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Genetic variants for personalised management of very low carbohydrate ketogenic diets

Lucia Aronica ,[1,2] Jeff Volek,[3] Angela Poff,[4] Dominic P D'agostino[4]

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[1]Nutrition Science, Metagenics Inc, Gig Harbor, Washington, USA
[2]Medicine, Stanford University, Stanford, California, USA
[3]Human Sciences, The Ohio State University, Columbus, Ohio, USA
[4]Medicine Molecular Pharmacology & Physiology, University of South Florida, Tampa, Florida, USA

Correspondence to
Dr Lucia Aronica, Medicine, Stanford University, Stanford, CA 94305, USA;
lucia.aronica@metagenics.com

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ABSTRACT

The ketogenic diet (KD) is a low-carbohydrate, high-fat, adequate-protein diet proven to be effective for the reversal of obesity, metabolic syndrome and type 2 diabetes, and holding therapeutic potential for the prevention and treatment of other chronic diseases. Genetic and dynamic markers of KD response may help to identify individuals most likely to benefit from KD and point to individuals at higher risk for adverse health outcomes. Here, we provide a clinician-friendly review of state-of-the-art research on biomarkers of KD response for a variety of outcomes including weight loss, body composition and cognitive performance drawing data from both intervention trials and case reports of rare inborn errors of metabolism. We also present a selection of the most promising candidate genes to evaluate in future studies and discuss key aspects of study design and variant interpretation that may help accelerate the implementation of these biomarkers in clinical practice.

INTRODUCTION

As the field of precision medicine is gaining traction, nutrition research is experiencing a 'gold rush' for biomarkers that may enable the selection of personalised dietary interventions to maximise an individual's likelihood of successful response. These biomarkers include both genetic factors and dynamic biomarkers that respond to lifestyle factors such as physiological markers,[1] epigenetics and transcriptomics,[2] metabolomics[3] and the microbiome.[4] The use of biomarkers of dietary response would be of particular clinical relevance for the selection and individualised risk–benefit analysis of therapeutic diets, such as the ketogenic diet (KD), which provide alternative or complementary therapies to standard-of-care treatments.

KD is a low-carbohydrate, high-fat, adequate-protein diet that was historically used to treat epilepsy and diabetes a century ago prior to the discovery of insulin and antiseizure medications.[5 6] The latest scientific evidence showing that ketones are both preferred fuels and potent signalling molecules has dramatically increased the number of investigations into the basic science of ketones and their applications across broad disciplines. Ketones demonstrate pleiotropic actions affecting gene expression and pathways regulating inflammation, oxidative stress, immune function, membrane health, cell signalling and antioxidant status[7] that manifest in reversal of metabolic disease and extended healthspan. The most remarkable therapeutic evidence of KDs is the rapid and sustained reversal of obesity, metabolic syndrome (MetS) and type 2 diabetes in a plethora of recent published research. Preclinical and other evidence point to KDs as a promising therapeutic intervention in other chronic diseases including Alzheimer's disease (AD) and certain type of cancers.[8] KD induces a metabolic switch from using glucose to using fat as primary fuel for the body. There are different variations of KD with the most commonly used protocols recommending 30–50 g or less of dietary carbohydrate or 25–30 g of 'net carbs' (calculated by total carbohydrate minus fibre) per day.[9 10] Beta-hydroxybutyrate (BHB) and acetoacetate are the two main ketone bodies in blood that are produced from partial oxidation of fatty acids. Both ketone bodies and fatty acids are important sources of energy for the brain, heart, muscle, kidney and other tissues when glucose availability is limited such as during periods of carbohydrate restriction and fasting or when energy demands are increased, such as during illness or vigorous exercise.

A personalised lifestyle approach to KD would enable to maximise both therapeutic effectiveness and long-term safety for patients. Although KD has proven to be effective and safe for most people in trials up to 2 years,[11] the degree of therapeutic response in terms of weight loss, metabolic changes and neurological effects varies across individuals.[8 12] In addition to physiological factors such as sex and age, this variability likely reflects the interaction of genetic and lifestyle-related

factors including diet, insulin resistance (IR) and activity level. As KD and personalised nutrition are becoming more and more popular, genetic variants for the prediction of KD response are increasingly discussed in media articles, blog posts and direct to consumer genetic reports. However, most of these variants have not been evaluated in intervention trials of KD, which can mislead and confuse consumers and their health practitioners.

