapter 7 of Erlich, ed., PCR Technology, Principles and Applications for DNA Amplification, W. H. Freeman and Co., New York (1992).

Generally, a target region to be analyzed by denaturing gradient gel electrophoresis is amplified using PCR primers flanking the target region. The amplified PCR product is applied to a polyacrylamide gel with a linear denaturing gradient as described in Myers et al., Meth. Enzymol. 155:501-527, 1986, and Myers et al., in Genomic Analysis, A Practical Approach, K. Davies Ed. IRL Press Limited, Oxford, pp. 95-139, 1988. The electrophoresis system is maintained at a temperature slightly below the T_m of the melting domains of the target sequences.

In an alternative method of denaturing gradient gel electrophoresis, the target sequences can be initially attached to a stretch of GC nucleotides, termed a GC clamp, as described in Chapter 7 of Erlich, supra. In one example, at least 80% of the nucleotides in the GC clamp are either guanine or cytosine. In another example, the GC clamp is at least 30 bases long. This method is particularly suited to target sequences with high T_m 's.

Generally, the target region is amplified by the polymerase chain reaction as described above. One of the oligonucleotide PCR primers carries at its 5' end, the GC clamp region, at least 30 bases of the GC rich sequence, which is incorporated into the 5' end of the target region during amplification. The resulting amplified target region

is run on an electrophoresis gel under denaturing gradient conditions as described above. DNA fragments differing by a single base change will migrate through the gel to different positions, which can be visualized by ethidium bromide staining.

Temperature Gradient Gel Electrophoresis

Temperature gradient gel electrophoresis (TGGE) is based on the same underlying principles as denaturing gradient gel electrophoresis, except the denaturing gradient is produced by differences in temperature instead of differences in the concentration of a chemical denaturant. Standard TGGE utilizes an electrophoresis apparatus with a temperature gradient running along the electrophoresis path. As samples migrate through a gel with a uniform concentration of a chemical denaturant, they encounter increasing temperatures. An alternative method of TGGE, temporal temperature gradient gel electrophoresis (TTGE or tTGGE) uses a steadily increasing temperature of the entire electrophoresis gel to achieve the same result. As the samples migrate through the gel the temperature of the entire gel increases, leading the samples to encounter increasing temperature as they migrate through the gel. Preparation of samples, including PCR amplification with incorporation of a GC clamp, and visualization of products are the same as for denaturing gradient gel electrophoresis.

Single-Strand Conformation Polymorphism Analysis

Target sequences or alleles can be differentiated using single-strand conformation polymorphism analysis, which identifies base differences by alteration in electrophoretic migration of single stranded PCR products, for example as described in Orita et al., Proc. Nat. Acad. Sci. 85:2766-2770, 1989. Amplified PCR products can be generated as described above, and heated or otherwise denatured, to form single stranded amplification products. Single-stranded nucleic acids can refold or form secondary structures which are partially dependent on the base sequence. Thus, electrophoretic mobility of single-stranded amplification products can detect base-sequence difference between alleles or target sequences.

Chemical or Enzymatic Cleavage of Mismatches

Differences between target sequences can also be detected by differential chemical cleavage of mismatched base pairs, for example as described in Grompe et al., Am. J. Hum. Genet. 48:212-222, 1991. In another method, differences between target sequences can be detected by enzymatic cleavage of mismatched base pairs, as described in Nelson et al., Nature Genetics 4:11-18, 1993. Briefly, genetic material from an animal and an affected family member can be used to generate mismatch free heterohybrid DNA duplexes. As used herein, 'heterohybrid' means a DNA duplex strand comprising one strand of DNA from one animal, and a second DNA strand from another animal, usually an animal differing in the phenotype for the trait of interest.

Non-gel Systems

Other possible techniques include non-gel systems such as TaqManTM (Perkin Elmer). In this system oligonucleotide PCR primers are designed that flank the mutation in question and allow PCR amplification of the region. A third oligonucleotide probe is then designed to hybridize to the region containing the base subject to change between different alleles of the gene. This probe is labeled with fluorescent dyes at both the 5' and 3' ends. These dyes are chosen such that while in this proximity to each other the fluorescence of one of them is quenched by the other and cannot be detected. Extension by Taq DNA polymerase from the PCR primer positioned 5' on the template relative to the probe leads to the cleavage of the dye attached to the 5' end of the annealed probe through the 5' nuclease activity of the Taq DNA polymerase. This removes the quenching effect allowing detection of the fluorescence from the dye at the 3' end of the probe. The discrimination between different DNA sequences arises through the fact that if the hybridization of the probe to the template molecule is not complete, i.e. there is a mismatch of some form, the cleavage of the dye does not take place. Thus only if the nucleotide sequence of the oligonucleotide probe is completely complimentary to the template molecule to which it is bound will quenching be removed. A reaction mix can contain two different probe sequences each designed against different alleles that might be present thus allowing the detection of both alleles in one reaction.

Many current methods for the detection of allelic variation are reviewed by Nollau et al, Clin. Chem. 43, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Sample

The test sample of nucleic acid is conveniently a sample of blood, mouth swab, biopsy, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation. The invention includes the detection of the polymorphism determined from a nucleic acid sample (which may be as defined above) that has already been removed from the individual.

Primers/Probes

According to one aspect of the present invention there is provided an allele-specific oligonucleotide primer or an allele-specific oligonucleotide probe capable of detecting a polymorphism (e.g., a SNP) in a *FAT4* gene (or its complementary strand) or a regulatory region thereof.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide primer or an allele-specific oligonucleotide probe capable of detecting a polymorphic position selected from:

- (i) position 101 of SEQ ID NO:1;
- (ii) position 101 of SEQ ID NO:2; or
- (iii) position 101 of SEQ ID NO:3.

It should be noted that reference to an allele-specific oligonucleotide primer or an allele-specific oligonucleotide probe which is capable of detecting a polymorphic position selected from position 101 of SEQ ID NO:1, position 101 of SEQ ID NO:2

or position 101 of SEQ ID NO:3 includes an allele-specific oligonucleotide primer or an allele-specific oligonucleotide probe which is capable of detecting the complement of a polymorphism at position 101 of SEQ ID NO:1, position 101 of SEQ ID NO:2 or position 101 of SEQ ID NO:3, respectively.

The allele-specific primers of the present invention are used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS(TM) assays. The allele-specific primers of the present invention are preferably 15-50 nucleotides, more preferably about 15-35 nucleotides, still more preferably about 17-30 nucleotides.

An allele-specific primer capable of detecting a polymorphism at position 101 of SEQ ID NO:1 may discriminate, in an amplification reaction such as a PCR reaction, between a sequence comprising base A at position 101 of SEQ ID NO:1 (or a sequence complementary to such a gene or fragment), and a sequence comprising base G at position 101 of SEQ ID NO:1 (or a sequence or fragment complementary to such a gene or fragment).

An allele-specific primer capable of detecting a polymorphism at position 101 of SEQ ID NO:2 may discriminate, in an amplification reaction such as a PCR reaction, between a sequence comprising base A at position 101 of SEQ ID NO:2 (or a sequence complementary to such a gene or fragment), and a sequence comprising base G at position 101 of SEQ ID NO:2 (or a sequence or fragment complementary to such a gene or fragment).

Similarly, an allele-specific primer capable of detecting a polymorphism at position 101 of SEQ ID NO:3 may discriminate, in an amplification reaction such as a PCR reaction, between a sequence comprising base A at position 101 of SEQ ID NO:3 (or a sequence complementary to such a gene or fragment), and a sequence comprising base G at position 101 of SEQ ID NO:3 (or a sequence or fragment complementary to such a gene or fragment).

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for

Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

The design of probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as, for example, up to 100 bases, up to 50 bases, up to 40 bases and up to 30 bases in length. For example, the probes may be 10 to 30 bases, preferably 18-30 bases in length. The probes may comprise base sequences entirely complementary to the target sequence. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

An allele-specific probe capable of detecting a polymorphism at position 101 of SEQ ID NO:1 may discriminate, in a hybridisation reaction, between a sequence comprising base A at position 101 of SEQ ID NO:1 (or a sequence complementary to such a gene or fragment), and sequence comprising base G at position 101 of SEQ ID NO:1 (or a sequence or fragment complementary to such a gene or fragment).

An allele-specific probe capable of detecting a polymorphism at position 101 of SEQ ID NO:2 may discriminate, in a hybridisation reaction, between a sequence comprising base A at position 101 of SEQ ID NO:2 (or a sequence complementary to such a gene or fragment), and sequence comprising base G at position 101 of SEQ ID NO:2 (or a sequence or fragment complementary to such a gene or fragment).

An allele-specific probe capable of detecting a polymorphism at position 101 of SEQ ID NO:3 may discriminate, in a hybridisation reaction, between a sequence comprising base A at position 101 of SEQ ID NO:3 (or a sequence complementary to such a gene or fragment), and sequence comprising base G at position 101 of SEQ ID NO:3 (or a sequence or fragment complementary to such a gene or fragment).

The primers and/or probes of the present invention will typically be in the form of nucleic acids (e.g. DNA or cDNA). Alternatively, the primers and/or probes may be in the form of nucleic acid analogues, for example PNA (Peptide Nucleic Acids), LNA (Locked Nucleic Acids) or BNA (Bridged Nucleic Acids). The primers or probes may be nucleic acids which have been substituted in part by LNA or PNA.

Primer Design Strategy

Increased use of polymerase chain reaction (PCR) methods has stimulated the development of many programs to aid in the design or selection of oligonucleotides used as primers for PCR. Four examples of such programs that are freely available via the Internet are: PRIMER by Mark Daly and Steve Lincoln of the Whitehead Institute (UNIX, VMS, DOS, and Macintosh), Oligonucleotide Selection Program (OSP) by Phil Green and LaDeana Hiller of Washington University in St. Louis (UNIX, VMS, DOS, and Macintosh), PGEN by Yoshi (DOS only), and Amplify by Bill Engels of the University of Wisconsin (Macintosh only).

Generally these programs help in the design of PCR primers by searching for bits of known repeated-sequence elements and then optimizing the T_m by analyzing the length and GC content of a putative primer. Commercial software is also available 35 and primer selection procedures are rapidly being included in most general sequence analysis packages.

Designing oligonucleotides for use primers requires selection of an appropriate sequence that specifically recognizes the target, and then testing the sequence to eliminate the possibility that the oligonucleotide will have a stable secondary structure. Inverted repeats in the sequence can be identified using a repeat-identification or RNA-folding programs.

If a possible stem structure is observed, the sequence of the primer can be shifted a few nucleotides in either direction to minimize the predicted secondary structure.

For PCR primers used to amplify genomic DNA, the primer sequence should be compared to the sequences in the GenBank database to determine if any significant matches occur. If the oligonucleotide sequence is present in any known DNA sequence or, more importantly, in any known repetitive elements, the primer sequence should be changed.

Kits

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele-specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

In one embodiment the diagnostic kit comprises (i) an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of G at position 101 of SEQ ID NO:1 and (ii) an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of A at position 101 of SEQ ID NO:2.

In one embodiment the diagnostic kit comprises (i) an allele-specific oligonucleotide primer capable of detecting the presence of G at position 101 of SEQ ID NO:1 and (ii) an allele-specific oligonucleotide primer capable of detecting the presence of A at position 101 of SEQ ID NO:2.

In one embodiment the diagnostic kit comprises (i) an allele-specific oligonucleotide probe capable of detecting the presence of G at position 101 of SEQ ID NO:1 and (ii) an allele-specific oligonucleotide probe capable of detecting the presence of A at position 101 of SEQ ID NO:2.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

***FAT4* gene**

Protocadherin Fat 4, also known as cadherin family member 14 (CDHF14) or FAT tumor suppressor homolog 4 (FAT4), is a protein that in humans is encoded by the *FAT4* gene. FAT4 is associated with the Hippo signaling pathway. The Hippo pathway has emerged as a conserved signaling pathway that is essential for the proper regulation of organ growth in *Drosophila* and vertebrates (Halder, 2011, Development, Jan;138(1):9-22).

The FAT4 gene referred to herein may contain coding regions, introns, untranslated regions and control regions.

Figure 4 shows the genomic sequence of *FAT4* gene (SEQ ID NO: 4).

Figures 5A, 5B and 5C show examples of three *FAT4* transcripts (SEQ ID NOs 5, 6 and 7)

Figure 6 A and 6B show examples of two protein sequences (SEQ ID NOs: 8 and 9) translated from the *FAT4* gene.

It has recently been shown that *Drosophila* Fat (Ft) cadherin has a direct role in regulating mitochondrial morphology and metabolism (Sing et al., 2014, Cell, 158, 1293-1308). It was shown that proteolytic cleavage of Ft releases a soluble fragment (Ft^{mito}) that is imported into the mitochondria and that such cleavage functions as a switch mechanism to coordinate cell cycle and metabolism. It was suggested that altering the levels of Ft^{mito} may allow an organism to directly adjust metabolic rates in accordance with changing energy requirements.

It will be understood that reference to *FAT4* is not limited to the exact sequences disclosed above but includes variants and derivatives thereof. Identification of polymorphisms e.g., SNPs in similar sequences are contemplated (i.e. SNPs at positions which the skilled person would consider correspond to the positions identified herein).

Subject

Preferably the subject is a mammal, preferably a human. The subject may alternatively be a non-human mammal, including for example, a horse, cow, sheep or pig. In one embodiment, the subject is a companion animal such as a dog or a cat.

Dietary Intervention

By the term “dietary intervention” is meant an external factor applied to a subject which causes a change in the subject’s diet. In one embodiment, the dietary intervention is a low calorie diet.

The low calorie diet is one which is adjusted to the starting body weight of the animal.

Preferably the low calorie diet comprises a calorie intake of about 600 to about 1500 kcal/day, more preferably about 600 to about 1200 kcal/day, most preferably about

800 kcal/day. In one embodiment, the low calorie diet may comprise a predetermined amount (in grams) of vegetables per day. Preferably up to about 400g vegetables/day, e.g. about 200g vegetables/day.

The low calorie diet may comprise administration of at least one diet product. The diet product may be a meal replacement product or a supplement product which may e.g. suppress the subject's appetite. The diet product can include food products, drinks, pet food products, food supplements, nutraceuticals, food additives or nutritional formulas.

In one embodiment, the diet may comprise a product such as Optifast® or Modifast®. This may be supplemented with three portions of non-starchy vegetables such that the total energy intake is about 2.5 MJ (600 kcal/day). This may be further supplemented with at least 2 L of water or other energy free beverages per day.

In another embodiment, the diet may comprise, for example, a composition which is 46.4% carbohydrate, 32.5% protein and 20.1% with fat, vitamins, minerals and trace elements; 2.1MJ per day (510 kcal/day); This may be supplemented with three portions of non-starchy vegetables such that the total energy intake is about 2.5 MJ (600 kcal/day). This may be further supplemented with at least 2 L of water or other energy free beverages per day.

In one embodiment, the low calorie diet has a duration of up to 12 weeks. Preferably the low calorie diet has a duration of between 6 and 12 weeks, preferably between 8 and 10 weeks, e.g. 8 weeks.

Combining the genotyping with anthropometric measures and/or lifestyle characteristics

In one embodiment, the present method further comprises combining the determination of the genotype as referred to herein (e.g., determining the presence of a polymorphic marker e.g., SNP/ determining the sequence of the *FAT4* gene or a regulatory element thereof) with one or more anthropometric measures and/or lifestyle characteristics of the subject.

As is known in the art, an anthropometric measure is a measurement of a subject. In one embodiment, the anthropometric measure is selected from the group consisting of gender, age (in years), weight (in kilograms), height (in centimetres), and body mass index (in kg/m^2). Other anthropometric measures will also be known to the skilled person in the art.

By the term “lifestyle characteristic” is meant any lifestyle choice made by a subject, this includes all dietary intake data, activity measures or data from questionnaires of lifestyle, motivation or preferences. In one embodiment, the lifestyle characteristic is whether the subject is a smoker or a non-smoker. This is also referred to herein as the smoking status of the subject.

Subject stratification

The predisposition to weight loss and/or weight maintenance predicted by the method of the present invention may be used to stratify subjects into categories.

Subjects may be stratified into categories which are indicative of the degree of predicted weight loss/maintenance. Such stratification is useful to determine which subjects would benefit most from certain interventions. In this way, dietary intervention and modification of lifestyle can be optimised, and realistic expectations of the weight loss to be achieved by the subject can be set.

In one embodiment, the categories include weight loss resistant subjects and weight loss sensitive subjects.

By the term “weight loss resistant” is meant a predicted degree of weight loss which is less than a predetermined value. In one embodiment, “weight loss resistant” is defined as a subject having a weight loss percentage inferior to a predetermined value e.g. a subject predicted to lose less weight than the 10th, 15th, 20th or 30th percentile of the expected weight loss.

By the term “weight loss sensitive” is meant a predicted degree of weight loss of more than a predetermined value. In one embodiment, “weight loss sensitive” is defined as a subject having a weight loss percentage superior to a predetermined threshold value.

For example a subject predicted to lose more weight than the 85th, 80th or 75th percentile of the expected weight loss.

The “expected weight loss” can be obtained from data of a population of subjects that have undergone the same dietary intervention as the one being tested.

In another embodiment, the categories include weight maintenance resistant subjects and weight maintenance sensitive subjects.

By the term “weight maintenance resistant” is meant a predicted degree of weight maintenance which is less than a predetermined value. In one embodiment, “weight maintenance resistant” is defined as a subject having a degree of weight maintenance inferior to a predetermined threshold value e.g. a subject predicted to maintain a lesser degree of the weight loss following a dietary intervention than the 10th, 15th, 20th or 30th percentile of the subject population.

By the term “weight maintenance sensitive” is meant a predicted degree of weight maintenance which is more than a predetermined value. In one embodiment, “weight maintenance sensitive” is defined as a subject having a degree of weight maintenance superior to a predetermined threshold value. e.g. a subject predicted to maintain a greater degree of the weight loss following a dietary intervention than the 10th, 15th, 20th or 30th percentile of the subject population.

The subject population can be obtained from data of a population of subjects that have undergone the same dietary intervention as the one being tested.

Method for selecting a modification of lifestyle of a subject

In a further aspect, the present invention provides a method for modifying the lifestyle of a subject. The modification in lifestyle in the subject may be any change as described herein, e.g. a change in diet, more exercise, a different working and/or living environment etc.

Preferably the modification is a dietary intervention as described herein. More preferably the dietary intervention includes the administration of at least one diet product. The diet product preferably has not previously been consumed or was consumed in different amounts by the subject. The diet product may be as described

herein. Modifying a lifestyle of the subject also includes indicating a need for the subject to change his/her lifestyle, e.g. prescribing more exercise or stopping smoking.

For example, if a subject is not predicted to lose weight on a low calorie diet, a modification may include more exercise in the subject's lifestyle.

Use of Diet Products

In one aspect, the present invention provides a diet product for use as part of a low calorie diet for weight loss. The diet product being administered to a subject that is predicted to achieve a weight loss by the methods described herein.

In another aspect, the present invention provides a diet product for use in treating obesity or an obesity-related disorder, wherein the diet product is administered to a subject that is predicted to attain weight loss by the methods described herein.

The obesity-related disorder may be selected from the group consisting of diabetes (e.g. type 2 diabetes), stroke, high cholesterol, cardiovascular disease, insulin resistance, coronary heart disease, metabolic syndrome, hypertension and fatty liver. In a further aspect, the present invention provides the use of a diet product in a low calorie diet for weight loss where the diet product is administered to a subject that is predicted to attain weight loss by the methods described herein.

Computer Program Product

The methods described herein may be implemented as a computer program running on general purpose hardware, such as one or more computer processors. In some embodiments, the functionality described herein may be implemented by a device such as a smartphone, a tablet terminal or a personal computer.