The main aim of this review is to provide clinicians and patients with a snapshot of clinically tested common single-nucleotide polymorphisms (SNPs) for the prediction of KD response, ranked by their strength of scientific evidence. These SNPs have been identified in intervention trials testing the effects of KD on various outcomes including weight loss, body composition and cognitive performance. In addition, we also provide a list of rare mutations with strong effects on KD response, either as a therapeutic indication or contraindication, and present a selection of the most promising candidate genes and approaches to evaluate in future studies. Finally, we discuss the importance of establishing common standards of study design and variant interpretation and delineate the steps we need to take towards the implementation of practice-based guidelines.

COMMON SNPS ASSOCIATED WITH METABOLIC OR NEUROLOGICAL OUTCOMES IN INTERVENTION STUDIES OF KD OR KETOGENIC AGENTS

Genetic variation may influence KD response by affecting the body's ability to process and use carbohydrates or fats. While rare mutations (frequency <1%) can produce strong effects on phenotype, common (SNPs, minor allele frequency ≥1%) result in more subtle effects that depend on the interaction with other genetic variants and environmental factors. Genome-wide association studies (GWAS) have identified many SNPs associated with interindividual differences in the response to high fat food intake. However, most of these GWAS were observational studies based on populations that consumed diets high in both fats and carbohydrates, often referred as to obesogenic high-fat diets (oHFD).[13] oHFD-associated SNPs, such as those in the PPARA gene,[14] are commonly but misleadingly listed as a contraindication to KD in many genetic interpretation tools available on the internet. Since the metabolic, hormonal and neurological effects of oHFDs are diametrically opposite to those of KD, we do not have any evidence for the use of oHFD-associated SNPs for the prediction of KD response.

Only a few intervention studies have tested the effects of oHFD-associated SNPs on weight loss or body composition in the context of a carbohydrate restriction sufficient to induce ketosis. In table 1, we provide a list of these SNPs as well as other SNPs that have been associated with interindividual differences in the neurological response to KD or ketogenic agents in other intervention studies. A strength of evidence score is provided for each of these SNPs based on recent guidelines for the interpretation of nutrigenetic variants.[15]

SNPs associated with weight loss and body composition outcomes

A study in 86 adults (53 overweight men and 33 normoweight/overweight women) found that SNPs in genes encoding for the metabolic enzymes gastric lipase (LIPF, rs814628-G), hepatic glycogen synthase (GYS2, rs2306179-C), cholesteryl ester transfer protein (rs5883-T) and galanin (rs694066-G) were significantly associated with a greater weight loss in response to KD (8%–13% CHO, 60%–63% fat and 28%–30% protein) over a period of 4–12 weeks.[16]

Another study compared the effects of KD (n=93, CHO ~12%) vs a low fat diet (LF, n=70, fat ~25%) on weight loss and body composition, and the association of these effects with genetic variants. The minor G allele of the rs5950584 SNP in the promoter region of the angiotensin II receptor type 2 (AGTR2) gene was associated with a greater reduction in body fat percentage in response to KD (CHO ~12%).[17] The AGTR2 gene has been implicated in the accumulation of fat and development of muscle cell IR induced by high fat, hypercaloric feeding in mice.[18] The AGTR2 gene is X-linked, thus men can have only one of either the T (major) or the G (minor) allele, while women may have zero, one or two copies of either allele. This means that the effect of this gene variant on fat loss with KD may be more prevalent and/or stronger in men than in women. The major G allele in the rs322695 SNP of the RARB gene was associated with greater reductions in body fat percentage within both the KD and LF groups. The RARB gene encodes the retinoic acid receptor beta, a thyroid–steroid hormone receptor which regulates energy production in the liver through modulation of gene expression. This observation is noteworthy in light of the putative role of the retinoic acid system in IR.[19] Other genetic variants were associated with a greater reduction in body fat percentage within the LF group only. These include the major G allele of the rs12691940 SNP in the HNMT gene, which has been implicated in appetite regulation in mice,[20] and the major G allele of the rs2838549 SNP in the PFKL gene, which produces a key regulatory enzyme for glycolysis known as hepatic phosphofructokinase.

SNPs associated with neurological outcomes

Using a discovery approach, a recent intervention study in epileptic children found that a SNP within the chromodomain Y 1 ligand, CDY1L, may affect the seizure KD response.[21] CDY1L is a chromodomain protein and histone acetyltransferase with important roles in the epigenetic regulation of gene expression in the brain. In this study, 232 epileptic children (age 3–9; male, n=131; female, n=121; 82% Caucasians) received KD for 3 months (classical KD, n=165; medium chain triglyceride (MCT) KD, n=48; modified KD, n=38). Children carrying at least one copy of the CDYL1 rs12204701-A allele, who

Table 1 SNPs for the selection of KD or exogenous ketone sources as therapeutic option with preliminary evidence from intervention studies

Clinical snapshot

Are these SNPs ready for clinical implementation?
These SNPs should be considered as candidate gene variants to evaluate and validate in clinical research rather than established predictors of KD response. The strength of scientific evidence for these SNPs is 'probable' or 'possible' using a scoring system based on recent guidelines for the interpretation of nutrigenetic variants.[15] A strength of evidence score is provided for each SNP in the table.