In one aspect, the present invention provides a computer program product comprising computer implementable instructions for causing a programmable computer to predict the degree of weight loss/maintenance based on the genotyping of the present invention and optionally anthropometric measures and/or lifestyle characteristics from the user. As described herein, anthropometric measures include age, weight, height, gender and body mass index and lifestyle characteristics include smoking status.

In a particularly preferred embodiment, the user inputs into the device the results of the genotyping of the present invention, optionally along with age, body mass index, gender and smoking status. The device then processes this information and provides a prediction on the degree of weight loss/maintenance attainable by the user from a dietary intervention.

The device may generally be a server on a network. However, any device may be used as long as it can process biomarker data and/or anthropometric and lifestyle data using a processor, a central processing unit (CPU) or the like. The device may, for example, be a smartphone, a tablet terminal or a personal computer and output information indicating the degree of weight loss attainable by the user.

Those skilled in the art will understand that they can freely combine all features of the present invention described herein, without departing from the scope of the invention as disclosed.

Various preferred features and embodiments of the present invention will now be described by way of non-limiting examples.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA* Methods in Enzymology, Academic Press; and E. M. Shevach and W. Strober,

1992 and periodic supplements, *Current Protocols in Immunology*, John Wiley & Sons, New York, NY. Each of these general texts is herein incorporated by reference.

EXAMPLES

Background information

Diogenes (Diet Genes and Obesity) is an intervention study in overweight/obese patients (with BMI between 27 and 45 kg/m²) (Larsen, T. M. *et al. N. Engl. J. Med.* **363**, 2102–2113 (2010)). To our knowledge, Diogenes is the largest and most comprehensive study on weight management.

Patients followed a low-caloric diet (about 800kcal/d) for 8 weeks. Then participants that had lost at least 8% of their initial body weight, were randomly assigned, in a two-by-two factorial design, to one of five ad libitum diets to prevent weight regain over a 26-week period: a low-protein and low-glycemic-index diet, a low-protein and high-glycemic-index diet, a high-protein and low-glycemic-index diet, a high-protein and high-glycemic-index diet, or a control diet.

The aim of our analyses was to analyze whether genetic markers (SNPs) would be classifiers of weight loss and weight maintenance.

Example 1 – Material and methods

Study design

Diogenes (ClinicalTrials.gov identifier: NCT00390637) is composed of two main periods:

- Weight loss period with a low-caloric diet (800kcal/d) for 8 weeks
- Weight maintenance diet for 26 weeks

A number of clinical measurements and biological samples were taken at different time points. Four time points are of notable interest for the present invention:

1. Screening visit
2. Clinical Intervention Day 1 (CID1): start of the weight loss period

3. CID2: end of the weight loss period, randomization into one weight maintenance diet and start of the weight maintenance period
4. CID3: end of the weight maintenance period

Genotype data

The Illumina 660-quad Bead chips were used to perform the Whole Genome Scan (Hypothesis-free approach). The Illumina technology is based on a DNA chip allowing the genotyping of approximately 660'000 single nucleotide polymorphisms (SNPs) per subject. SNPs are distributed over all the chromosomes and are used as tagging markers of the corresponding genomic area. The details of the process and experimental protocol followed the manufacturer recommendations (www.illumina.com).

Quality control of the genotype data

SNPs were excluded from the analysis, if they fulfilled at least one of the following:

- Low call rate (<95%)
- Violating Hardy-Weinberg equilibrium (FDR < 20%)
- Minor allele frequency < 0.28%

Subjects were excluded from the analysis, if they fulfilled at least one of the following:

- Low call rate (<95%)
- Abnormal autosomal heterozygosity (FDR<1%)
- For pairs of subjects with Identity-By-State >0.95, the subject with the lowest call rate was excluded.
- Individuals that were outliers in terms of genetic structure: 6 standard deviation distant from the Diogenes cluster's centroid, based on a principal component analysis (2 first components) performed on genotypes from Diogenes and the Hapmap project (with CEU, YRI and CHB+JPT panels)
- Individuals having gender discrepancies between records from the clinical database and inferred gender from genotype data

The final genotype dataset, after QC, included 516'636 SNPs and 869 subjects.

About genomic coordinates

All genomic coordinates (SNPs, genes) used in this document correspond to the Human genome assembly hg19.

Genome-wide association tests

Genome-wide scan was performed using the following linear model:

$$BMI3 \sim BMI2 + SNP + center + gender + age + \varepsilon$$

In this model, BMI2 and BMI3 refer to Body Mass Index (BMI) measured respectively before and after the weight maintenance period (i.e. CID2 and CID3). Center refers to one the Diogenes center. Age refers to the subject's age measured at the screening visit. ε corresponds to the model's residuals term.

Association was tested independently for each SNP using the *GenABEL* *qtscore* function (Aulchenko et al., *Genetics* **177**, 577–585 (2007)). The method first regresses on the trait of interest for co-variables then test association between the obtained residuals and a SNP using least-square methods. This function implements both allelic and genotypic tests. Since allelic tests are more powerful than genotypic tests, only allelic tests were considered for data interpretation.

The initial number of subjects (n=869) becomes much smaller when removing subjects with missing values either for BMI or any covariate of the regression tests (including the SNP being modelled). The average number of subjects, without any missing values, that was used for each of the 516'636 models was n=461.

TagSNP identification

We used the *LD select* methodology proposed by Carlson (Carlson *et al.*, *Am. J. Hum. Genet.* **74**, 106–120 (2004)). Tag SNP analysis was performed with the following settings: MAF threshold 10% and R-square threshold 50%.

Haplotype phasing

Genotype data were phased using the *MACH* software (Li, et al., *Genet. Epidemiol.* **34**, 816–834 (2010)) with the following parameters: --rounds 50 --states 200 --phase.

Haplotype association tests

Subsequently to the phasing with *MACH*, association between change in BMI during weight maintenance and all haplotype blocks was tested using the following model:

$$BMI3 \sim BMI2 + center + gender + age + AAC + AAT + AGC + GAC + GAT + GGC + \varepsilon$$

Center refers to one the Diogenes center. Age refers to the subject's age measured at the screening visit. ε corresponds to the model's residuals term.

Both additive (number of haplotypes: 0, 1 or 2) and dominant (absence/presence) genetic models were tested. Backward selection was then performed on the fitted model (*stepAIC* function in R (R Development Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>)).

Additional linear models

Change in a given trait over a period of interest (weight maintenance or weight loss period) was tested using linear models.

These models were used to test either single-SNP or epistatic effect (interaction between two SNPs) with several weight-related phenotypes.

Those models can be written in the Wilkinson-Roger notation as follows:

$$Trait_at_time(i) \sim Trait_at_time(i-1) + genetic\ effect + center + gender + age + \varepsilon$$

The trait can be any quantitative trait like BMI; waist or hip circumferences; fat-free mass or fat mass weight. The time points (i-1) and (i) refer to before and after a given

period (e.g. weight maintenance or weight loss period). Such modeling allows predicting the trait at time (i) while adjusting for baseline values at time (i-1).

Center refers to one the Diogenes center. Age refers to the subject's age measured at the screening visit. ε corresponds to the model's residuals term.

The genetic effect can be either a single SNP or the interaction between two SNPs (SNP1 and SNP2). For single-SNP effect, genotypic models were used. When testing SNP interaction, SNP1 and SNP2, were each coded for presence/absence of a given allele. The ε term corresponds to residuals of the model.

Significance of each term in the model was tested using a type III ANOVA (Fox & Weisberg, *An R Companion to Applied Regression*. (Sage, 2011). at <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>).

Dichotomizing changes in BMI over time

A recurrent challenge in weight maintenance studies is to define a threshold that allows classifying patients into groups of good and bad weight maintainers. Frequently such classification is made with the two most extreme groups of patients (for e.g. patients that have a response below the 10th percentile or above the 90th percentiles). Yet the decision on which percentile to use is an entirely arbitrary one. One cannot exclude the risk (prior doing the analyses) that different conclusions might be reached depending on the chosen cutoff. Instead of making such an arbitrary decision, we decided to use several cutoffs as explained below.

First, we expressed change in BMI during weight maintenance as:

$$\Delta = 100 * \frac{BMI_2 - BMI_3}{BMI_2}$$

where BMI2 and BMI3 stands for BMI before and after the weight maintenance period (i.e. CID2 and CID3). This difference (Δ) corresponds to the percentage of BMI change during weight maintenance relative to the baseline BMI (BMI before weight maintenance).

Interpretation of Δ is as follows:

- $\Delta < 0$ implies that $BMI2 < BMI3$, therefore the patient regained weight (i.e. bad weight maintenance profile)
- $\Delta > 0$ implies that $BMI2 > BMI3$, therefore the patient continued to lose weight (i.e. good weight maintenance profile)

Next, we computed dichotomized Δ into groups of “good” and “bad” weight maintainer profiles. Such dichotomization was made by selecting the patients with the most extreme Δ values. This is achieved by selecting Δ above or below a given percentile. For example, using the 10th and 90th percentiles, we defined the following groups:

- “Bad weight maintainers” as patients with a Δ below the 10th percentile
- “Good weight maintainers” as patients with a Δ above the 90th percentile

In this example, subjects with Δ between the 10th and 90th percentiles are not included in the analysis. These subjects can be referred to as those with “an intermediate profile” and for which one cannot decide whether they should be classified to one group or another.

To avoid bias due to the choice of an arbitrary percentile threshold, we analyzed data from several classification schemes:

- Patients with Δ below the 10th percentile or above the 90th percentile
- Patients with Δ below the 25th percentile or above the 75th percentile
- Patients with Δ below the 33rd percentile or above the 66th percentile
- Patients with Δ below the 50th percentile or above the 50th percentile

It can be noted that the first three schemes discard patients with intermediate profiles (e.g. Δ between the 10th and 90th percentiles) and thus only use a subset of the data, while the last scheme classifies all patients into two distinct groups. Therefore classification using the first three schemes can be challenged by the fact only a limited subset of the data is used. In contrast, the fourth scheme uses all available data but the two groups would each have high heterogeneity and since patients with “intermediate profiles” are not discarded, the distinction between those two groups might not be

clear. Therefore this last classification scheme is expected to be the most complex case.

Similar dichotomization was applied on BMI during the weight loss period, using the following formula:

$$\Delta = 100 * \frac{BMI_1 - BMI_2}{BMI_1}$$

In this formula, BMI₁ and BMI₂ correspond respectively to before and after the weight loss period (i.e. CID1 and CID2).

Classification analysis

Following dichotomization of the BMI into groups of “bad” and “good” weight maintainers, we assessed the classification performance with simple contingency tables like:

	Bad weight maintainers	Poor weight maintainers
Protective genotypes	a	b
Non-protective genotypes	c	d

Where a, b, c and d correspond to the number of patients from a given category.

From such table, one can compute the accuracy of classification:

$$ACC = 100 * \frac{a + d}{a + b + c + d}$$

Ninety-five confidence intervals (95% CI) for the classification accuracy are then derived with a binomial test (*binom.test* function in R (R Development Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>)). A one-sided binomial test is also performed to test whether the classification accuracy is better than the "no information rate," which is taken to be the largest class percentage in the data (see the *confusionMatrix* function in the caret package (Kuhn *J. Stat. Softw.* 28, 1–26 (2008))).

By non-protective genotypes, we aim at identifying subjects with a low Δ (patients regaining weight). Conversely, with protective genotypes, we aim at identifying subjects with high Δ (patients regaining less weight, or even still losing weight).

Protective and non-protective genotypes were defined as follows:

SNP(s)	Protective genotype	Non-protective genotype
rs1509289	A carriers	G/G
rs1509290	A carriers	G/G
rs953211	G carriers	A/A
rs953211 and rs1509290	rs953211 G carriers ; rs1509290 A carriers	Any other genotypes

The same methodology was applied on BMI changes during a weight loss period.

Analysis of imputed SNPs

Genotype from additional SNPs located nearby FAT4 were obtained using imputation. Imputation was performed using the *MACH* software (Li et al., *Genet. Epidemiol.* **34**, 816–834 (2010)) and using subjects of European ancestry from the 1000 Genome Project (Consortium, T. 1000 G. P. *Nature* 491, 56–65 (2012)) (phase II) as a reference data. Imputation was performed following recommended steps from the MACH software. Analysis of allele dosage value was performed using ProbABEL (Aulchenko, et al., *BMC Bioinformatics* 11, 134 (2010)) and using the following model:

$$BMI3 \sim BMI2 + center + gender + age + SNP + \varepsilon$$

BMI3 and BMI2 refer to BMI measured respectively at CID3 and CID2. Center refers to one the Diogenes center. Age refers to the subject's age measured at the screening visit. ε corresponds to the model's residuals term. The SNP term corresponds to the imputed allele dosage values.

Example 2 – Detailed Results

GWA scan for weight maintenance

From the genome-wide analysis, SNPs located on chromosome 4 were found significantly associated with BMI trajectories during the weight maintenance period. In particular, the two following SNPs: rs953211 and rs1509289 (Table 1), both intronic variants in the *FAT4* gene, emerged within the genome-wide list as top ranking SNPs. Since these two SNPs are only in moderate linkage disequilibrium (LD) ($R^2=50.61\%$) and given that there were other nearby SNPs with association p-values less than 5% (Figure 1), we searched whether the observed association could be due to a haplotype.

Haplotype tests for weight maintenance

To restrict the list of SNPs to be used for haplotype phasing, a tagSNP search was performed. This search identified the following three SNPs: rs953211, rs1509290 and rs13136889 as best tag SNPs for other SNPs found marginally associated with BMI during weight maintenance (p-values < 5%, see Figure 1). Six distinct haplotypes can be defined using these three SNPs: AAT, GAC, GGC, AAC, GAT and AGC (The order of SNPs in each haplotype is rs953211, rs1509290 and rs13136889).

Next, these haplotypes were tested for association with change in BMI during the weight maintenance. Both dominant and additive genetic models were considered. Results are shown in Table 2-5. These analyses identified presence of the GAC and GAT haplotypes as significantly associated with a more favourable BMI after the weight maintenance intervention. In particular, patients carrying at least one copy of either haplotypes are less prone to regain weight compared to patients not carrying any of these haplotypes. These results were reproduced using univariate approaches (ranksum tests) and with the *Plink* software (Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007)).

Since favourable weight maintenance is observed with both the GAC and GAT haplotypes, the third SNP does not seem to contribute much and therefore the

protective haplotype can be simplified as rs953211-G and rs1509290-A. Conversely, this result also implies the rs953211-A and rs1509290-G haplotype as a deleterious haplotype.

Epistasy tests for weight maintenance

In the light of the haplotype results, we defined the following genotype groups:

- rs953211-G carriers and rs1509290-A carriers
- rs953211-G carriers and rs1509290- G/G
- rs953211-A/A and rs1509290- A carriers
- rs953211-A/A and rs1509290- G/G

The interaction between rs953211 and rs1509290, as defined with the above groups, was found significantly associated with change in BMI during weight maintenance ($p = 7.96E-05$, for details about the methodology see Additional linear models). Full results from the linear model and from the subsequent ANOVA are shown respectively in Table 6 and Table 7. These results show that rs953211-G carriers and rs1509290-A carriers have significantly better BMI profiles than the three remaining genotype groups.

To facilitate genetic diagnostic, the four genotype groups can be simplified as “rs953211-G carriers and rs1509290-A carriers” and subjects from the three remaining group pooled together. Such genetic dichotomization is also significantly associated with the BMI trajectories during the weight maintenance period. Results from the linear model are shown in Table 8. This table also includes the 95% confidence intervals (CI) on the regression coefficient for each term of the model. These 95% CI were obtained by performing bootstrap (Davison. & Hinkley *Bootstrap Methods and Their Applications*. (Cambridge University Press, 1997). at <http://statwww.epfl.ch/davison/BMA>) of the linear model (with $n=1'000$ re-sampling and using the *boot* package (Canty, A. & Ripley, B. D. *boot: Bootstrap R (S-Plus) Functions*. (2014)) in R). Interpretation of these coefficients is as follows: when adjusting for covariates such as age, gender, participating center and BMI before weight maintenance; the effect of being “rs953211-G carriers and rs1509290-A carriers” is -0.76 kg/m^2 on the BMI after weight maintenance. In other words, the

effect on BMI after a weight maintenance period is -0.76 kg/m^2 (with 95% CI = $[-1.1, -0.43]$) for subjects that are “rs953211-G carriers and rs1509290-A carriers” compared to subjects from any other genotype combination.

An even simpler way to look at these results is to only consider the change in BMI, before and after weight maintenance and without adjusting for covariates (age, gender, etc....). Without any genetic stratification (i.e. not knowing the genotype of a subject), the median change in BMI is $+0.38 \text{ kg/m}^2$ (Table 9). This means that subjects tend regain weight. When the genotype is available for rs953211 and rs1509290; subjects from these three genotype groups: “rs953211 A/A ; rs1509290 A carriers” ; “rs953211 A/A ; rs1509290 G/G” and “rs953211 G carriers ; rs1509290 G/G” all regain weight (median BMI changes are respectively $+0.57$, $+0.63$ and $+0.68 \text{ kg/m}^2$). By contrast, subjects that are “rs953211 G carriers ; rs1509290 A carriers”, do not tend to regain weight (median BMI change = 0).

Therefore this combination of these two genetic markers:

- Identifies a group of patients (“rs953211 G carriers ; rs1509290 A carriers”) that has better weight maintenance than the three others groups
- Demonstrates that knowing the genotype of these two markers brings additional information compared to not knowing the genotype. The BMI change for each of the four genotype groups is different from the average BMI change of the global population.

Epistasy tests for weight loss

The same combination of SNPs was found associated with BMI changes during the weight loss period ($p=0.0243$, for details about the methodology see Additional linear models). Results from the linear model and its subsequent ANOVA are shown respectively in Table 10 and Table 11.

As done for the weight maintenance analyses, the combination of these two SNPs was dichotomized into “rs953211-G carriers and rs1509290-A carriers” and all remaining subjects pooled together. Results from a linear model modeling BMI changes during the weight loss period are shown in Table 12. This table shows that subjects

“rs953211 G carriers ; rs1509290 A carriers” have a stronger weight loss (BMI change = -0.20 kg/m² with 95% CI [-0.34, -0.06]) compared to subjects with other genotype combinations.

Computing the median change in BMI before and after the weight loss period, one can observe that “rs953211 G carriers ; rs1509290 A carriers” lose more weight (median BMI change = -3.69 kg/m²) than the other genetic groups (see Table 13).

Epistasy tests for baseline BMI

No difference in BMI at baseline (CID1) was found between the different genotype groups, as defined by rs953211 and rs1509290 (ranksum p=0.93). This indicates that this combination of markers is predictive of weight upon an intervention (e.g. weight loss or weight maintenance) but is not prognostic.

Association between FAT4 SNPs and weight-related phenotypes

Since *FAT4* SNPs were associated with change in BMI, we tested whether the same SNPs would also be associated with additional weight-related phenotypes such as change in fat mass, fat-free mass, hip circumference and waist circumference (for details about the methodology see Additional linear models). And indeed, those SNPs: rs1509289, rs1509290, rs953211; alone or in combination, are significantly associated with change in weight-related phenotypes during weight maintenance (Table 14).

During the weight loss period, several SNPs (rs1509289, rs1509290 and the interaction between rs953211 and rs1509290) were found significantly associated with change in BMI and hip circumference (Table 15).

These analyses demonstrate that *FAT4* SNPs are associated with both BMI and several additional weight-related phenotypes both for the weight maintenance and weight loss periods.

Using *FAT4* SNPs as classifiers of dichotomized BMI changes

In order to estimate the performance at predicting the outcome of a weight loss or weight maintenance intervention, we computed the percentage of change in BMI and dichotomized this value into groups of “good” and “bad” profile (For methodological details, see section Dichotomizing changes in BMI over time). We also dichotomized the genotypes of our genetic markers into combinations that are expected to identify subjects with either a “good” or “bad” profile (see Classification analysis).

Although analyzing dichotomized values results in significant loss of statistical power, such simple scheme gives very conservative estimates about the prediction performance.

We performed such analyses for BMI during both weight maintenance and weight loss periods. Also to avoid introducing biases due to the choice of the cutoff to dichotomize the BMI, we repeated the analysis with several cutoffs.

The predictive performance for the different genetic markers is shown in Table 16 and Table 17. These tables present results respectively for BMI outcome after weight maintenance or weight loss intervention.

These tables include odds ratios with their 95% confidence intervals, and p-value from a Fisher’s exact test performed on the contingency table (derived from the classification). These values assess the association between the classifiers (genotypes) and the true outcome (dichotomized percentage of BMI change). A p-value below 5% indicates a significant association between the classifier and outcome.

These tables also include performance metrics such as the classification accuracy (i.e. fraction of correctly classified subjects) and the 95% confidence interval for the accuracy. A random classifier (e.g. tossing a coin to predict the outcome) would have 50% of accuracy. When the lowest confidence interval is above 50%, it means that the classifier is doing significantly better than the random classifier. Another metric to evaluate whether the accuracy is significantly better than a random classifier is referred as the accuracy’s p-value. This one-sided p-value, obtained with a binomial

test, assesses whether the observed accuracy is better than the no information rate (Kuhn, *J. Stat. Softw.* **28**, 1–26 (2008)).

These analyses shows that FAT4 genetic markers (rs1509289, rs150929 and rs953211), used independently or in combination have a significantly higher accuracy than a random classifier at predicting the BMI outcome, after a weight maintenance period (all accuracy pvalues are $< 5\%$, see Table 16).

Regarding the BMI outcome after weight loss period, the classification accuracy for rs953211 is not better than a random classifier (accuracy pvalues $> 5\%$, see Table 17). This was expected because rs953211 was not found significantly associated with BMI change during weight loss.

For the two other SNPs (rs1509289 and rs150929) and for the combination of rs953211 and rs1509290, the classification accuracies are significantly better than the random classifier for the majority of the dichotomization schemes (Table 17).

Therefore, FAT4 markers used alone or in combination, can be used as classifier of weight outcome following weight loss or weight maintenance period.

Finding additional SNPs as potentially better classifiers

Providing that some candidate regions have been identified, it is straight-forward to refine association signals. Extensive reviews have been written to describe the process of refining association signals (McCarthy & Hirschhorn *Hum. Mol. Genet.* **17**, R156–R165 (2008); Ioannidis et al., *Nat. Rev. Genet.* **10**, 318–329 (2009); and McCarthy *et al. Nat. Rev. Genet.* **9**, 356–369 (2008)). A few strategies are summarized below.

Gene re-sequencing and additional genotyping

With the advent of next-generation sequencing (NGS), it is now possible to sequence either exome or full genome of large cohorts. Following experimental protocols from vendors and using recent analytical pipelines from the community (McKenna *et al. Genome Res.* **20**, 1297–1303 (2010)), additional SNPs, not assayed with classical SNP arrays, can be identified. Genotyping additional markers is possible with a wide

range of technologies. This includes and is not limited to sequencing (exome-sequencing, full-genome sequencing, targeted sequencing, RNA-sequencing, pyrosequencing); genotyping assays (either high-density SNP arrays, custom SNP-arrays, targeted genotyping with Taqman, Fluidigm), PCR-based assays, mass-spectrometry genotyping techniques, etc.

Once additional markers have been genotyped, it is straight-forward to test each of those for association with a trait (e.g. change in BMI) and identify the causal marker(s). Notably by genotyping additional markers located nearby our identified SNPs (for e.g. +/- 100Kb) and repeating the same analyses described above, one may identify further markers or combination of markers useful at predicting change in weight-related phenotypes during a weight loss or weight maintenance period.

Using imputed SNPs

Obtaining additional markers can also be achieved using imputation techniques (Marchini & Howie, *Nat. Rev. Genet.* **11**, 499–511 (2010)). With the availability of public reference datasets (Consortium, T. I. H. 3., *Nature* 467, 52–58 (2010); Consortium, T. 1000 G. P. *Nature* 491, 56–65 (2012); Gibbs *et al.*, *Nature* 426, 789–796 (2003); The International HapMap Consortium. A haplotype map of the human genome. *Nature* 437, 1299–1320 (2005)) and well-established imputation tools (Li *et al.*, *Genet. Epidemiol.* 34, 816–834 (2010); Marchini & Howie, *Nat. Rev. Genet.* **11**, 499–511 (2010)), this strategy is commonly used in genome-wide association studies, to increase the number of markers to be tested.

Such a strategy was applied to our data, leading to the availability of genotype data for 475 SNPs located nearby the *FAT4* gene (+/- 10Kb) (see section Analysis of imputed SNPs). Subsequently, these SNPs were tested for association with BMI change during weight maintenance. The results are displayed in Figure 2. Ninety-two SNPs have a p-value smaller than 5%, indicating that other genetic variants, located nearby *FAT4*, are associated with weight management (see list in Figure 7). Yet none of these SNPs have a stronger association (lower p-value) than the already detected SNPs (Figure 2). This suggests that although those imputed SNPs could act as proxy

SNPs for rs953211, rs1509290 or rs1509289; they are unlikely to have a significantly better predictive value.

Meta-analyses

Meta-analyses aim at combining results from multiple studies so that consistent patterns can be found across those studies. Specifically meta-analysis of GWA studies aim at identifying SNPs associated with a trait of interest that could be missed when analyzing a single study. Statistical methodologies to perform genetic meta-analysis are well established (Ioannidis et al., *Nat. Rev. Genet.* **10**, 318–329 (2009); Evangelou & Ioannidis *Nat. Rev. Genet.* **14**, 379–389 (2013)) and are frequently used in large consortia (Yang et al. *Nat. Genet.* **44**, 369–375, S1–3 (2012); DIABetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium et al. *Nat. Genet.* **46**, 234–244 (2014)) to identify additional markers.

By combining, results from several studies and focusing on SNPs located within and nearby the *FAT4* gene (for e.g. +/- 100Kb), additional SNPs may be found.

Multi-dimensional reduction analyses

Another approach, once a region of interest has been detected is to screen all possible 2-SNPs or even higher order SNP interactions (e.g. 3-SNPs interaction) using a multi-dimensional reduction approach (Moore *Adv. Genet.* **72**, 101–116 (2010); Pan et al., *Methods Mol. Biol. Clifton NJ* **1019**, 465–477 (2013)). Multi-dimensional reduction approach (MDR) can be used to test SNP-SNP interactions in case-control studies. MDR is non-parametric, model-free method that aims at identifying the combination of SNPs with the best accuracy at predicting a binary class. When using MDR, cross-validation are recommended to evaluate the accuracies on a training dataset and on a validation dataset. These accuracies are referred to as classification accuracy and prediction accuracy respectively for the training and validation datasets (for additional details see this review by Motsinger and Ritchie (*Hum. Genomics* **2**, 318–328 (2006))). Cross-validation consistency can also be evaluated. That is when doing several random split of the data into training and validation set, how many times the same best classifier (combination of SNPs) would be identified.

We applied such technique onto SNPs located within the *FAT4* gene (+/- 10kb), and aimed at identifying the best SNP or best combination of two SNPs to predict dichotomized change in BMI during weight maintenance or weight loss (see section: Dichotomizing changes in BMI over time). Results are shown in Table 18 and Table 19 respectively for BMI changes during weight maintenance or during weight loss. These analyses essentially found the rs1509289, rs953211, rs1509290 SNPs as being the best classifiers for BMI changes. This is consistent with our previous analyses and shows that these are the preferred markers for predicting weight loss / maintenance.

Example 3 – Summary of preferred embodiment

FAT4 SNPs and change in BMI during weight maintenance

Our various analyses demonstrated that SNPs located nearby *FAT4* were associated with change in BMI during a weight maintenance period. These results can be summarized as follows:

- Patients tend to regain weight during the weight maintenance phase. This observation can be made irrespectively of the genotype of any SNPs
- When a skilled person knows the genotype at *FAT4* SNPs, in our preferred embodiment at the rs953211 and rs1509290 SNPs, the following predictions can be made:
 - Patients that are “rs953211 G carriers ; rs1509290 A carriers” tend to have neutral change in BMI (median change in BMI close to 0)
 - Patients from any other genotype groups tend to regain significantly more weight than “rs953211 G carriers ; rs1509290 A carriers”; and also more weight than the average (or median) change in the population (i.e. all patients pooled together irrespectively of their genotypes)

Therefore knowing the genotype of *FAT4* SNPs, the following predictions can be made:

Group	Predicted outcome after weight maintenance
Overall population (not knowing the	Regain weight (i.e. increase in BMI)

genotypes)	
“rs953211 A/A ; rs1509290 A carriers”	Regain weight
“rs953211 A/A ; rs1509290 G/G”	Regain weight
“rs953211 G carriers ; rs1509290 A carriers”	Neutral change in weight (i.e. median BMI change is 0)
“rs953211 G carriers ; rs1509290 G/G”	Regain weight

FAT4 SNPs and change in BMI during weight loss

Our analyses also found that the same FAT4 SNPs that predict weight maintenance outcome could also be used to predict weight loss outcome. The results can be used as follow:

Group	Predicted outcome after weight loss
Overall population (not knowing the genotypes)	Lose weight
“rs953211 A/A ; rs1509290 A carriers”	Lose weight
“rs953211 A/A ; rs1509290 G/G”	Lose weight
“rs953211 G carriers ; rs1509290 A carriers”	Lose more weight than all other groups
“rs953211 G carriers ; rs1509290 G/G”	Lose weight

FAT4 SNPs and change in other weight-related phenotypes during weight maintenance

FAT4 SNPs were found associated with change during weight maintenance in additional weight-related phenotypes, such as hip and waist circumference, fat mass and fat-free mass. Median changes for those phenotypes stratified per genotype group are shown in Table 20. These results can be used as follows:

Group	Fat mass	Fat free mass	Waist circumference	Hip circumference
Overall population (not knowing the genotypes)	Neutral change	Increase fat free mass	Increase waist circumference	Neutral change
“rs953211 A/A ; rs1509290 A carriers”	Neutral change	Increase fat free mass	Increase waist circumference	Neutral change
“rs953211 A/A ; rs1509290 G/G”	Neutral change	Increase fat free mass	Increase waist circumference	Increase hip circumference
“rs953211 G carriers ; rs1509290 A carriers”	Lose fat mass	Increase fat free mass	Neutral change	Neutral change
“rs953211 G carriers ; rs1509290 G/G”	Neutral change	Increase fat free mass	Increase waist circumference	Neutral change

FAT4 SNPs and change in other weight-related phenotypes during weight loss

FAT4 SNPs were found significantly associated with change in hip circumference during weight maintenance. Median changes for hip and other weight-related phenotypes stratified per genotype group are shown in Table 21. Results for hip circumference can be summarized as follows:

Group	hip circumference
Overall population (not knowing the genotypes)	Reduce hip circumference
“rs953211 A/A ; rs1509290 A carriers”	Reduce hip circumference
“rs953211 A/A ; rs1509290 G/G”	Reduce hip circumference
“rs953211 G carriers ; rs1509290 A carriers”	Reduce hip circumference (slightly bigger change than change occurring in the other groups)
“rs953211 G carriers ; rs1509290 G/G”	Reduce hip circumference

Tables

SNP	chr	Position	allele A	allele B	beta coefficient (allele B)	Beta standard error	allelic test p- value	allelic test adjusted for genomic control	SNP call rate	MAF	Overlapping gene
rs953211	4	126351049	A	G	-0.59	0.13	2.74E-06	3.73E-06	100.00%	36.66%	FAT4, intron
rs1509289	4	126308835	G	A	-0.64	0.14	3.00E-06	4.06E-06	100.00%	25.60%	FAT4, intron

Table 1 Top SNPs on chromosome 4 identified with the genome-wide analysis

	Estimate	Std. Error	t value	Pr(> t)
Intercept	2.997204	0.957387	3.131	0.001858
BMI2	0.928099	0.018983	48.892	< 2e-16
center	-0.122483	0.031351	-3.907	0.000108
gender	-0.256245	0.178685	-1.434	0.152248
age	0.001246	0.013732	0.091	0.927753
AAC	0.201121	0.224391	0.896	0.370574
AAT	0.917214	0.820991	1.117	0.264502
AGC	0.358763	0.206106	1.741	0.082423
GAC	-0.803172	0.293798	-2.734	0.006508
GAT	-0.261936	0.233207	-1.123	0.261953
GGC*	NA	NA	NA	NA

Table 2 FAT4 haplotype additive analysis (rs953211, rs1509290, rs13136889), results from the following linear regression: BMI3 ~ BMI2 + center + gender + age + AAC + AAT + AGC + GAC + GAT + GGC + ε

* Coefficients for the GGC haplotypes could not be estimated due to singularity in the data.

	Estimate	Std. Error	t value	Pr(> t)
Intercept	2.97142	0.6078	4.889	1.41E-06
BMI2	0.92619	0.01891	48.974	< 2e-16
center	-0.12471	0.03117	-4	7.38E-05
AGC	0.21733	0.14177	1.533	0.125973
GAC	-0.96774	0.25632	-3.775	0.000181
GAT	-0.39107	0.17892	-2.186	0.02934

Table 3 FAT4 haplotype additive analysis (rs953211, rs1509290, rs13136889), results from backward selection on the following linear regression: BMI3 ~ BMI2 + center + gender + age + AAC + AAT + AGC + GAC + GAT + GGC + ε

	Estimate	Std. Error	t value	Pr(> t)
Intercept	3.5225218	0.9816641	3.588	0.000369
BMI2	0.9276853	0.0190143	48.789	< 2e-16
center	-0.1244909	0.0314629	-3.957	8.83E-05
gender	-0.249386	0.1786981	-1.396	0.163532
age	0.0009298	0.0137588	0.068	0.946152
AACpresent	-0.0634121	0.2412081	-0.263	0.792753
AATpresent	0.6913093	0.824904	0.838	0.402449
AGCpresent	0.1547387	0.2616326	0.591	0.554526
GACpresent	-1.1271817	0.2835424	-3.975	8.18E-05
GATpresent	-0.5652319	0.225268	-2.509	0.012452
GGCpresent	-0.2576433	0.2480019	-1.039	0.29942

Table 4 FAT4 haplotype dominant analysis (rs953211, rs1509290, rs13136889), results from the following linear regression: BMI3 ~ BMI2 + center + gender + age + AAC + AAT + AGC + GAC + GAT + GGC + ε

	Estimate	Std. Error	t value	Pr(> t)
Intercept	3.6015	0.64308	5.6	3.71E-08
BMI2	0.92823	0.01892	49.065	< 2e-16
center	-0.12568	0.03123	-4.025	6.68E-05
gender	-0.25106	0.1768	-1.42	0.15628
GACpresent	-1.11961	0.25072	-4.466	1.01E-05
GATpresent	-0.57589	0.17441	-3.302	0.00104

Table 5 FAT4 haplotype dominant analysis (rs953211, rs1509290, rs13136889), results from backward selection on the following linear regression: BMI3 ~ BMI2 + center + gender + age + AAC + AAT + AGC + GAC + GAT + GGC + ε

	Estimate	Std. Error	t value	Pr(> t)
Intercept	3.621	0.9464	3.826	0.0001485
age	0.0002888	0.01382	0.02089	0.9833
BMI2	0.9271	0.01919	48.32	1.94E-180
genotype_group (rs953211 A/A ; rs1509290 G/G)	0.247	0.2678	0.9223	0.3569
genotype_group (rs953211 G carriers ; rs1509290 A carriers)	-0.6924	0.2286	-3.028	0.002599
genotype_group (rs953211 G carriers ; rs1509290 G/G)	-0.1202	0.3093	-0.3886	0.6977
gender	-0.2954	0.1797	-1.644	0.1009
center	-0.121	0.03158	-3.833	0.0001446

Table 6 FAT4 epistasis analysis (rs953211, rs1509290), results from the following linear regression: BMI3 ~ BMI2 + center + gender + age + genotype_group + ε

In this model, the term “genotype_group” has the following levels: “rs953211 A/A ; rs1509290 A carriers”, “rs953211 A/A ; rs1509290 G/G”, “rs953211 G carriers ; rs1509290 A carriers”, “rs953211 G carriers ; rs1509290 G/G”

	F value	Pr(>F)
Intercept	14.64	0.0001485
age	0.0004366	0.9833
BMI2	2335	1.94E-180
genotype_group	7.362	7.96E-05
gender	2.702	0.1009
center	14.69	0.0001446

Table 7 FAT4 epistasis analysis (rs953211, rs1509290), results from a type III Anova on the following linear regression: BMI3 ~ BMI2 + center + gender + age + genotype_group + ε

In this model, the term “genotype_group” has the following levels: “rs953211 A/A ; rs1509290 A carriers”, “rs953211 A/A ; rs1509290 G/G”, “rs953211 G carriers ; rs1509290 A carriers”, “rs953211 G carriers ; rs1509290 G/G”

PARAM	Estimate	Std. Error	t value	Pr(> t)	95% bootstrap CI estimates
(Intercept)	3.68	0.93	3.97	0.0001	[1.64 , 6.12]
genotype_group	-0.76	0.17	-4.52	0.0000	[-1.1 , -0.43]
BMI2	0.93	0.02	48.39	0.0000	[0.88 , 0.97]
center	-0.12	0.03	-3.85	0.0001	[-0.18 , -0.05]
gender	-0.30	0.18	-1.65	0.1000	[-0.62 , 0.04]
age	0.00	0.01	0.01	0.9942	[-0.03 , 0.03]

Table 8 FAT4 epistasis analysis (rs953211, rs1509290), results from the following linear regression: BMI3 ~ BMI2 + center + gender + age + genotype_group + ε.

Estimates of the different terms have been assessed with bootstrap(with n=1000 resampling). In this model, the term “genotype_group“ has the following levels: “other”, “rs953211-G carriers and rs1509290-A carriers”.

group	median change (BMI3-BMI2)	95% bootstrap CI estimates
Any	0.38	[0.19, 0.53]
rs953211 A/A ; rs1509290 A carriers	0.57	[0.22, 0.93]
rs953211 A/A ; rs1509290 G/G	0.63	[0.3, 0.95]
rs953211 G carriers ; rs1509290 A carriers	0.00	[-0.35, 0.32]
rs953211 G carriers ; rs1509290 G/G	0.68	[0.32, 0.91]

Table 9 Median change and 95% bootstrap confidence interval (from n=1000 replicates) on BMI difference between CID3 and CID2

PARAM	Estimate	Std. Error	t value	Pr(> t)
Intercept	-0.9409	0.4187	-2.247	0.02495
age	0.009702	0.00606	1.601	0.1099
BMI1	0.8855	0.00766	115.6	0
genotype_group (rs953211 A/A ; rs1509290 G/G)	0.1496	0.1197	1.25	0.2119
genotype_group (rs953211 G carriers ; rs1509290 A carriers)	-0.09215	0.1019	-0.9041	0.3663
genotype_group (rs953211 G carriers ; rs1509290 G/G)	0.1945	0.1386	1.403	0.161
gender	0.3858	0.07871	4.901	1.21E-06
center	0.01319	0.014	0.9422	0.3465

Table 10 FAT4 epistasis analysis (rs953211, rs1509290), results from the following linear regression: BMI2 ~ BMI1 + center + gender + age + genotype_group + ε.