How can clinicians use these SNPs?
Rather than as a predictive test, clinicians may use these SNPs to set possible/probable expectations with patients for target outcomes such as weight loss, seizure reduction, or cognitive performance and develop an individualised protocol to achieve those goals (eg, macronutrient composition, total energy intake, use of exogenous ketone sources, duration of the intervention, etc). These SNPs may also be used as an explorative tool in clinic-based research for the design of personalised weight loss strategies using KD. Please refer to the 'KD response in intervention studies' column for further details.

How can clinicians help accelerate the clinical implementation of these SNPs?
Clinicians can contribute to the building of a nutrigenomics knowledge base and accelerate the clinical implementation of these SNPs by testing them in clinic-based research, keeping records of their research data, and promoting the establishment of curated databases of nutrigenetic SNPs where they can submit their research data.

SNPs associated with weight loss/body composition outcomes in intervention studies of response to KD

Effect allele	Allele frequency	Enzyme function	Outcome	Response to KD
LIPF rs814628-G		Gastric lipase is secreted by the mucosa of the stomach and hydrolyzes dietary triglycerides in the gastrointestinal tract. It is responsible for 30% of fat digestion in humans. The minor G allele of this SNP determines an amino acid change in the protein sequence (Ala161 >Thr), with possible decrease in enzymatic function.	Weight loss	Individuals with the minor G allele lost more weight than those homozygous for the major A allele in response to KD (8%–13% CHO, 60%–63% fat and 28%–30% protein) over a period of 4–12 weeks in a study in 86 adults. This suggests that decreased gastric fat breakdown on KD may enhance weight loss.[16] Strength of evidence: Possible.
GYS2 rs2306179-C		Hepatic glycogen synthase two catalyses the formation of glycogen from glucose in the liver. The minor C allele of this SNP is an intronic variant with unknown consequences on protein function.	Weight loss	Carriers of the minor C allele lost more weight than those homozygous for the major T allele in response to KD (8%–13% CHO, 60%–63% fat and 28%–30% protein) over a period of 4–12 weeks in a study in 86 adults. This suggest that the hepatic glycogen response to carbohydrate restriction may influence the weight loss response to KD.[16] Strength of evidence: Possible.
CETP rs5883-T		CETP regulates the reverse cholesterol transport, a process by which excess cholesterol is removed from peripheral tissues and returned to the liver. CETP may mediate the triglyceride lowering and remodelling effects of LDL and HDL observed with low carbohydrate diets (PMID: 15930434).	Weight loss	Carriers of the minor T allele lost more weight than those homozygous for the major C allele in response to KD (8%–13% CHO, 60%–63% fat, and 28%–30% protein) over a period of 4–12 weeks in a study in 86 adults. This suggest that the weight loss response to KD may depend on the metabolism of circulating lipoproteins.[16] Strength of evidence: Possible.
GAL rs694066-G		Galanin is an appetite hormone that stimulates food consumption, particularly fat intake. The minor A-allele of this SNP is an intronic variant with unknown consequences on protein function.	Weight loss	GG genotype increased weight loss compared with those who carried the minor A allele in response to KD (8%–13% CHO, 60%–63% fat, and 28%–30% protein) over a period of 4–12 weeks in a study in 86 adults. This suggest that a role of fat-mediated appetite hormones in determining the response to carbohydrate restriction.[16] Strength of evidence: Possible .