In this model, the term “genotype_group” has the following levels: “rs953211 A/A ; rs1509290 A carriers”, “rs953211 A/A ; rs1509290 G/G”, “rs953211 G carriers ; rs1509290 A carriers”, “rs953211 G carriers ; rs1509290 G/G”

PARAM	F value	Pr(>F)
Intercept	5.051	0.02495
age	2.563	0.1099
BMI1	1.34E+04	0
genotype_group	3.157	0.02434
gender	24.02	1.21E-06
center	0.8877	0.3465

Table 11 FAT4 epistasis analysis (rs953211, rs1509290), results from a type III Anova on the following linear regression: BMI2 ~ BMI1 + center + gender + age + genotype_group + ε

In this model, the term “genotype_group” has the following levels: “rs953211 A/A ; rs1509290 A carriers”, “rs953211 A/A ; rs1509290 G/G”, “rs953211 G carriers ; rs1509290 A carriers”, “rs953211 G carriers ; rs1509290 G/G”

PARAM	Estimate	Std. Error	t value	Pr(> t)	95% bootstrap CI estimates
Intercept	-0.83	0.41	-2.01	0.0450	[-1.6 , -0.08]
genotype_group	-0.20	0.07	-2.65	0.0082	[-0.34 , -0.06]
BMI1	0.89	0.01	115.62	0.0000	[0.87 , 0.9]
center	0.01	0.01	0.93	0.3534	[-0.01 , 0.04]
gender	0.38	0.08	4.86	0.0000	[0.23 , 0.56]
age	0.01	0.01	1.61	0.1078	[0 , 0.02]

Table 12 FAT4 epistasis analysis (rs953211, rs1509290), results from the following linear regression: BMI2 ~ BMI1 + center + gender + age + genotype_group + ε. Estimates of the different terms have been assessed with bootstrap(with n=1000 resampling).

In this model, the term “genotype_group” has the following levels: “other”, “rs953211-G carriers and rs1509290-A carriers”.

group	median change (BMI2-BMI1)	95% bootstrap CI estimates
Any	-3.59	[-3.66, -3.5]
rs953211 A/A ; rs1509290 A carriers	-3.47	[-3.7, -3.31]
rs953211 A/A ; rs1509290 G/G	-3.57	[-3.72, -3.39]
rs953211 G carriers ; rs1509290 A carriers	-3.69	[-3.83, -3.58]
rs953211 G carriers ; rs1509290 G/G	-3.48	[-3.64, -3.17]

Table 13 Median change and 95% bootstrap confidence interval (from n=1000 replicates) on BMI difference between CID2 and CID1

phenotype	rs953211 * rs1509290	rs1509289	rs1509290	rs953211
BMI (kg/m2)	7.34E-05	0.000167	0.004939	0.0002769
Fat free mass (kg)	0.008892	0.006536	0.02874	0.1644
Fat mass (kg)	0.01184	0.04789	0.4854	0.00613
Hip (cm)	0.001831	0.002953	0.03591	0.002632
Waist (cm)	2.38E-05	0.006161	0.02719	0.007947

Table 14 Anova p-values assessing significance of a genetic term from the following linear regression: trait_at_CID3 ~ trait_at_CID2 + center + gender + age + genetic_component + ε.

P values smaller than 5% (10%) are shown in bold (italic). Genotypic models were used in these analyses.

phenotype	rs953211 * rs1509290	rs1509289	rs1509290	rs953211
BMI (kg/m2)	0.02755	0.04307	0.04041	0.438
Fat free mass (kg)	0.7917	0.8083	0.5731	0.2791
Fat mass (kg)	0.3087	0.5614	0.6296	0.4783
Hip (cm)	0.008625	0.01202	0.09093	0.08627
Waist (cm)	0.4302	0.5895	0.2597	0.6784

Table 15 Anova p-values assessing significance of a genetic term from the following linear regression: trait_at_CID2 ~ trait_at_CID1 + center + gender + age + genetic_component + ε.

P values smaller than 5% (10%) are shown in bold (italic). Genotypic models were used in these analyses.

genetic marker	percentile dichotomization	for	Odds ratio and 95% CI	Fisher's exact test p value	Accuracy and 95% CI	Accuracy value	P	Sensitivity	Specificity
rs953211 and rs1509290	0.1		3.36 [1.33 ; 8.85]	0.0063	0.65 [0.54 ; 0.75]	0.0042		0.67	0.63
rs953211 and rs1509290	0.25		2.41 [1.37 ; 4.29]	0.0014	0.61 [0.54 ; 0.67]	0.0013		0.59	0.62
rs953211 and rs1509290	0.33		2.41 [1.48 ; 3.95]	0.0002	0.61 [0.55 ; 0.66]	0.0002		0.59	0.62
rs953211 and rs1509290	0.5		1.99 [1.35 ; 2.95]	0.0003	0.58 [0.54 ; 0.63]	0.0003		0.6	0.57
rs1509289	0.1		0.27 [0.1 ; 0.69]	0.0032	0.66 [0.55 ; 0.76]	0.0022		0.69	0.63
rs1509289	0.25		0.5 [0.28 ; 0.88]	0.0117	0.59 [0.52 ; 0.65]	0.0069		0.59	0.58
rs1509289	0.33		0.46 [0.28 ; 0.75]	0.0012	0.59 [0.54 ; 0.65]	0.0007		0.61	0.58
rs1509289	0.5		0.53 [0.36 ; 0.78]	0.0009	0.58 [0.53 ; 0.62]	0.0006		0.63	0.52
rs1509290	0.1		0.37 [0.13 ; 1.02]	0.0404	0.6 [0.5 ; 0.71]	0.037		0.4	0.8
rs1509290	0.25		0.43 [0.23 ; 0.82]	0.0076	0.59 [0.52 ; 0.65]	0.0097		0.37	0.8
rs1509290	0.33		0.5 [0.29 ; 0.86]	0.0104	0.57 [0.51 ; 0.63]	0.0119		0.36	0.78
rs1509290	0.5		0.59 [0.39 ; 0.9]	0.0123	0.56 [0.51 ; 0.6]	0.0106		0.39	0.73
rs953211	0.1		2.87 [1.12 ; 7.61]	0.0193	0.63 [0.52 ; 0.73]	0.0135		0.53	0.72
rs953211	0.25		2.44 [1.36 ; 4.44]	0.0016	0.6 [0.54 ; 0.67]	0.0014		0.48	0.73
rs953211	0.33		2.31 [1.4 ; 3.85]	0.0006	0.6 [0.54 ; 0.65]	0.0005		0.48	0.72
rs953211	0.5		1.66 [1.12 ; 2.47]	0.0097	0.56 [0.51 ; 0.61]	0.0064		0.46	0.66

Table 16 Performance of genetic markers at predicting dichotomized percentage of BMI change during weight maintenance

Levels of the genetic markers are encoded as follows:

- rs953211 and rs1509290 : "other" vs "rs953211 G carriers ; rs1509290 A carriers)"
- rs1509289: "G/G" vs "A carriers"
- rs1509290: "G/G" vs "A carriers"
- rs953211: "A/A" vs "G carriers"

genetic marker	percentile dichotomization	Odds ratio and 95% CI	Fisher's exact test p value	Accuracy and 95% CI	Accuracy value	P	Sensitivity	Specificity
rs953211 and rs1509290	0.1	1.6 [0.75 ; 3.45]	0.2149	0.56 [0.47 ; 0.65]	0.1243		0.52	0.59
rs953211 and rs1509290	0.25	1.61 [1.01 ; 2.58]	0.0433	0.56 [0.5 ; 0.62]	0.0189		0.55	0.57
rs953211 and rs1509290	0.33	1.68 [1.12 ; 2.51]	0.0111	0.56 [0.52 ; 0.61]	0.0048		0.55	0.58
rs953211 and rs1509290	0.5	1.47 [1.06 ; 2.04]	0.0173	0.55 [0.51 ; 0.59]	0.0096		0.56	0.54
rs1509289	0.1	0.51 [0.24 ; 1.1]	0.0764	0.58 [0.49 ; 0.67]	0.0457		0.6	0.56
rs1509289	0.25	0.64 [0.4 ; 1.02]	0.0562	0.55 [0.5 ; 0.61]	0.0322		0.6	0.51
rs1509289	0.33	0.61 [0.41 ; 0.92]	0.0146	0.56 [0.51 ; 0.61]	0.0084		0.59	0.53
rs1509289	0.5	0.7 [0.51 ; 0.98]	0.0315	0.54 [0.5 ; 0.58]	0.0178		0.59	0.49
rs1509290	0.1	0.43 [0.18 ; 0.99]	0.0353	0.59 [0.5 ; 0.68]	0.0309		0.4	0.78
rs1509290	0.25	0.55 [0.33 ; 0.92]	0.0208	0.56 [0.51 ; 0.62]	0.0143		0.37	0.75
rs1509290	0.33	0.6 [0.38 ; 0.93]	0.0203	0.55 [0.51 ; 0.6]	0.014		0.37	0.74
rs1509290	0.5	0.8 [0.57 ; 1.13]	0.2048	0.52 [0.48 ; 0.56]	0.1249		0.35	0.7
rs953211	0.1	1.26 [0.57 ; 2.83]	0.5769	0.53 [0.44 ; 0.62]	0.3288		0.37	0.69
rs953211	0.25	1.19 [0.73 ; 1.93]	0.4862	0.52 [0.46 ; 0.58]	0.269		0.38	0.66
rs953211	0.33	1.34 [0.88 ; 2.05]	0.1562	0.53 [0.49 ; 0.58]	0.0941		0.4	0.67
rs953211	0.5	1.31 [0.94 ; 1.82]	0.105	0.53 [0.49 ; 0.57]	0.0611		0.42	0.64

Table 17 Performance of genetic markers at predicting dichotomized percentage of BMI change during weight loss

Levels of the genetic markers are encoded as follows:

- rs953211 and rs1509290 : "other" vs "rs953211 G carriers ; rs1509290 A carriers)"
- rs1509289: "G/G" vs "A carriers"
- rs1509290: "G/G" vs "A carriers"
- rs953211: "A/A" vs "G carriers"

best combination of SNPs	percentile for dichotomization	classification accuracy	prediction accuracy	cross validation consistency (max=5)
rs1509289	0.1	66.77	63.92	4
rs953211	0.25	60.25	60.42	3
rs1509289	0.33	60.59	55.05	2
rs1509289	0.5	58.01	57.11	4

Table 18 Genetic markers identified with multi-dimensional reduction analyses for predicting dichotomized percentage of BMI change during weight maintenance

best combination of SNPs	percentile for dichotomization	classification accuracy	prediction accuracy	cross validation consistency (max=5)
rs1509290	0.1	64.67	62.69	4
rs1509290	0.25	56.99	54.02	2
rs2710559, rs1509290	0.33	58.84	54.51	3
rs1509289	0.5	55.18	50.82	4

Table 19 Genetic markers identified with multi-dimensional reduction analyses for predicting dichotomized percentage of BMI change during weight loss

genotype	BMI (kg/m2)	fat mass (kg)	fat free mass (kg)	waist (cm)	hip (cm)
Overall population (not knowing the genotypes)	0.38 [0.20 , 0.51]	-0.30 [-0.61 , 0.20]	1.16 [0.84 , 1.3]	0.70 [0.15 , 1.35]	0.150 [-0.25 , 0.75]
rs953211 A/A ; rs1509290 A carriers	0.57 [0.24 , 0.90]	0.45 [-1.10 , 1.10]	0.84 [0.20 , 1.3]	1.50 [0.00 , 3.50]	0.550 [-0.45 , 1.50]
rs953211 A/A ; rs1509290 G/G	0.63 [0.25 , 0.95]	0.10 [-0.40 , 1.40]	1.31 [0.98 , 1.8]	1.38 [0.25 , 2.25]	1.425 [0.50 , 2.33]
rs953211 G carriers ; rs1509290 A carriers	0.00 [-0.35 , 0.32]	-0.66 [-1.60 , -0.08]	0.96 [0.52 , 1.4]	-0.75 [-1.50 , 0.98]	-0.450 [-1.50 , 0.30]
rs953211 G carriers ; rs1509290 G/G	0.68 [0.32 , 0.91]	0.15 [-1.85 , 1.72]	1.45 [0.83 , 2.0]	1.80 [0.10 , 4.90]	-0.087 [-1.00 , 1.50]

Table 20 Median change during weight maintenance and 95% bootstrap confidence interval (from n=1000 replicates) on various weight-related phenotypes stratified by FAT4 genotypes

genotype	BMI (kg/m2)	fat mass (kg)	fat free mass (kg)	waist (cm)	hip (cm)
Overall population (not knowing the genotypes)	-3.6 [-3.7 , -3.5]	-7.3 [-7.5 , -7.0]	-3.0 [-3.3 , -2.7]	-9.1 [-9.6 , -8.8]	-6.9 [-7.0 , -6.5]
rs953211 A/A ; rs1509290 A carriers	-3.5 [-3.7 , -3.3]	-7.0 [-7.7 , -6.5]	-3.1 [-4.0 , -2.3]	-9.3 [-11.5 , -8.3]	-6.0 [-6.7 , -5.3]
rs953211 A/A ; rs1509290 G/G	-3.6 [-3.7 , -3.4]	-7.2 [-7.7 , -6.5]	-3.2 [-3.8 , -2.6]	-9.1 [-9.9 , -8.0]	-6.5 [-7.2 , -6.1]
rs953211 G carriers ; rs1509290 A carriers	-3.7 [-3.8 , -3.6]	-7.3 [-7.9 , -6.9]	-2.8 [-3.3 , -2.5]	-9.2 [-10.0 , -8.5]	-7.0 [-7.8 , -6.9]
rs953211 G carriers ; rs1509290 G/G	-3.5 [-3.6 , -3.2]	-7.6 [-8.4 , -6.7]	-2.9 [-3.7 , -2.2]	-9.0 [-10.0 , -8.0]	-6.8 [-7.8 , -5.8]

Table 21 Median change during weight loss and 95% bootstrap confidence interval (from n=1000 replicates) on various weight-related phenotypes stratified by FAT4 genotypes

CLAIMS

1. A method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions to a subject and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the nucleotide of the subject at one or more polymorphic positions selected from:

(i) position 101 of SEQ ID NO:1 (rs953211)

(ii) position 101 of SEQ ID NO:2 (rs1509290)

(iii) position 101 of SEQ ID NO:3 (rs1509289)

and/or detecting one or more biomarkers genetically linked to said polymorphic positions.

2. The method according to claim 1 wherein the one or more biomarkers are within the *FAT4* gene or a regulatory element thereof.

3. A method according to claim 1 or 2 wherein the method comprises determining the presence of A or G at position 101 of SEQ ID NO:1, and/or A or G at position 101 of SEQ ID NO:2, and/or A or G at position 101 of SEQ ID NO:3.

4. A method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions according to claim 1 or 2 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:1 and wherein the weight loss is represented by a change in body mass index (BMI), fat mass, hip circumference or waist circumference.

5. A method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions according to claim 1 or 2 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:2 and wherein the weight loss is represented by a change in BMI, fat free mass, hip circumference or waist circumference.

6. A method for assessing the predisposition of a subject to maintenance of a weight loss by applying one or more dietary interventions according to claim 1 or 2 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:3 and wherein the weight loss is represented by a change in BMI, fat mass, fat free mass, hip circumference or waist
5 circumference.

7. A method for assessing the predisposition of a subject to maintenance of weight loss by applying one or more dietary interventions according to claim 1 or 2 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:1 and the presence of A or
10 G at position 101 of SEQ ID NO:2.

8. A method according to claim 7 wherein the weight loss is represented by BMI, fat mass, fat free mass, hip circumference or waist circumference.

15 9. A method according to claim 7 or 8 which comprises determining the presence of a genotype comprising a G at position 101 of SEQ ID NO:1 and an A at position 101 of SEQ ID NO:2 and wherein the presence of said genotype indicates that a subject has a predisposition to maintain weight loss following a dietary intervention.

20 10. A method according to any one of claims 7 to 9 which comprises determining the presence of a genotype selected from:

(i) G/G (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

(ii) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position
25 101 of SEQ ID NO:2;

(iii) G/A (heterozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2; and

(iv) G/A (heterozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

30 and wherein the presence of said genotype indicates that a subject has a predisposition to maintain weight loss following a dietary intervention.

11. A method according to claim 7 or 8 which comprises determining the presence of a genotype selected from:

(i) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

5 (ii) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/G (heterozygous) at position 101 of SEQ ID NO:2;

(iii) A/A (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

10 (iv) A/G (heterozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2; and

(v) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

and wherein the presence of said genotype indicates that a subject has a predisposition not to maintain weight loss following a dietary intervention.

15

12. A method according to any one of claims 8 to 11 wherein the weight loss is represented by a change in BMI.

13. A method for assessing the predisposition of a subject to weight loss attainable by
20 applying on or more dietary interventions according to claim 1 or 2 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:1 and wherein the weight loss is represented by a change in hip circumference.

14. A method for assessing the predisposition of a subject to weight loss attainable by
25 applying on or more dietary interventions according to claim 1 or 2 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:2 and wherein the weight loss is represented by a change in BMI or hip circumference.

15. A method for assessing the predisposition of a subject to weight loss attainable by
30 applying one or more dietary interventions according to claim 1 which method comprises determining the presence of A or G at position 101 of SEQ ID NO:3 and wherein the weight loss is represented by a change in BMI or hip circumference.

16. A method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions according to claim 1 or 2 which method comprises detecting the presence of A or G at position 101 of SEQ ID NO:1 and the presence of A or G at position 101 of SEQ ID NO:2.

5

17. A method according to claim 16 wherein the weight loss is represented by a change in BMI or a change in hip circumference.

18. A method according to claim 16 or 17 which comprises determining the presence of a
10 genotype comprising a G at position 101 of SEQ ID NO:1 and an A at position 101 of SEQ ID NO:2 and wherein the presence of said genotype indicates that a subject has a predisposition to lose weight following a dietary intervention.

19. A method according to any one of claims 16 to 18 which comprises determining the
15 presence of a genotype selected from:

(i) G/G (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

(ii) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2;

20 (iii) G/A (heterozygous) at position 101 of SEQ ID NO:1 and G/A (heterozygous) at position 101 of SEQ ID NO:2; and

(iv) G/A (heterozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

25 and wherein the presence of said genotype indicates that a subject has a predisposition to lose weight following a dietary intervention.

20. A method according to claim 16 or 17 which comprises determining the presence of a genotype selected from:

30 (i) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/A (homozygous) at position 101 of SEQ ID NO:2;

(ii) A/A (homozygous) at position 101 of SEQ ID NO:1 and A/G (heterozygous) at position 101 of SEQ ID NO:2;

(iii) A/A (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

(iv) A/G (heterozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2; and

5 (v) G/G (homozygous) at position 101 of SEQ ID NO:1 and G/G (homozygous) at position 101 of SEQ ID NO:2;

and wherein the presence of said genotype indicates that a subject has a predisposition not to lose weight following a dietary intervention.

10 21. A method for assessing the predisposition of a subject to weight loss attainable by applying one or more dietary interventions and/or the predisposition of a subject to maintenance of weight loss following one or more dietary interventions which method comprises determining the sequence of FAT4 gene or a regulatory element thereof.

15 22. The method according to any one of claims 1 to 21, wherein the dietary intervention is a low calorie diet.

23. The method according to claim 22, wherein the low calorie diet comprises a calorie intake of about 600 to about 1200 kcal/day.

20

24. The method according to claim 22 or 23, wherein the low calorie diet comprises administration of at least one diet product.

25 25. The method of any one of claims 22 to 24, wherein the low calorie diet has a duration of 6 to 12 weeks.

26. The method according to any one of claims 1 to 25, wherein the method further comprises combining determining the nucleotide at the one or more polymorphic positions, the biomarker and/or the FAT4 sequence with one or more anthropometric measures and/or
30 lifestyle characteristics of the subject.

27. The method according to claim 26, wherein the anthropometric measure is selected from the group consisting of gender, weight, height, age and body mass index, and wherein the lifestyle characteristic is whether the subject is a smoker or a non-smoker.

- 5 28. A method for optimizing one or more dietary interventions for a subject comprising:
assessing the predisposition of a subject to weight loss attainable by one or more
dietary interventions and/or the predisposition of a subject to maintenance of weight
loss following one or more dietary interventions according to the method of any one of
claims 1 to 27; and
10 applying one or more dietary interventions to the subject.