Continued

Table 1 Continued

Clinical snapshot				
AGTR2 rs5950584-G		The angiotensin 2 receptor is located primarily in the brain, adrenal medulla, heart and uterus where it counterbalance the effects of angiotensin II, a potent vasopressor hormone and a primary regulator of aldosterone secretion. In mice, AGTR2 has been implicated in the accumulation of fat and development of insulin resistance induced by high fat, hypercaloric feeding.	Weight loss response and reduction of body fat	The minor G allele was significantly associated with a greater reduction in body fat percentage in response to KD with ~12% of total energy from carbohydrates in a study with 93 adults.[17] Given that the AGTR2 gene is X-linked, its effects on fat loss with KD may be more prevalent and/or stronger in men than in women. Strength of evidence: Possible.
SNPs associated with neurological outcomes in intervention studies of response to KD/exogenous ketone sources				
Effect allele	**Allele frequency**	**Enzyme function**	**Outcome**	**Response to KD/exogenous ketone sources**
CDY1L rs12204701-A		CDY1L is a chromodomain protein and histone acetyltransferase acting as gene repressor and critical for the maintenance of cell identity. It has been implicated in seizure-related neurodevelopmental disorders.	Seizure reduction	The A allele of rs12204701 may alter the levels or function of CDYL1 with effects on gene expression regulation in the brain. Drug resistant epileptic patients with AA and AG genotype may experience lower (<50%) seizure reduction in response to KD with lower blood BHB (–10%), free carnitine (–23%), and lower acetylcarnitine (12%). Strength of evidence: Probable.
APOE rs429358-C		ApoE is the principal cholesterol carrier in the brain and helps clear both cholesterol and triglycerides from the bloodstream. There are at least three alleles of the APOE gene, called e2, e3, and e4, which produce three protein isoforms that differ in only two amino acid sites ApoE2 (Cys112/Cys158), ApoE3 (Cys112/Arg158) and ApoE4 (Arg112/Arg158). These structural differences result in different effects on lipid and glucose metabolism and chronic disease risk. The e4 allele, which is found in 30% of the general population, is associated with a 4-fold to 15-fold increase in Alzheimer's disease (AD) risk in the context of a Westernised lifestyle.	Cognitive performance	Ketones may function as an alternative fuel for neurons,[21] bypass the defects in cerebral glucose metabolism, and improve cognitive symptoms in AD patients. KD and MMKD may improve cognitive performance, AD biomarkers, and MetS in both e4 carriers and e3 homozygous.[28 29] With regard to exogenous ketone sources, e4 carriers experience smaller improvements in cognitive performance on MCT supplementation compared with e4 non-carriers, despite showing prolonged elevations of BHB levels (24% higher levels 2 hours after MCT administration). This suggests that e4 carrier may have a lower ability to utilise exogenous ketone sources, and thus derive cognitive benefits, compared with e4 non-carriers. Strength of evidence: Probable.[31 32]

SNP identification numbers (noted as "rs…") are the unique SNP identifiers from the NCBI dbSNP database.
AGTR2, angiotensin II receptor type 2; APOE, apolipoprotein E; BHB, beta-hydroxybutyrate; CDYL1, chromodomain Y-like protein 1; CETP, cholesteryl ester transfer protein; GAL, galanin; GYS2, glycogen synthase 2; HDL, low-density lipoprotein; KD, ketogenic diet; LDL, low-density lipoprotein; LIPF, lipase F; MetS, metabolic syndrome; MMKD, modified Mediterranean-ketogenic diet; NCBI, National Center for Biotechnology Information; SNP, single-nucleotide polymorphism.

represent 17% of the general population and almost 50% of Africans, experienced lower (≤50%) seizure reduction when following KD compared with non-carriers. The underlying mechanisms of this gene–diet interaction are unknown but might involve differences in KD-mediated gene expression changes in the brain.

Other neurological studies have used a candidate-gene approach to investigate the effects of genetic variation of the apolipoprotein E gene (APOE) on cognitive performance in response to KD or ketogenic agents in AD patients. ApoE is the principal cholesterol carrier in the brain and helps clear both cholesterol and triglycerides from the bloodstream. About 30% of people have at least one copy of the e4 allele, which has been associated with a 4-fold to 15-fold increase in AD risk compared with individuals with the more common e3 allele, while the e2 allele is related to decreased risk.[22] However, lifestyle plays a major role in modifying e4-associated AD risk as illustrated by the observation that, although the e4 allele is most frequently seen in African populations, the incidence of AD in elderly Nigerians is extremely low.[23] E4 carriers are more likely to develop AD if they have high IR,[24 25] which has been proposed to interact with the e4 variant to produce impaired glucose metabolism, insulin sensitivity and lipid transport in the brain leading to accumulation of amyloid beta and ultimately cognitive failure.[26] The underlying mechanisms of this interaction are not fully understood and may reflect biochemical differences between different ApoE isoforms such as susceptibility to glycosylation, effects on brain glucose metabolism through regulation of cytosolic hexokinase, and delivery of lipids to the brain.[27] The KD could alleviate the effects of these alterations and improve cognitive symptoms in patients with AD by reducing insulin signalling in peripheral tissues and the brain and providing ketones as an alternative energy source.