29. A method for selecting a modification of lifestyle of a subject, the method comprising:

- (a) performing a method as described in any of claims 1 to 27; and
(b) selecting a suitable modification in lifestyle based upon the predicted weight
15 loss attainable and/or predicted maintenance of weight loss from (a).

30. The method according to claim 29, wherein the modification of lifestyle in the subject comprises a dietary intervention.

31. The method according to claim 30, wherein the dietary intervention is as defined in any one of claims 22 to 25.

- 20 32. A diet product for use as part of a low calorie diet for weight loss, wherein the diet product is administered to a subject that is predicted to attain weight loss and/or weight maintenance by the method of any one of claims 1 to 27.

33. A diet product for use in treating obesity or an obesity-related disorder, wherein the diet product is administered to a subject that is predicted to attain weight maintenance and/or
25 weight loss by the method of any one of claims 1 to 27.

34. An allele-specific oligonucleotide probe capable of detecting a polymorphic position selected from:

- (i) position 101 of SEQ ID NO:1;
(ii) position 101 of SEQ ID NO:2; or

(iii) position 101 of SEQ ID NO:3.

35. An allele-specific oligonucleotide primer capable of detecting a polymorphic position selected from:

- 5 (i) position 101 of SEQ ID NO:1;
 (ii) position 101 of SEQ ID NO:2; or
 (iii) position 101 of SEQ ID NO:3.

36. A diagnostic kit comprising an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe as defined in claim 34 or 35.

10

37. A diagnostic kit comprising (i) an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of G at position 101 of SEQ ID NO:1 and (ii) an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of A at position 101 of SEQ ID NO:2.

15

38. A diagnostic kit according to claim 37 further comprising an allele-specific oligonucleotide primer and/or an allele-specific oligonucleotide probe capable of detecting the presence of A at position 101 of SEQ ID NO:3.

20

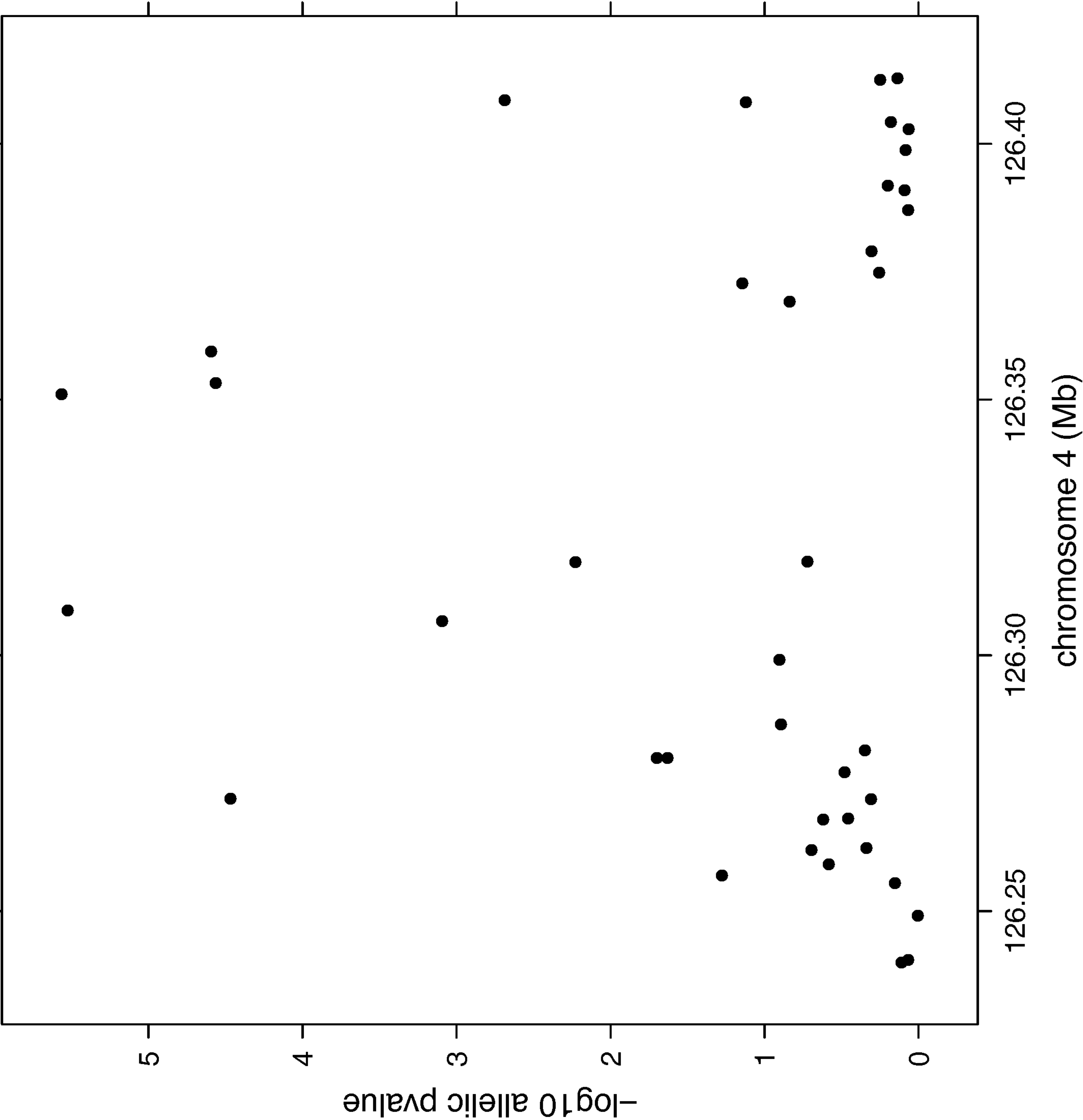


Figure 1

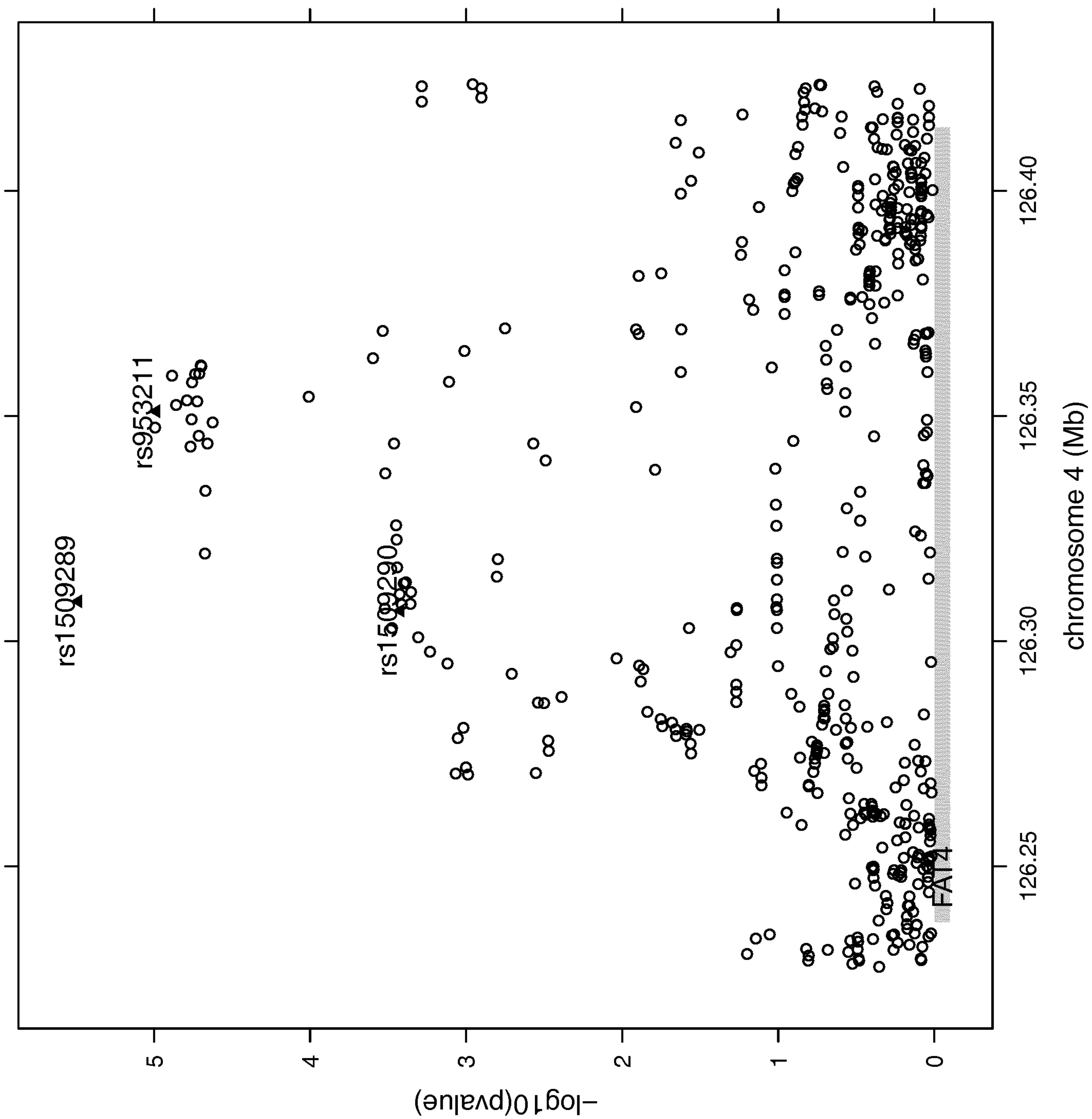


Figure 2

Figure 3**SEQ ID NO: 1 - rs953211 (chr4 126351049)**

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SEQ ID NO: 2 - rs1509290 (chr4 126306772)

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SEQ ID NO: 3 - rs1509289 (chr4 126308835)

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Figure 4 (SEQ ID NO: 4)

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 [Source:HGNC Symbol;Acc:23109]|HGNC Symbol|protein_coding

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Figure 5A (SEQ ID NO: 5)

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Figure 5B (SEQ ID NO: 6)

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 ATTTTACTCTAAACATCTACAGTGTGCAGATCAGTGAAGGGGTCCCAATAGGAACTCAT
 GTGACCTTTGTGAGTGCCTTTGACTCAGACTCCATCCCCAGCTGGAGCAGGTTTTCTTAC
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 GTTGATTCAGGGACCCCTCAGCTACAGGTAGTGCCTCTTTATTAGTCACCTTGGAAAGAT
 ATAAATGATAACGGGGCCCATGCTGACTGTGAGTGAAGGAGAAGTCATGGAAAACAAACGG
 CCAGGCACTTTGGTGATGACCCTTCAGTCCACTGACCCTGATCTCCCTCCAAATCAAGGT
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 GCTGGAGTTCTGAGCACAACCAGAGAGATTGACAGAGAGCAGATTGCAGACTTCTATCTG
 TCTGTGGTTACCAAGGATTCTGGTGTTCTCAAATGTCTTCCACAGGAACTGTGCATATC
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 TATTATGGTAACCTTGTTTCCCGGTGGGATTTTAGGCTCTGTGAAGCCACAGGATCCAGAT
 GTGTTAGACAGCTTCCACTGCTCCCTTACTTCAGGAGTTACCAGCCTCTTCAGTATTCCA
 GGGGGTACTTGTGATCTGAATTTCCAGCCAAGGTCCACAGATGGCACGTTTGATCTGACT
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 GGATTTTCCAATGCCACAGTGGATAACAGCATCTTACTTCGTCTCGGCGTACCAACAGTA
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 GGCTTAGGGACTGCTGTGCAACTGTACAGTGCATATGAAGAGAACAATAGAACGTTTCTT
 TTGGCAGCTGTGAAGCGAAATCATAATCAGTATGTGAATCCCAGTGGCGTAGCCACCTTC
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 GTGAGCTCCGTATTAAGAAAGCCGTGAGAGTCTTCCAGTCATCATCGTGGCAAATGAACCT
 CTGCAGCCTTTCTTATGCAAGTGTCTGCCAGGATATGCGGGTAGCTGGTGTGAAATAGAT
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 GAGTGCCTGCAGAGTCTTGCAAGAATGGTGCCATCTGCCAGAATTTTCCAGGAAGCTTC
 AACTGTGTTTGCAAACTGGATACACAGGTGTCTTTGGAAAACACTGCGAGTTGAACAGT
 TATGGATTTGAGGAGTTATCATACATGGAATTTCCAAGCTTGGACCCCAATAACAACCTAT
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 TCTTATAATTTAGGCAGTGGTACATATAAGCTCACCACCATGAAGAAGGTGTCAGATGGA
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 TGGACTCTTGATGTTTCAAGCAATAGAGTTACAGTTGGAGGTATCAGATCTCTAGAACCA
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 CAGTATGGCGATTTTATTTCTTACTGTTTTAAAGAAAAAAATGCAAAAAAGTATGCTTC
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 CCTTTTGGTGTGAACAGTCTGGAAGTAAAATTTAGGACCAGAAGCGAGAATGGCGTTTTA
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 TTTACATCCGATGCAGGAATTGCTGGGAAAGTGGAGAGAAATATTCCTGAAGTATATGTT
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 GTGCTTACTATATCACTTGGAGGAATTCCACCCAATCAAGCACATCGAGATGCCCCAAACA
 GCAGGTTTTGATGGCTGCATTGCTTCTATGTGGTATGGTGGAGAAAGTCTTCCTTTCAGC
 GGGAAAGCATAGCTTGGCCTCCATCTCAAAAACAGATCCCTCAGTGAAGATTGGCTGCCGT
 GGCCCGAACATTTGTGCCAGCAACCCCTGCTGGGGTGATTTGCTGTGCATTAATCAGTGG
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 AGCTGTGAGCCAGGCCTGCACTCCGGCTTCACCTGTAGCTGCCCAGACTCGCACACGGGA
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 GTCCTTAGCCTGATCCTGTGTAACCAGTGCAGGGGGAAGAAGGCCAAAAATCCCAAAGAG
 GAGAAGAAACCGAAGGAGAAGAAGAAAAAGGGAAGTGAGAACGTTGCTTTTGATGACCCT
 GACAATATCCCTCCCTATGGGGATGACATGACTGTGAGGAAGCAGCCTGAAGGGAACCCA
 AAACCAGATATCATTGAAAGGGAAAACCCCTACCTTATCTATGATGAAACTGATATTCCT
 CACAACCTCAGAAACCATCCCCAGCGCCCTTTGGCATCTCCAGAGCAGGAGATAGAGCAC
 TATGACATTGACAACGCCAGCAGCATCGCCCTTCGGATGCAGACATCATTCAACACTAC
 AAGCAGTTCGCGAGCCACACACCAAAATTTTCAATCCAGAGGCACAGTCCCCTAGGCTTT
 GCAAGGCAATCCCCCATGCCCTTAGGAGCAAGCAGTTTGACTTACCAGCCTTCATATGGT
 CAAGGTTTGAGAACCAGCTCCCTAAGCCACTCAGCATGCCCAACTCCCAACCCTCTGTCT
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 GCATCACATGGTTCTAGACCAGGGAGTCGCCTAAAGCAGCCGATTGGGCAGATTCCACTG
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 CCTAGAAACCCAAGTATCTGCAGTGCAGACCATGGGAGGTCTTCTTCAGAGGAGGACTGC
 AGAAGGCCACTGTCTAGAACAAGGAATCCAGCGGATGGCATTCCAGCTCCAGAATCCTCT
 TCTGATAGTGACTCCCATGAATCTTTCACTTGCTCAGAAATGGAATATGACAGGGAGAAG
 CCAATGGTATATACTTCCAGAATGCCCAAATTATCTCAAGTCAATGAATCTGATGCAGAT
 GATGAAGATAATTATGGAGCCAGACTGAAGCCTCGAAGGTACCACGGTCGCAGGGCCGAG
 GGAGGACCTGTGGGCACCCAGGCAGCAGCACCAGGCACTGCTGACAACACACTGCCCATG
 AAGCTAGGGCAGCAAGCAGGGACTTTCAACTGGGACAACCTTTTGAAGTGGGGCCCTGGC
 TTTGGCCATTATGTAGATGTTTTTAAAGATTTGGCATCTCTTCCAGAAAAAGCAGCAGCA
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 TATGTGTGA

Figure 5C (SEQ ID NO:7)

>ENSG00000196159 || ENST00000509444 | FAT4

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 GTAAATAGAAGTTACGATATTAATACTATATGACATATCCCTATTTCTGCTTTCTGCTTT
 AGTTATAGGATCATCGGTGGAAACTCTCAGTTACGATCAACCCATCGACAGGACAAATC
 ATCACCAGCGCATTGTTAGATAGGGAAACAAAAGATAATTATACTTTGGTAGTGGTCTGC
 AGTGATGCGGGATCCCCAGAGCCTCTTTCCAGTTCCACCAGTGTGCTTGTCACTGTGACT
 GATGTCAATGACAATCCACCAAGATTTCCAGCATCACCCATATGTCACTCACATCCCATCT
 CCTACTCTTCCAGGTTCTTTGTCTTTGCGGTTACAGTCACAGATGCTGATATTGGACCA
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Figure 6A (SEQ ID NO: 8)

>ENSG00000196159|ENSP00000377862|ENST00000394329|FAT4

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 VRVLVRDLNDNAPVFPDPSIVVTFKEDSSSGRQVILDTATDSDIGSNGVDHRSYRIIRGN
 EAGRFRLDITLNPSGEGAFHLVSKGGLDREVTPQYQLLVEVEDKGEPKRRGYLQVNVTV
 QDINDNPPVFGSSHYQAGVPEDAVVGSSVLQVAAADADEGTNADIRYRLQDEGTPFQMDP
 ETGLITVREPLDFEARQYSLTVQAMDRGVPSLTGRAEALIQLLDVNDNDPVVKFRYFPA
 TSTRYASVDENAQVGTVVALLTVTADADSPAANGNISVQILGGNEQRHFEVQSSKVPNLSLI
 KVASALDRERIPSYNLTVSVSDNYGAPPGAQAVQARSSVASLVIFVNDINDHPPVFSQQVY
 RVNLSEEAPPGSYVSGISATDGDGSLNANLRYSTIVSGNGLGWFHISEHSLVTTGSSGGL
 DRELASQIVLNIARDQGVHPKVSQAQLVVTLLDVNDEKPVFSQPEGYDVSVENAPTGT
 ELLMLRATDGDLDGNGTVRFSLQEAETDRRSFRLDPVSGRLSTISSLDREEQAFYSLLVL
 ATDLGSPQSSMARINVSLLDINDNSPVFYPVQYFAHIKENEPGGSYITTVSATDPDLGT
 NGTVKYSISAGDRSRFQVNAQSGVISTRMALDREEKTAYQLQIVATDGGNLQSPNQAIIVT
 ITVLDTQDNPPVFSQVAYSFVVFENVALGYHVGSVSASTMDLNSNISYLITTGDQKGMFA
 INQVTGQLTTANVIDREEQSFYQLKVVASGGTVTGD TMVNITVKDLNDNSPHFLQAIESV
 NVVENWQAGHSIFQAKAVDPDEGVNGMVLVSLKQNPKNLFAINEKNGTISLLGPLDVHAG
 SYQIEILASDMGVPQLSSSVILTVYVHDVNDNSPVFDQLSYEVTLSSEPVNSRFFKVQA
 SDKDSGANGEIAYTIAEGNTGDAFGIFPDGQLYIKSELRELQDRYVLMVVASDRAVEPL
 SATVNVTVILEDVNDNRPLFNSTNYTFYFEEEQRAGSFVGKVS AVDKDFGPNGEVRYSE
 MVQPDFELHAISGEITNTHQFDRESLMRRRGTA VFSFTVIATDQGIPQPLKDQATVHVYM
 KDINDNAPKFLKDFYQATISESAANLTQVLRVSASDVDEGNGLIHYSIIKGNEERQFAI
 DSTSGQVTLIGKLDYEATPAYSLVIQAVDSGTIPLNSTCTLNIDILDENDNTPSFPKSTL
 FVDVLENMRIGELVSSVTATDSDSGDNADLYYSITGTNNHGTFSISPNTGSIFLAKKLDF
 ETQSLYKLNITAKDQGRPPRSSTMSVVIHVRDFDNDNPPSFPPGDIFKSIVENIPIGTSVI
 SVTAHDPDADINGQLSYTIIQQMPRGNHFTIDEVKGTIYTNAEIDREFANLFELTVKAND
 QAVPIETRRYALKNVITILVTDLNDNVPMFISQNALAADPSAVIGSVLTTIMAADPDEGAN
 GEIEYEIINGD TDTFIVDRYSGDLRVASALVPSQLIYNLIVSATDLGPERRKSTTELTII
 LQGLDGPVFTQPKYITILKEGEPITGNVISIEAASPRGSEAPVEYYIVSVRCEEKTVGRL
 FTIGRHTGIIQTAAILDREQGACLYLVDVYAIEKSTAFPRTQRAEVEITLQDINDNPPVF
 PTDMLDLTVEENIGDGSKIMQLTAMDADEGANALVTYTTIISGADDSFRIDPESGDLIATR
 RLDRERRSKYSLLVRADDGLQSSDMRINITVSDVNDHTPKFSRPVYSFDIPEDTIPGSLV
 AAILATDDDSGVNGEITYIVNEDDEDGIFFLN PITGVFNLTRLLDYE VQYYILTVRAED
 GGGQFTTIRVYFNILDVNDNPPIFSLNSYSTSLMENLPVGSTVLVFNVT DADDGINSQLT
 YSIASGDSLQFTVDKNGVLKVLKALDRESQS FYNLVVQVHDL PQIPASRFTSTAQVSII
 LLDVNDNPPTFLSPKLTYPENTPIDTVVFKAQATDPDSGPN SYIEYTL LNPLGNKFSIG
 TIDGEVRLTGELDREEVSNYTLTVVATDKGQPSLSSSTE VVMVLDINDNNPIFAQALYK
 VEINENTLTGTDIIQVFAADGDEGTNGQVRYGIVNGNTNQEFRI DSVTGAITVAKPLDRE
 KTPTYHLTVQATDRGSTPRTDTSTVSIVLLDINDFVPVFELSPYSVNV PENLGTLPRTIL
 QVVARDDDRGSNSKLSYVLFGGNEDNAFTLSASGELGVTQSLDRET KERFVLMITATDSG
 SPALTGTGTINVIVDDVNDNVPTFASKAYFTTIPEDAPTGT DVLLVNASDADASKNAVIR
 IIGGNSQFTINPSTGQIITSALLDRETKDNYTLVVVCSDAGSPEPLSSSTS VLVTVTDVN
 DNPPRFQHHPYVTHIPSP TLPGSFVFAVTVDADIGPNSELHYSLSGRNSEKFHIDPLRG
 AIMAAGPLNGASEVTFSVHVKDGGSF PKTDSTTVTVRFVNKADFPKVRAKEQTFMF PENQ
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 ARDGGFPFPSSYEKLDITVLDVNDNAPIFKEDPFISEILENLSPRKILTVSAMDKDSGPN
 GQLDYEIVNGNMENSFSINHATGEIRSVRPLDREKVSHYVLT IKSSDKGSPSQSTSVKVM
 INILDENDNAPRFSQIFSAHVPENSPLGYTVTRVTTSD EDIGINAISRYSIMDASLPFTI
 NPSTGDIVISRPLNREDTD RYRIRVSAHDSGWTVSTDVTIFVTDINDNAPRFSRTSY YLD
 CPELTEIGSKVTQVFATDPDEGSNGQVFYFIKSQSEYFRINATTGEIFNKQILKYQNV TG
 FSNVNINRHSFIVTSSDRGKPSL ISETTVTINIVDSNDNAPQFLKSKYFTPVTKNVKVGT
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 AKDKGNPPLSSQATVHITVTEENYHTPEFSQSHMSATIPESH SIGSIVRTVSARDRDAAM
 NGLIKYSISSGNEEGIFAINSSTGILTLAKALDYELCQKHEMTISAIDGGWVARTGYCSV
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 KLYYFEISEAAPKGTIVGEVFASDRDLGTDGEVHYLIFGNSRKKGFQINKKTGQIYVSGI
 LDREKEERVSLKVLAKNFGSIRGADIDEVTNVTVLDANDPPIFTLNIYSVQISEGVPIG
 THVTFVSAFDSDSIPSWSRFSYFIGSGNENGAFSINPQTGQITVTAELDRETLPIYNLSV
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QGPFTYYLLSTGPATSYFSLSTAGVLSTTREIDREQIADFYL SVVTKDSGVPQMSSTGTV
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 IPGGTCDLNSQPRSTDGTFDLTVLSNDGVHSTVTSNIRVFFAGFSNATVDNSILLRLGVP
 TVKDFLTNHYLHFLRIASSQLTGLGTAVQLYSAYEENRTFLLAAVKRNHNQYVNPSPGVA
 TFFESIKEILLRQSGVKVESVDHDSVHGPCQNGGSCLRRLAVSSVLKSRESLPVIIVAN
 EPLQPFLCKCLPGYAGSWCEIDIDECLPSPCHSGGTCHNLVGGFSCSCPDGFTGRACERD
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 YCHCPFGVFGKHCELSYGFEELSYMEFPSLDPNNNYIYVKFATIKSHALLLYNYDNQTG
 DRAEFLALEIAEERLRFSYNLGSPTYKLTTMKKVSDGHFHTVIARRAGMAASLTVDSCE
 NQEPGYCTVSNVAVSDDWTLDVQPNRVTVGIRSLLEPILQRRGHVESHDVFGCIMEFAVN
 GRPLEPSQALAAQGILDQCPRLEGACTRSPCQHGGTCMDYWSWQQCHCKEGLTGKYCEKS
 VTPDTALSLEGKGRLDYHMSQNEKREYLLRQSLRGAMLEPFGVNSLEVKFRTRESENGVLI
 HIQESSNYTTVKIKNGKVYFTSDAGIAGKVERNIPEVYVADGHWHTFLIGKNGTATVLSV
 DRIYNRDIHPTQDFGGLDVLTLISLGGIPPNQAHQDAQTAGFDGCIASMWYGGESLPFSG
 KHSLASISKTDPSVKIGCRGNICASNPCWGDLLCINQWYAYRCVPPGDCASHPCQNGGS
 CEPGLHSGFTCSCPDSHTGRTCEMVVACLGVLCPOGKVCKAGSPAGHVCVLSQGPEEISL
 PLWAVPAIVGSCATVLAALLVLSLILCNQCRGKKAKNPKEKKPKKEKKKKGSENVAFDDPD
 NIPPYGDDMTVRKQPEGNPKPDIIERENPYLIYDETDIPHNSSETIPSAPLASPEQEIEHY
 DIDNASSIAPSDADIIQHYKQFRSHTPKFSIQRHSPLGFARQSPMPLGASSLTYQPSYGG
 GLRTSSLSHSACPTPNPLSRHSPAPFSKSSTFYRNSPARELHLPIRDGNTLEMHGDTCP
 GIFNYATRLGRRSKSPQAMASHGSRPGSRLKQPIGQIPLLESSPPVGLSIEEVERLNTPRP
 RNPSICSADHGRSSSEEDCRRPLSRTRNPADGIPAPESSSDSDSHESFTCSEMEYDREKP
 MVYTSRMPKLSQVNESDADDEDNYGARLKPRRYHGRRAEGGPVGTQAAAPGTADNTLPMK
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 V*

Figure 6B (SEQ ID NO: 9)

>ENSG00000196159|ENSP00000335169|ENST00000335110|FAT4

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 SDMRINITVSDVNDHTPKFSRPVYSFDIPEDTIPGSLVAAILATDDDSGVNGEITYIVNE
 DDEGDIFFLNPITGVFNLTRLLDYEYQYYILTVRAEDGGGQFTTIRVYFNILDVNDNPP
 IFSLNSYSTSLMENLPVGSTVLVFNVTADDDGINSQLTYSIASGDSLQFTVDKNGVLKV
 LKALDRESQSFYNLVVQVHDLQPASPASFTSTAQVSIILLDVNDNPPTFLSPKLTYPEN
 TPIDTVVFKAQATDPDSGPNSYIEYTLNPLGNKFSIGTIDGEVRLTGELDREEVSNYTL
 TVVATDKGQPSLSSSTEVVVMVLDINDNNPIFAQALYKVEINENTLTGTDIIQVFAADGD
 EGTNGQVRYGIVNGNTNQEFRIDSVTGAITVAKPLDREKTPTYHLTVQATDRGSTPRTDT
 STVSIVLLDINDFVPVFEELSPYSVNVNENLGLTPRTILQVVARDDDRGSNSKLSYVLFGG
 NEDNAFTLSASGELGVTQSLDRETKEFVLMITATDSGSPALTGTGTINVIVDDVNDNVP
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 LDRETKDNYTLVVVCS DAGSPEPLSSSTS VLVTVDVNDNPPRFQHHYPVTHIPSPTLPG
 SFVFAVTVDADIGPNSELHYSLSGRNSEKFHIDPLRGAIMAAGPLNGASEVTFSVHVKD
 GGSFPKTDSTTVTRFVNKADFVKVRAKEQTFMFENQPVSSLVTTITGSSLRGEPMSYY
 IASGNLGNTFQIDQLTGQVSIISQPLDFEKIQKYVWVIEARDGGFPFSSYEKLDITVLDV
 NDNAPIFKEDPFISEILENLSRKILTVSAMDKDSGPNGQLDYEIVNGNMENFSINHAT
 GEIRSVRPLDREKVSHYVLTIKSSDKGSPSQSTSVKVMINILDENDNAPRFSQIFSAHVP
 ENSPLGYTVTRVTTSDDEDIGINAISRYSIMDASLPFTINPSTGDIVISRPLNREDTDRYR
 IRVSAHDSGWTVSTDVTIFVTDINDNAPRFSRTSYLDCPELTEIGSKVTQVFATDPDEG
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 ISNDNHLGKFKLDNDTGWISVASSLISDLNQNFITVTAKDKGNPPLSSQATVHITVTEE
 NYHTPEFSQSHMSATIPESHSIGSIVRTVSARDRDAAMNGLIKYSISSGNEEGIFAINSS
 TGILTLAKALDYELCQKHEMTISAIDGGWVARTGYCSVTNVNVIDVNDNSPVFLSDDYFPT
 VLENAPSGTTVIHLNATDADSGTNAVIAAYTVQSSSDSLFVIDPNTGVITTQGFDFETKQ
 SYHLTVKAFNVPDEERCSFATVNIQLKGTNEYVPRFVSKLYYFEISEAAPKGTIVGEVFA
 SDRDLGTDGEVHYLIFGNSRKKGFQINKKTGQIYVSGILDREKEERVSLKVLAKNFGSIR
 GADIDEVTNVNVTVDANDPPIFTLNIYSVQISEGVPIGTHVTFVSAFSDSDSIPSWRFSY
 FIGSGNENGAFSINPQTGQITVTAELDRETLPIYNLSVLAVDSGTPSATGSASLLVLTLED
 INDNGPMLTVSEGEVMENKRPGLTLMTLQSTDPDLPPNQGPFTYYLLSTGPATSYFSLST
 AGVLSTTREIDREQIADFYL SVVTKDSGVPQMSSTGTVHITVIDQNDNPSQSRTVEIFV
 YGNYLFPGGILGSVKPQDPDVLDSFHCSLTSGVTSLSFSIPGGTCDLNSQPRSTDGTFDLT
 VLNDGVHSTVTSNIRVFFAGFSNATVDNSILLRLGVPTVKDFLTNHYLHFLRIASSQLT
 GLGTAVQLYSAYEENRTFLLAAVKRNHNQYVNPSPGVATFFESIKEILLRQSGVKVESVD
 HDSCVHGPCQNGGSCLRRLAVSSVLKSRESLPVIIVANEPLQPFLCKCLPGYAGSWCEID

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IDECLPSPCHSGGTCHNLVGGFSCSCPDGFTGRACERDINECLQSPCKNGAICQNFPGSF
NCVCKTGYTG VFGKHCELSYGFEELSYMEFPSLDPNNNYIYVKFATIKSHALLLYNYDN
QTGDRAEFLALEIAEERLRFSYNLGSGTYKLTTMKKVSDGHFHTVIARRAGMAASLTVDS
CSENQEPGYCTVSNVAVSDDWTLDVQPNRVTVGGIRSLEPILQRRGHVESHD FVGCIMEF
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SQNEKREYLLRQSLRGAMLEPFGVNSLEV KFRTRSENGVLIHIQESSNYTTVKIKNGKVY
FTSDAGIAGKVERNIP EYVADGHWHTFLIGKNGTATVLSVDRIYNRDIIHPTQDFGGLD
VLTISLGGIPPNQAHRDAQTAGFDGCIASMWYGGESLPFSGKHSLASISKTDPSVKIGCR
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VLSLILCNQCRGKKAKNPKEEKKPKEKKKKGSENVAFDDPDNI PPYGDDMTVRKQPEGNP
KPDIIERENPYLIYDETDIPH NSETIPSAPLASPEQEIEHYDIDNASSIAPSDADIIQHY
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ASHGSRPGSRLKQPIGQIPLESSPPVGLSIEEVERLNTPRPRNPSICSADHGRSSSEEDC
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DEDNYGARLKPRRYHGRRAEGGPVGTQAAAPGTADNTLPMKLGQQAGTFNWDNLLNWGPG
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Figure 7

Proxy SNPs (chromosome position/strand/rsidsequence)

chr4 126347460 + rs6842220

ATATTCCAGAAAGATCACATTTTGTCAATATTATTTGTGGGAATGTATGATAGAAAAGTTTGCCTTGTCTGTTAT
GATCAACATTTTTAATAATGAAAAT[C/T]GCTTGAAAACCTTTTAAGGCCCAATAATTCTATAAATCAAATCATG
CTAGAGTTGCCTTCAATTACCAGACATGAAATGCATTTGACTTAATTTTATTTT (SEQ ID NO:10)

chr4 126359002 + rs7656978

ACATTAAGAGTAGAACCTTGGGAATTAAGTTGTGTGGAATTGAATCCTGACTCTACTATTACTATTGCATTAGT
ATGGGCAAATTAACATTTCTGGACCC[A/G]GTTTTTCAGTGGATTAAGCAGAGTAATAATGAACTAACTTTAAA
GAGAGTAAGTAAAATAATTTAAGTGAGCTGGTTAGAATAGCACCAGGAACAAAAAA (SEQ ID NO:11)

chr4 126352482 + rs13141820

GAATTACAGCACAATGTGTATATCAGAAATATTCAGATCTTTCTTGAAATCTCACTGTAAGTCTTAAAATACCA
CATTTAAAAATGTCCAATTTACTATG[C/T]CTTCCATAAAGCTGTGAATTTTTAGAGAGCTAAATGTCTCATGTA
TTTTTACCACAAGTGAATCATTGAGATGAATTATTATTCAAATATTTTCCTGAA (SEQ ID NO:12)

chr4 126353466 + rs10021618

GCAGTACCTTCCAGCTTCTTGTGAGTGGTAAAGACTCCAGGGCTGCTTTACATGGGTACATCTCAGTGACATT
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GAGATGACAGCAGTGCTGATTTATGGGGATTAGATAAGACAGTTTATGTAACTTC (SEQ ID NO:13)

chr4 126343279 + rs11931967

ATAAATGAAACATTCAACTGCTATTTTTCTTTCCCCAAATAAATAAATGTTTGAAAACATTTTTAAATGAAGCCA
AATTAAGTGGAATCAGTAATTTCAA[A/T]ATTGGTATTTGAAAATAAGCTTAAATTTAGACATTGTTTAAATTT
AATTGTAAATAAAGAAGATATAAAGAATTTGTTAGGCTTTATGCCTAGAAGAT (SEQ ID NO:14)

chr4 126349342 + rs28694074

CAGGAGTTCGAGACTAGCCTGACCGACAAGGTGAAACCCCGTCTCTACTAAAAACACAAAAATTAGCCAAGTA
TGGTGACACATGCCTGTAATCCCAGCT[A/G]CTTGGAAGGTTGAGCCAGGAGAATCGCTTGAACCCGGGAGG
CGGAGGTTGCAGTGAGCCGAGATCACACCGCTGCTCTTCAGCCTGGGCAACAAGAGCGA (SEQ ID NO:15)

chr4 126357469 + rs35589914

GGACGTGAGATCAAATGAAAGAGAGAAATCAGAATCATCTAAGTTTTCCACTCAAACACCTAGGCAGATCATG
ATATGATATCATTACTGAAATGGGTA[A/G]CATTGAAGGAGGAACAGTTTTGTGAGAAGTCAGAAGTATTCT
ATTTGAATACAGTTTCAAATGACTGTTACTCATTATTAGAGATACCAGTTAGGAAG (SEQ ID NO:16)

chr4 126359365 + rs17803313

GCAGATGCTGTTTCTTAGACAGTATATAGAATATTACCAATCAGATATAGGGAAATTCTCTTGTATGAGATGTA
TAAATTCCTGAATATGAAGTTGAATG[C/T]TTACAACTTGATCCTGTCTTCCATTGAGTCATTCATTTATTTCTA
TAATAAAGTAAATGCTGTGATGACCTGCTGTTTTTTCTAGTGCATGTTAAAGA (SEQ ID NO:17)

chr4 126353280 + rs7677254

AGAAATTTAAAATCTATGCCAAGATTATTGTGCTCAAATGTCCCACAACTTAAGCTGGCCAGTAGTGAAGGT
CCCTCCCTGTTGGGCCTCTTCACCTCC[C/T]GCCTGGTGCTCTTCTAGGCTCAGCGACAGAGCTGCTTGTCCAG
ACCCCCAAAGCAGCGCAGGAAGCAGCACTGTTCCCCCTACTGGCAGTACCTTCCAGC (SEQ ID NO:18)

chr4 126345585 + rs28453293

ATGGTTCTATAAGGGCTTCACCCCACTTCACTCTGCACATCTCATTTTTCTCTCTCCTGCTGCCTTGTGAATAAG
GACATGTTTGCTTCTGATTCTGCCA[C/T]GATTATAAGTTTCCTGAGGCCTCCCCAGCTCTGGGTAAATTGTGAG
TCAATTAAACCTCTTTCCTTTATAAATTGCCAGTCCCAGGTATGGCCTTATAG (SEQ ID NO:19)

chr4 126359456 + rs1395241

AGTTGAATGTTTACAACTTGATCCTGTCTTCCATTGAGTCATTCATTTATTTCTATAATAAAGTAAATGCTGTG
ATGACCTGCTGTTTTTTCTAGTGC[A/G]TGTTAAAGATTCATTGCAAAATGCTATCCTATGCTTGTGAATTATG
CTTGTAATTTGGTCTAGAAAAATTGTATTCATACTCTCAAACCTGTTTTACAAA (SEQ ID NO:20)

chr4 126361331 + rs9307564

TATTCTTCTCATATCATCTTTTTACTCCTAATATATTCTTTCTTTTACAATATCTGGTACATAACAGTGGTTGTCA
CTTAAATAAATGCATTGAATGAA[C/T]GATTTTAATATTCTCTGGAATGGCTGCTTTTACTCATTCTATTTTACT
ACAACATTCTTGAAAGCTTCACAACCTGTTTTCTCTGACCATTATTCTG (SEQ ID NO:21)

chr4 126361095 + rs9307563

TTTATTTGCCTCCCATGGCCTAGGAAAGTCATTTTAGTGCCTCAAAGGCTTAGTTTCTGCATCAATAAAAATGA
ACATAAAAATTTATACTTTACAGTTT[A/G]TGAGACAAACATTTACTAACCACCTACTATGTATCAAACAGTATG
TATGGAATCAGTTAATATCCATAAATGTCTCTGGCATAATTATTAGGACATAGAA (SEQ ID NO:22)

chr4 126319493 + rs7697927

GATACGAGTGGAAATAGAAAGAAAAGATGTATTTTCTTCCCATTAAGGTTGAGCGTGGACTTTAAAGGGG
GAAATTCTGAGTGATTCTTAAACAACA[G/T]GTGAACTTTTTTATTTCCCCCAGACATTAGTTGCCAATATGTA
AACATTTCAAGCAAGAAATTTTATACCACCTCCTCTGTTTGTGAGGCAGCATTTAATG (SEQ ID NO:23)

chr4 126333397 + rs4623022

TGAGCTTAGTATTTTTCAGTATACTTCAAATATTCCGGGAGAAATTTGAGTTTTGCTATGTCTGCATGTATTTT
TAGATGTAGTGTATGTGGCCAAAGT[C/T]ACTTTTACTTGTGAGAAATGAAAAGTTATTTTCTTTACAGTA
AAGTAAATGCAGATGGATTCCATGACCTGTAATTGCAGCAATTAATTTTCAGTTC (SEQ ID NO:24)

chr4 126343978 + rs2136345

AGTAGGGCACTGCTGTAAAAATACCAAAGTGACTTTGGAAGTGGGTAAGTGGCAGAGACTGGAACAGTTTGG
AGGGTTCAGAATAAGAGAGAAAAATGTT[C/G]GAAAGTTTGGAAGTTCCTACAGACTTTTTGAATGGCTTTGA
CCAAAATGCTGAGGTGTTATGGAAAATGAAGTCCAGGCTGAGGTGGTCACAGATGGAGA (SEQ ID NO:25)

chr4 126348639 + rs11098812

ATATAGTGTTTTTGATAGATTCCTATGCAAAAATAATGAAAAAATATTAAGTGTGTATATATCTAATTTATATA
AGCATATATGTAGAACTAATTAGCA[A/G]CAATACATTATTTTATAAAACAGAATTTTAAAATACAGAAGTGT
TGTTAATATTTAAATTGTTACATACCTTTTGTGTTTATGTATTTTTGTATTCAA (SEQ ID NO:26)

chr4 126354307 + rs1395233

ACTAGGCCACGAGTAATTACAATCTAAGAACATAGAAGAATGATTGAATAGTGGTAAAGGAAATACCAGCAA
ATCACTTGACTATTAGAAAATAATATGG[G/T]AAGGTTGTGAATCTCAATACCATTCTGTGATAATGAGGGCAC
TCCATAAACATTTTACAAATAAATGAATGAGTGGCTTTATTTACAGAATATTGAATGG (SEQ ID NO:27)

chr4 126362855 + rs17803397

AGCAAAGCTGGAGTCAGTGAATTAAAGGGCAATGGCAGAAGTATGCATCAACATGAGCATCTCTTGGCTCTG
CTTTAACAATTATACTTCCTTCCTCAAA[A/G]TCTTTCAATTCCAGTTTGCCTATGGTATAACCCCCAGTCTTCAA
ATTCTAGTCACCCCATAAATGGCCCCAGCTAATTCTGCAAACTCTGCAACATATG (SEQ ID NO:28)

chr4 126368878 + rs13108706

AAAGATATTTTACTTTTGTACCGTTTCTTCTGTAGGGGTGAGAGTGCAGGTTTGGGAATGGTTTCTGAATGAG
TAGATTGAAAATATGTCCTTATGGCA[A/G]CTTTTCTGCATATTTTACGACAATCTGTTTCATTGGCACTCAGT
CTAAGCACTAACTTTTGGAGTCCAAGTTGAGTCCATTATCCCTCAATTCTTATTT (SEQ ID NO:29)

chr4 126307341 + rs13146586

GTTACCTCCTTCTTAATTTGCGTGTGTGTGTAACAATAAAATGTACTAGTGCAATGAACAACTGGTTTTTACA
TCATTGAATAAATGTACATTAAATG[C/T]CTTCTGTACCCTAGGCGTTATATTGAACTGATCTAAATGTTTCAC
TGTTTGAACTTAGAAAAGAAGCAGGCTTTCTGAGTTTTGTTTTGTTTTGTTTT (SEQ ID NO:30)

chr4 126302984 + rs7669346

TGACTGTCATGAAAGATTTCTCTGTAGCATTCAATACTGTTTGAGAGCATTTTACACATAGTAGAATGTCATTC
AAACTGGAGTCAATCCTCTCAAACC[C/T]TGCCACTCTTTATCAGCTAAGTTGATTTATTATTCTAAATATTTT
GTTGTCATTTCAACGGTATTTACAGCATCTTCACCAGGAATAGGTTCCATCTCA (SEQ ID NO:31)

chr4 126325798 + rs62322574

ATTTGATACGCTTTTATTTTATTTTATTGTGATAAGAACATTAATGTGAGATCTGCCCTTTTAAAAGATTTCTAA
ATGTACAATACAGTATTGTAACT[A/C]TAGGCACGATGTTGTACAGTAGATCTAGAAATTATTCCTCTTGCA
AAGTGAAGATGTATACCCATGGCTTATAAGCTCCTGATTTCTCCCTACTGTCTG (SEQ ID NO:32)

chr4 126316472 + rs1986721

TATCTAAGGAACTGTGGAGTAGAGAAAAGGGAATGGGCAATTAAGGAAAAATATCTAGATTTTCAAGAAATG
GAATATGAAGTTATTCACCTTTCTTTTT[A/C/T]ATTCTTTTTTGAGACAGAGTCTCACTCTGTCGCTGAGGCTGG
AGTGCAGTGATGCCATCTTGGCTCACTGCAACCTCTGCATCCTGGGTTCAAGCGATT (SEQ ID NO:33)

chr4 126310531 + rs4349616

TTAAAAACAGAATTATTCCAATTCCCATATTGTATGTTACTTCATCAAATATTACCATTATATCATGTTTCTTGTA
CTAAAGTGTTTCCTGTGAAATAAC[A/G]TAATAAAATGGCCTTTAGTATGCATGATAATTTAAATACGCATT
TTCTAGTAAATATAATGCTTATTCATTTTTTTCTTCTAGTTCTAAAGAAAAAA (SEQ ID NO:34)

chr4 126308245 + rs885272

AACCTAGATCCTTCGCATGCACCATTCACAATAGGGTCCACCCTTCTTTGAGAATCTAATGCCGCAGCTGATCT
GACAGGAGGCAGAGCTCAGGCAGTAA[C/T]GCTCACTTGCCCGCCCCCTCACCTGCTGCTATGGGCCGGGTTC
TAACAGGCCACAAACCAGTACCGGTCTGTGCCAGGGGTGGAGACCCCTGTTATAG (SEQ ID NO:35)

chr4 126312920 + rs1509292

AAAGAAAAAATAGTAAGTCATTATTATGAGTAGTTACTGAATTATCATAAAATATCATTTTCATAACATAAAAA
TTATATATGATAGCATGTTCCACAC[A/C]AAAAGTAGGAAATCAATAGGTTTGAATTGCTTTAAATATCATT
GTAATAGTGGATAACTTTGATTAATACATCTTTATATGCCAGTAGAATGCCTATGT (SEQ ID NO:36)

chr4 126313149 + rs1912044

TTAATAAAAATGTAAAAATGACCTGGATTTGCTTAAAAACAGAAATATTTCTAGAGGAGAATTTACATGCTAG
ACATGGATAAGTTGTCAGAATGAGCAG[A/G]ACACATTATTGCTCTTGGAATCTCAGAATATAGAAGTCTCTA
ATTTAAGTAATATAAATGATCTTCAGTATCCTGAAGATATTTGCAATTACCTATGTTG (SEQ ID NO:37)

chr4 126300866 + rs6855908

CACCATGTCCAGCTAATTTTTGTATTTTAGTGGAGACATGGTTTCACCATGTTGGCCAGGCTGGTATTGAACT
CGTGACCTCAGGTAATCCACCCACCT[C/T]GGCCTCCCAAAGTGCTTGGATTACAGGTATGAGCCACCGCGCTT
GGCCAGACATTGATATTTAACTCTTTCTTAATCAAATTTTGAAGTTATTTTCATACC (SEQ ID NO:38)

chr4 126297735 + rs34874968

AGGAGAAATACCTAATGTAAATGACGAGTTAATGGGTGCAGTAAACCAACATGGCATATGTATACATATGTAA
CAAACCTGCACGTTGTGCACGTGAACC[C/G]TAGATCTTAAGTATAATTAATAAATAAATAAATAGGCCT
GGCGCGGTGGCTCATGCCTGTAATCCCAGTACTTTGGGAGGCTGAGATAGGCGGATCA (SEQ ID NO:39)

chr4 126295062 + rs1396694

TCAATAGGGCTACGGCATTTCATGCCTTCCTTTAAAAATTAATTGAGGGCAAGAAACCATTGTTTAATATGTAAT
TTCAAACATCTAGATGAAGAAAATAT[A/C]TGTATTAGAAAATAATAAGGACTATGTATATATGATTTTCAGACG
ATTCCTATTTAAATAAATTAGGATAAATGACATATTTGTTATAGGGTGAAATGTT (SEQ ID NO:40)

chr4 126270636 + rs7697665

AGAGTTTCAAATGAGTTTGTGAATGGATATCATACCCTTCCACAAATGACAGAGATGGACTATTAAATCAGAG
TTGGTAGTTTTTGTCTTTGTTTACTGT[A/G]TTTCTCATTACCCTAAATGGTCCTATCTATAACAAGCTTTGGAA
AAGCACCATGCACCTGAAATTCTTGCATTCTGATGTTTCATACCTAGAGAGATTTA (SEQ ID NO:41)

chr4 126272004 + rs9307560

GCTGATTCCTTGAAAGTACTCCCTCCTCATTCTCTACAGTAAGCCATTGAATGTTACTAGGGATGGACACTAA
GCAGAAAGATTTCTGAGTATCTATTC[A/G]TCTTCCCAATCAGACAGTCCTTTAAATTTAAAGAAGAATGCCT
GACCCTTAAGACAGGGAGGTTTCATCATATTTCCATTCTGACAGTTTGGAATGAAA (SEQ ID NO:42)

chr4 126270451 + rs10032352

CATTTGTTTTGAATTTTCCAGTATATTAACAATGGCTAACAATTATAGTTGAGAGAAATATATTTAGGAAAA
GTAATGCATTATTCAGCATTCTATTA[A/T]CTATACTATATTAACACATGTGTTATGGGCTGAAATTACACCAGT
ATCACATGAGGGTAAATGGGTTGAAAGTGTAAGTTTAAAAGAGTTTCAAATGAGT (SEQ ID NO:43)

chr4 126338088 + rs6534478

CTACTCAGAAGGCTGAGGCAGGAGAATCGCTTGAAACCAGGAGGCGGAGGTTGCAGTGAGCCGAGATCACA
CCACTGTTCTCCAGCCTGGGCAACAAGAA[C/T]GAAACTCGTCTCAAAATAAACAAATAAATTAAATAATAATA
ATTTCTTCATAATATGAGTATGAAGTATTTTCTGTTTGCCATTAAATGAAATATTTGTT (SEQ ID NO:44)

chr4 126282761 + rs1396685

AGAGGTGATCTGACAAGTGTTCACTCAAACTCTGCAACCTTGATTGAGACTGTGCTAGATGATATAAAAATAC
ATATGCCCTAACAGGGAGGATAGGCAA[A/C]CAAACAATTAATTTTAAAATTGTGAATTTACAACTGTGGGT
TATTATATGCTACAGAAGCCACACAGAGAAAGAGTTATTTGCTCTGTTTCAGAAAATA (SEQ ID NO:45)

chr4 126281130 + rs17193280

TCCAGCCTGGATCTCTGGGAGGGAGAAGTAGTTCCAAAGATTACAGTGCTTAATCTTAACTCTGAAAACACTA
CTTATTGACTCTCCACTGGATTCCACT[C/T]GCCCAAATTCCCTAGATGGATTTTCCCTCAGGATGAAACAACCT
AAATTCAAGTGTCTAACTGTCTTCTCCTCCTGCCATTGCCTTTCCTTGTT (SEQ ID NO:46)

chr4 126281923 + rs2663251

TACTGAACAATAACATATGCACTTAATTGTAGTCATCCCCCTGACTATACTCACAAAACCTTAACATG
TCTTTAGGGGAGGAGGATGGATTGCAGATAT[A/G]CTATTAGAGGCATAGCCATCTTTACTTCATTAAGTTT
AGTTTACTAAAATTTGCAAATGTTTAAAATATGGTCTTGCTTAATTCACAAATTCTGGGA (SEQ ID NO:47)

chr4 126280485 + rs62321342

TAGTCAGGTGGAGATAATTCACCCCACGTCTTAAGGCCAAATGGAAAAAATCTTCTTACTCTGGAATTAGGTG
GTTAAGTATCTTGATTTTAATGGATGT[C/T]GTTGTTTTCTAATAATTGAGTTATTTGTATTAAAATGCTAACT
GGCAATATATGCCGATGGCCAATTTACCTCCAAATATATTGAGTTCATACATATT (SEQ ID NO:48)

chr4 126278969 + rs62321341

ACACTGCTGGCATGTGCTTTGTGAATATGTTTGAATCAAGGGCCTTTACAAAGGGCTCAGACCTTCCTCCTAGG
GGGTTGTCATTTACCGTGGATGCTCT[C/T]GTTTGTGTGGTAAACAGCCTCAGAAAACGTCTTGACATTGTCA
ATTCCAACAGATTAATGAAGTAACTGGGCCATACATCCCTTCCCCTTTACATATGA (SEQ ID NO:49)

chr4 126279303 + rs2663295

GGGCGGGGGAACTCCAGTGTGATTATAAGTGAGTATATAGGAAAAAAGAAATGAAATTAACCTTTTTTTCTT
TAGAAATCTTGTGTTTAATTTTATGTT[C/T]CAGTGTTTTAAATAACTGAAACCTTATTATGGACAAATTTACCTG
TTATGTTAGGAGAAATGAGTTTCATTTACCATCTTCATCAGCGATCCCCAACCTT (SEQ ID NO:50)

chr4 126280623 + rs2710556

GCTAAACTGGCAATATATGCCGATGGCCAATTTACCTCCAAATATATTGAGTTCATACATATTCATTAAGTGGG
ATATTTTGGAGAATATACTGCTTATA[C/T]GTTCAAATGCAAATAATTTAATTCGCTGTGATTTATAATACTGTT
ACTCAGTAAATGGACTAAAGGCAGGGTAAGATTTATCAGTCTTAAAAATGTAAGT (SEQ ID NO:51)

chr4 126280021 + rs2710559

TGGGGACCCCTTATCTACATCCACGTTTTCTATGTTTGTGAAGATTTGATGTGGGGTTATGGGTTTGAGGAGCT
AAAAATTATAGTTAGTAGAAGGAAAG[A/G]CAGCTAGAAGTGGAAGTAAAATCACTTACATTGTACTTTTAC
TAGGACCTATATTGTAGGTATTATAAATCATACAAACAAGTTTGTTATACAAAACAT (SEQ ID NO:52)

chr4 126280006 + rs2710560

CTGTGGCCCAGGGGTTGGGGACCCCTTATCTACATCCACGTTTTCTATGTTTGTGAAGATTTGATGTGGGGTTA
TGGGTTTGAGGAGCTAAAAATTATAG[C/T]TAGTAGAAGGAAAGACAGCTAGAAGTGGAAGTAAAATCACT
TACATTGTACTTTTACTAGGACCTATATTGTAGGTATTATAAATCATACAAACAAGTT (SEQ ID NO:53)

chr4 126277240 + rs2940782

GTTTTTTTTCTGTCGGCAGTGTATCACAAGTATTTTCCTCAGTGGCCTAACAGACCCTGAGTGGAAGGCTGGT
AAAAGCAACCTCGTCTGCATGCATCA[C/T]TTTGAATGATTTCAAAGAGGAAAGAAGCAGCTGTGACAATGTT
CACATGAACCACTCAGAGTGGCCTTATCTTTGTAGTCTAACAATAATAGTACACCCT (SEQ ID NO:54)

chr4 126275098 + rs2036725

GTGAAGTTTGAGAATTTGCAAAGCCATAATGATAGAATCAACCCAAAGGTCCCAGAAATTTTCAATAATCTAT
CATCCTTATTTAAAATTATTATACAAT[A/T]AAAAATTGTGTTTTAATCGCATGAGTAGACAGATGTTTATGCTG
TTATTCAGCGAATCTTGCAAAAGTGAGTGTAATTCATAGAATAAAACAATGTTGGA (SEQ ID NO:55)

chr4 126408586 + rs17009825

AATATGTTTTACAGATTAAGAATGGCAAAGTATATTTTACATCCGATGCAGGAATTGCTGGGAAAGTGGAGAG
AAATATTCCTGAAGTATATGTTGCAGA[C/T]GGCCACTGGCACACTTTTCTAATTGGGAAAAATGGAACAGCA
ACAGTATTGTCTGTTGACAGAATATATAACAGAGATATTATCCACCCTACTCAGGACT (SEQ ID NO:56)

chr4 126280338 + rs2710558

ATAGAAGCAGCAGCATAAATATCTATATAGAATTAGCCAGGTTCTAGTCTGGCACATCATCACATAGTAGCAC
ATTTAATGCCCCCAATTATTCTATGAT[A/G]TAGATACTACTAGTTTCCCCATTTTCCTAATGAGAAAAGTGAGT
CATAGTCAGGTGGAGATAATTCACCCACGTCTTAAGGCCAAATGGAAAAAATCTT (SEQ ID NO:57)

chr4 126297549 + rs10011289

ATTTAAATTTAAATCAAACAGTTCTCACTGAAATAATTAGGGGTAAAATGGACCTCTGTTGATGCTATTTGAAA
AAACTATTTGAACAATGAGAACAATT[A/G]GACACAGGGTGGGAAACATCACACACCGGGGCCTGTCGTGAC
CGGGGCCTGTCGTGGGGTGAGGGGAGGGGAGAGGAATAGCATTAGGAGAAATACCTAA (SEQ ID NO:58)

chr4 126423687 + rs1395227

ATTTCAAAGCTTATTATTTTCCATTACTACCTAACACTTGTGTATCTCTTCTTCAGCTTTCAAAGAGTTTTCAGG
TGAGTTATCTAATTTGAACATTAC[A/G]TTACATCTGTGAGGTTGATTACAGAAAAGTAATTAACACATTTTAG
AGAAAGGAAAACATAAATGAAAAGAGACTTACTCAAAGTCACAAAGTAACGGTG (SEQ ID NO:59)

chr4 126422859 + rs9995724

AAATAACTCATACAATTCACTTTAAGCTGTTTCTTTGATATGCAATTCTTAGCCATTACTCTTTGAGCCTGTCGTA
CTACTGTCATTATTATCATTATTT[G/T]TGACCCTGCCTTTTGCAATCCGCATCATTACACATTCTTTAGTTCAAA
GTGGAGAACTAATGTCTTCCTGCTTCAAGCGAGGCTGTCTTGTCAGCTCTTT (SEQ ID NO:60)

chr4 126314379 + rs13146027

AACAAAAGATTGTTTTCTTCTTTATTTGAGAACCATTTTATGTAGATTGCAGGAAGGTTGTGAATTAAAATAT
GTTTAGATTTTTTCATTCCGTTTCATT[A/G]AGAAACATTTGATTTAAGCTGCAGAGAGGATATGCATTTAAGAA
TATGTTTAGAATTGTGGTTGAGGTGGCAACCACCATGCAATTATCAGTTTATTAAC (SEQ ID NO:61)

chr4 126318288 + rs13136889

TGCCAGTTGAACCTGTATGTGGGACTGGAATTCTGATTTTTAAAAATACCTTTTCTCAAAGCCTTTCTCTGGCTC
TAAGAAGCTTCCAGATCTGTCCTAA[C/T]AGCTTGAAAACCAAGATCTTGACTTTTTATAGAATACTTCACTCTG
TGCCAGAAACTGTGTTAAGCATTTTCTTTACCTATATCATCAGGATTTAGTCTT (SEQ ID NO:62)

chr4 126292769 + rs2663256

TCTGCTATAAGTGCTATAAGTAATAATATAGAAATAGTTGACCTCACAGTTAATTTATTAAGCAAATAGTGGAG
AAGATGCTAGTAATTTCTAGACCTTG[C/T]CTCAGGGGCTGTGAATATAGGAGGGGGATAAGATAAAAAGTGG
CTCTTTCATTGGAGTATCAATTGTGGGGAGAGATTGAATATCCTCAATAATATCTTAA (SEQ ID NO:63)

chr4 126270773 + rs7676058

GCTTTGGAAAAGCACCATGCACCTGAAATTCTTGCATTCTGATGTTTCATACCTAGAGAGATTTATGGTGACATA
AGCAGCCAAATAGCACAAAGTGAAATT[G/T]TATATGAAGTATATGACATGCATGATTCAATATATATGCCCACT
TTTAAATATACCAATAGTATATTTGGACTATAATAATGAATTCATTTCAAATATTG (SEQ ID NO:64)

chr4 126286443 + rs2663252

AATAAATTAGTTCAGGACCTTGTTAAATGTGAGGTCCTCTGAGATACCAGTGGAAATATCCAGCAGGCAGAT
GGATATGTGAACTGGAGCACAAACAGAG[A/T]AGTCTAGATTAAAATTTGCATTTGGAGTGTAGTATCCATGAA
AGTAGCCACGAGCTTGGATATGTTGTCCAAAAGAATGTGGACTCTCTTCTCATCATTC (SEQ ID NO:65)

chr4 126286314 + rs9307561

TTGATGACGAATTGAATGTAGATGTGGAAGATGATGGAAGAACCAAGGAAGATGGCTTAGTTTCTGCCTTGA
GCAACTAAGTGGATGATGAGTTAGGGAA[C/T]ACCGGATGAGGAGTCATAACAAGAAGACAATAAATTAGTT
CAGGACCTTGTTAAATGTGAGGTCCTCTGAGATACCAGTGGAAATATCCAGCAGGCAGA (SEQ ID NO:66)

chr4 126287648 + rs2952841

CTCCAGCTCCATCCATGTTCTGCAAAGGACATGATCTTGTTCTTTTAATGGCTGCGTAGTATTCCATGGTGTGT
ATGTACTATATATATATTTTTTAATC[C/T]GGTCTACCATTGATGGGTATTTGGGTGATTCCACGTCTTTGCCATT
GTGAATAGTGTTGCAATGAACATAACATGTGCATCTTTACAATAGAATGATGC (SEQ ID NO:67)

chr4 126296175 + rs2710582

ACACATAGAAAAAATGTAGGCTTGTTAGCATCAAGTAAGTTGCCAAATATGATATTGCCCCTTATATAACTTG
AATAACTTTAGGTGTTACTTAAATTC[A/G]TAGGCACATATTGGCATCTTCAGAGCAAGATGAATTAACACTCT
GGAGTTTTTATTTTATTTATTTAAAAATAGATTATCACTTGAGAAAAGACAGCATC (SEQ ID NO:68)

chr4 126369279 + rs6846428

ATTGGAAGATATTTTAAGACACACTAGAGCAACGGTATCATTGCATTGGGTAGAGGCATAGCACTTTGCAGAG
CTCGCATATTATGTTTCTTGGAATGTT[G/T]TTTTATGTGCAGATTTTCTCCTAGAACTATGATTTTTGAACGA
TTAGCAAGTTCTTTCCCTAGGCTTCTCTCAAAGGCATACTGAAAAGTATTAAAT (SEQ ID NO:69)

chr4 126351995 + rs17009641

GGAGAAAGTTCTTGGCTTGAAAACAGACTCATATAGATGTAGAATCACCTATGGGGCTGATCGTGGCTTAAG
GATCATCTGCCAGTCTTGAGAATCATTT[A/G]CTAATCTCAAGGATCAACAAATGATCCTCAAGCCAGAATTGT
CTGTTGTCACCTGAGTGCTTTGAAAATCATCTGGAAAAATAAGTTGATATTCTTTGGT (SEQ ID NO:70)

chr4 126368263 + rs13129279

GTTTTCAAAAAGTTTAATAAATGGATAATTCTTGTTAATATTTATTTTATTCCAGGTTTTACAAATGAAAAGTGT
TCTGTTAGCAAGTTTATGGATCCAT[A/T]TATGAATCATTTTCATATTGACAATATGCATTTATCCTTCTGTATCT
CTTTAAATTTAGTTTGACATTTGCTAAGAAAGGCATACTCTAAGAACTGCACA (SEQ ID NO:71)

chr4 126294586 + rs62321346

TTTTTATGTTGTTTTATCTTAATTACTCTAACAGAGACAAATTCATCTAAAGAGGTGATATCTAAAGGAAATG
TAGAGATCAGAATATAGGACAGATT[G/T]TTTTAAAAAGTTTGATATTTATTATTAGGTGCCTAATAAATGCTC
CTTCTAGTTTATGAAGCTTTATTTCTAAACTATAATCTATGTTTATGTGTGTTT (SEQ ID NO:72)

chr4 126291032 + rs6854625

TCCTCCAATCGGCAAGCTCCAGGCCACCACTGTTCTGCTTTCTGTTCCCATGAAGTTGACTACTTTTGCTAACTG
ATATAAATGGAATCATACATATTTG[C/T]TTTTCTGTGTCTGGCTTATTTTACTTAGCATAATGTCCTCCAGGTA
GATCCAAGTTGTAGTAAATGTCAGAATTCCTTTTTTTTTTAAAGACCGAATAGT (SEQ ID NO:73)

chr4 126293819 + rs62321345

GAACATATTACTGGTGAATGGGGAAGCTGGCTTAAAAAAAAGGGAGGGTCTTCTCAGACAGTTCTCTTTTAC
ATACGTGGCACAATGATTCATGATTTG[C/T]TATATTGTGAATGTTGAATGTGGAATATTTATAGGAATGCAAA
TCACATTTCTTTACACAATAGTATTAACTGATTTCAAAGTGTTTTGATATATGCT (SEQ ID NO:74)

chr4 126284351 + rs6817722

AATTATCAAATCATTTTTCACACAATAAAAAATTGTATACAAGGCAAACCTATGTACATATATGTGGGTGTCCTT
AGGAATAAATGCATAATTTCAAAGAC[A/G]TGGAATTTGTAAGTGTTAGTCATGTTCTCACCTGTAATAAAGCA
ATGTGTTAATGAAATTGGGTGCTTATATATATTTATATATAATTTCTTAGTTTGAG (SEQ ID NO:75)

chr4 126275717 + rs79129861

TCTTTGCTGATGGAATATTGAACACGCTCTCACTCTGATGTACAGTTATAGTGTATACTAGGTAAGAAAAGAG
AGTCCCAGGCCAATTTAAACAATGATA[A/G]GGTTTGCTTTTCTGAATTCTGAGAAGCTAGTTCAGGGGAAGA
AATGAAACCACAGTGTAGAGGGTCGAATGGTGAAGGTGAAAAATTAGTATCAAGCCTC (SEQ ID NO:76)

chr4 126277903 + rs79626418

GTGACAAGACTAATATAAGTAGAATATAGTTGAGTTACAGAATATGTTGTAATAGTCTTTACAATGCACATCCA
ATGAACATCATATAGACAGATATTAA[C/T]ATCAATATATCTACACATAATTGATATATAGTATATATTATAATA
AATATAAGATATGATAATATAACAGATAATATAATAGAAATCATAGATATCTGTA (SEQ ID NO:77)

chr4 126278572 + rs78188548

CAGTCTCTCGATTGATAATAAAATTTGTCATTTTCTCAGTTAAATTGATACAGATTCTTGTAGAGCAAAAAGAC
CTAAAGATAAGATTTGGGATAGATTT[A/T]TACCTTAACCCAGGTAATAAATAGATTATTCGGGTACTTTAATA
GTAACAAAGGAATCAAATGGAATTTTAATTGAAATGATTTTACTAATATGGTCATT (SEQ ID NO:78)

chr4 126280842 + rs74646069

TATTATCTTCTAATTATCCTTTTAAATCAAAAACATACTTTCATGTTGGTATGAGTATCATAACCAAAAGAGAAG
AGAACTTCAAAGAGAAAACCTGAAT[C/T]AGTGGAGAGTAACTTAATTTCAGAAGAAAATCGTACTTTCATAT
AAATTCATAGTGAAAGGTAACCTATGTGATACCAGCATTGAGGGAACAAATGCCCTT (SEQ ID NO:79)

chr4 126302971 + rs79145530

CTTTGATTGAGTCTGACTGTCATGAAAGATTTCTCTGTAGCATTCAATACTGTTTGAGAGCATTTTACACATAGT
AGAATGTCATTCAAAACTGGAGTCA[A/G]TCCTCTCAAACCCTGCCACTCTTTTATCAGCTAAGTTGATTTATTA
TTCTAAATATTTTGTGTCATTTCAACGGTATTTACAGCATCTTCACCAGGAAT (SEQ ID NO:80)

chr4 126308282 + rs78474635

CCACCCTTCTTTGAGAATCTAATGCCGCAGCTGATCTGACAGGAGGCAGAGCTCAGGCAGTAACGCTCACTTG
CCCGCCCCTCACCTGCTGCTATGGGCC[A/G]GGTTCCTAACAGGCCACAAACCAGTACCGGTCTGTGCCCAGG
GGTTGGAGACCCCTGTTATAGACTAATAACCAACTGAAATAGAATAGCATAGCTATGA (SEQ ID NO:81)

chr4 126310980 + rs79031396

CATCAAGATGAGACAGTAAACTATTATTAGAGTTACCACAAGTTTGAACAAAGTTGATTATACATCGCAAACA
AATAAATACTTAAACCTTGTTTCATAG[A/G]GATCAGGCCTTGCATACCTACATAGTCCAAACATTACATTCCTG
ACATTTAGAACATTTAAATGCAAACCTGTTCTTTGTTAGGGCTTTCTCATAACTGAA (SEQ ID NO:82)

chr4 126322550 + rs74733937

GTTGTTGTTTTAGTTGCTTATAATAAATATTAATGCTTTTAGATAAACCTTTGTTTTATTTTCTACTTTATTCTCA
GCTCCTTACCTCTTAGCCAAATT[A/T]TGGGGAAAATATTTTAAAAATTTTCTCTCTTTTCAAATCCTGGCTCTGT
TTTCTTTCTTCATCTAGTTTGAGTAAGACTCGAGATTATGTAGGTCATCAG (SEQ ID NO:83)

chr4 126337327 + rs74829391

TTTTGAATTTTGTTCACAAGGATAATATCTGCACATAGTAAAAAACTAAAATGATGGAAATGTTTGAAACTT
CGTTATAAATATTGTTCTACTCTTTT[C/G]CTTCATATATTTAACTACCTAGGTGAAACTGCTCTTAAAAATTTT
ATGAAGCCTTCCAAAAATGCTGTGCATTAGCTTTACACATATTTTACACAAAT (SEQ ID NO:84)

chr4 126340181 + rs74340824

CAAGTAGCAGGAAATTATCAGGGAAACTAGAAGAGTATACTTTAAAAAATGAATTCAGATAATTAATTTAGAA
ATGGAAGTATATCATGTAGTTAAATAC[A/G]TAGACTTTATAGTTAAACCCAGTTCACATCCTACCTCTGACAT
TCCCAGCTATATGACCTTCAACAACCTTTACTATTCCTTCATGTTTGTATCTATGAT (SEQ ID NO:85)

chr4 126343877 + rs74856924

TGGGTCAATTAAACCTCTTTCCTTTATAAATTACCCAGTCTTGGGTATGTCTTTATTAGCTGCATGACAGCAGAC
TAATACAGGTTACTGATATCAGAGA[A/G]AGTAGGGCACTGCTGTAAAAATACCAAAGTGACTTTGGAAGTGG
GTAAGTGGCAGAGACTGGAACAGTTTGGAGGGTTCAGAATAAGAGAGAGAAAAATGTT (SEQ ID NO:86)

chr4 126357601 + rs77217742

AGAAGTATTCTATTTGAATACAGTTTCAAATGACTGTTACTCATTCATTTAGAGATACCAGTTAGGAAGCTGGA
GATATGAGTGTGGAGCTTAGGGTTGG[A/G]GTTAGGGCTGGATATACACATTTGAGAGTCATCAGTGTTTAG
AGGACATTTAAAACCAATAAACTGAATGAGAACACGTTTGCGGAGTGTATTAGTCTGT (SEQ ID NO:87)

chr4 126359840 + rs74501747

TTAAAATGAAAATTGAAGCTATTTTTGTCTTGTGAATAAAAAACATTCAATTCACCTTGCAAATTAACAGGGCAA
ACAATTATTGCAGCTTAAATAGAAAA[C/T]ATTTTCACAGACAAAGTAAATAGTTATTATAACCTCAACTTTATT
TACTCTGGTTTGATAAAATCTTAATGGAATATAATGTATTTTATGGTTGGTGTTA (SEQ ID NO:88)

chr4 126364496 + rs79033360

GTTTATGCAAATAATTTGAAATAGTAATGTATTCTCAAATTAGAAAATAATTTAACGTGTTTATTGATTTTCATT
GAACTAAATGTGAACAAATTCTATA[C/T]TGACACAACCTTGAGCAAGAGTCCATGTTGGCCTTCGCAATTGGTA
CATTTTCCGTCTTCACTACATTCCCTCAGTTGAGTTTTACCTTCCTTCTACTGTG (SEQ ID NO:89)

chr4 126369230 + rs76891791

ATAAATGGTAATGAACATGATTTTTGTTGACAAGATTACATATAAAGTGATTGGAAGATATTTTAAGACACACT
AGAGCAACGGTATCATTGCATTGGGT[A/G]GAGGCATAGCACTTTGCAGAGCTCGCATATTATGTTTCTTGGA
ATGTTGTTTTATGTGCAGATTTTTCTCCTAGAACTATGATTTTTGAACGATTAGCA (SEQ ID NO:90)

chr4 126369554 + rs115240175

TGTGAACCTCGGAGAAAATATAGGAGATACTGTTCTCCCTAGTAATCATGAGAAAATAAGGTCACATCAAGCA
GCAATAGAGTGTCTTATATGCACTTAT[C/T]TGCTACCAAATATTTTATTTAAATTCCACGTGAGTATAACTGCA
CACTTTCTCTTTTATAGGTTCCCTTTGTCTTTGCGGTTACAGTCACAGATGCTGATA (SEQ ID NO:91)

chr4 126381199 + rs115962084

TAAAAAAGCATAACTTTCCAAACCTGAGAAATATGCATTTAGAAGCTTTCTTTTGATTCTAAGTAAAATTATG
GAATCCTTTTCTCACACAAAAGACAC[A/C]GGTGTTTTAAATAATAATTTAAATTTAAATCTGTTTTCCTACTAT
GTCTTTGAATTCAAGTATCTGCTATGGCAACCTCTGAGTTCATGAATTGTAAAAA (SEQ ID NO:92)

chr4 126381679 + rs141880214

CTCCCTCCTCCCACCCTCCACCCTCCAGTAGACCCCAGTGTCTGTTGTTTCCTTCTCTGTGTCCATGTGTTCTCAT
CATTAGCTCCCACTTATAAGTGA[A/G]AACAGGTGGTATTTGGTTTTCTGTTCTATGTTAGTTTGCTGAGGAT
AATAGCCTCCAGCTCCATCCATGTTCCCACAAAAGACATAATCTTGTTCTTTT (SEQ ID NO:93)

chr4 126399393 + rs114235331

GAGATTACCAGAAATAAACTATTAAAGGCAAAGCTAGGAATACTTTCTAATGTTTGTAACCTAAGTATTTTAA
TAACATTTTCTATTTGCCTTTCTTTT[C/T]CTACATGTTGTTACTGTTTTGTTACAAAGTCACACTAGTTCGTTATT
ATTATTAATCAAACAGTACAGAAAAACAAAGGATTGGGATTGCCACCATTGTT (SEQ ID NO:94)

chr4 126402260 + rs114783000

ATACTGATTATACATGATGATAAATTATATGTGATGATTACTGAAAATAAAGTTAGTTACCGATGTAAATACTT
GATTGAACACTTCTTTGTCCTAATTT[A/G]TAGCATACTAGAGACTAAAGATTACAAATCATTTTAACCATGCAA
ACGTATATTTTGGAAACACAGTGAAAATGATTACAAACAAATTTTCAAGTACAT (SEQ ID NO:95)

chr4 126410669 + rs115415133

TGCTCCCCAAGAACCTATGTTCCACACCTTTGGTGTATTATCACCTTCAATGGGAATGCATGATTTAGTAGTTG
AAGCCATTGGCCTATTTTCATGTTCTT[C/T]ACAAATTTAAAAAATATATATTTTATTTGTATGTATGCAATTTTGT
GTATATGGGATGTAAAAAGGAAGGAAATTGATAAATAAATGAGTAAGAAAAAGA (SEQ ID NO:96)

chr4 126415682 + rs115572680

ATTATGACCTGAGAATTTAATCCATCCCCTGTCAAGTTTTATTGGATTTCCCTGGAATACAGTTACTCTACTCTG
TAGAGGGCAGAGCAACTCCATCTTG[G/T]ATGCTCATCTTCCATGTCTACTTCTGATTAAACCCAGTTCTGGGA
ATGCCTCTAATATTTCCAGTATATCTATAGTTCCTTATGTAAGAGCTTGTAATAA (SEQ ID NO:97)

chr4 126419863 + rs78127669

TAAGTTAGCCTGTAAGTCTTGCTATTGCATCCACTTTTTAAATTTGTTCAACACCAGAGCTACTGTGTGCCAAGC
ACCATATTTAGATAAGTGAGAGATG[A/G]CTCTTGCCTTCAAGAGGTTTGCAGTTTTTGGTGACTAGACTTTCC
CTTTTCCAAAGTAGTTTAAGATTTGCATAGCTCTGTTTGCAGAGAATTATTTAAA (SEQ ID NO:98)

chr4 126420770 + rs148385122

CTCTATAATATTAATTTTCATATTACATCTTAAGACTTTCATTTAAGCAAATTACATAACTCCTAAAAGGTGCTGC
ATTGAGGCCAGGCACGGTGGCTCAT[A/G]CCTGTAATCCCAGCACTTTGGGAGGCCGAGACAGGTGGATCAC
CTGAGGTCAGGAGTTTCAGACCAGCCTGGCTAACATGGTGAAACCCCATTTGTACTA (SEQ ID NO:99)

chr4 126423297 + rs79771845

CAATAAATTTGTTACCTTTATATTCAGAAACCTAATTGAATCGGAGTAGACGAGGACTAAAAGCATAAGGAAT
AATAATCACTAATCTTCACTATTTTTC[C/T]TTGTTTGTGTTGCAAATATTGTGTGTTGGCTGGTGTAGGCATG
GTGAAATATATGAAAATGATATTAATATAAAACAATGTCCAAGTCCTAGAGGCAAC (SEQ ID NO:100)

chr4 126343915 + rs115504303

TCTTGGGTATGTCTTTATTAGCTGCATGACAGCAGACTAATACAGGTTACTGATATCAGAGAGAGTAGGGCAC
TGCTGTAAAAATACCAAAGTGACTTTG[A/G]AACTGGGTAACTGGCAGAGACTGGAACAGTTTGGAGGGTTC
AGAATAAGAGAGAAAAATGTTGGAAAGTTTGGAACCTCCTACAGACTTTTTGAATGGCT (SEQ ID NO:101)