Preliminary evidence indicates that APOE genotype may affect cognitive performance in response to elevations in blood ketones depending on the modality of how ketosis is achieved (ie, through KD or ketogenic supplements), which can affect the kinetics of cellular uptake of ketone bodies and their blood levels. In a randomised, double-blind, cross-over trial comparing a modified Mediterranean-ketogenic diet (MMKD) vs American Heart Association Diet over a period of 6 weeks, MMKD improved AD biomarkers such as accumulation of brain amyloid in both e4 carriers and e3 homozygous with mild cognitive impairment (MCI) (n=17; age 58–70 y; 11 subjects with MCI and 6 cognitive normal) with no significant difference based on APOE genotype.[28] This is in line with a case report of an e4 heterozygous 71-year-old woman with mild AD and MetS who experienced significant improvements in both cognitive performance and MetS biomarkers (HOMA-IR, −75%; triglycerides, −50%; very low-density lipoprotein (LDL), −50%; Hemoglobin A1C (HgA1c), from 5.7% to 4.9%) after a 10-week protocol of KD, time-restricted eating and physical/cognitive exercise.[29] In contrast, greater carbohydrate intake has been associated with poorer performance in attention in e4 carriers and poorer performance in verbal memory in e4 non-carriers.[30]

On the other hand, the e4 variant may have an effect on cognitive performance in response to ketogenic agents in patients with AD. In a placebo-controlled trial in 20 patients with AD or MCI, the administration of MCT, 40 mL blended with 152 mL heavy whipping cream produced improvements in cognitive performance as measured by AD Assessment Scale-Cognitive Subscale (ADAS-cog) only in e3 homozygous but not in e4 carriers.[31] At the same time, e4 carriers experienced, counterintuitively, more prolonged elevations in ketone levels in response to MCT administration suggesting that their lower cognitive response may reflect lower cellular uptake or utilisation of ketogenic agents compared with e4 non-carriers. These findings were replicated in a larger randomised, double-blind, placebo-controlled study in 152 AD patients, which tested the cognitive effects of an MCT-based supplement composed of glycerin and caprylic acid (AC1202, 10 gr) administered over a period of 3 months. While AC1202 induced significant improvement in ADAS-cog in both e4 carriers and non-carriers, these effects were greater and significantly correlated with blood BHB levels only in e4 non-carriers.[32]

SNPs associated with blood lipids and cardiovascular health outcomes

One of the most clinically relevant and frequently asked question among health practitioners and patients is whether some genetic variant may predispose to cardiovascular disease (CAD) such as high LDL cholesterol (LDL-C) in the context of KD. Although several cholesterol-raising genetic variants have been identified and associated with higher CAD risk in the content of oHFD, there is still no evidence about whether these variants may constitute a contraindication to KD. Box 1 provides a brief discussion of this topic along with some practical tips for clinicians managing patients who experience an increase in LDL-C in response to KD.

INBORN GENETIC CONDITIONS WITH EFFECTS ON KD RESPONSE

While only a few common SNPs have been shown to affect KD response in clinical trials, several rare mutations (frequency ≤ 1%) can cause inborn errors of metabolism (IEM) and have strong effects on KD response, representing either an indication or contra-indication for its use. Online supplemental tables S1 and S2 provide an overview of these conditions and a selection of their most frequently reported mutations with a focus on their effects on KD response and levels of ketones and glucose in the blood.

KD is contraindicated and potentially lethal in individuals affected by IEMs that prevent the body from using ketones or fatty acids as fuel such as inborn defects of ketone metabolism as well as fatty acid oxidation disorders

Box 1 Cholesterol-raising genetic variants: a contraindication for ketogenic diet (KD)?

Approximately one in four people experience increases in low-density lipoprotein cholesterol (LDL-C) when following KD. These individuals are often referred as to 'high responders'. While the biological mechanisms of this response are still unclear, we know that certain genetic variants may predispose some people to produce more cholesterol or reduce its blood clearance on a high fat diet. Several LDL-C-raising common single-nucleotide polymorphisms (SNPs) and rare mutations have been identified and linked to metabolic and cardiovascular disease in the context a Westernised obesogenic high fat diet (oHFD) high both in fats and refined carbohydrates. Examples of well-characterised common SNPs associated with higher LDL-C and/or adverse metabolic traits in the context of oHFD include lipase C, hepatic type (LIPC) rs1800588 (high LDL/high-density lipoprotein (HDL) ratio),[50–52] APOA2 rs5082 (high body mass index),[37 53–55] APOA4 rs675 (higher LDL-C and ApoB),[56] PPAR-alpha rs1800206 (high LDL-C and triglycerides (TG) with high saturated fat intake and low polyunsaturated fat intake),[14 51 57 58] as well as non-coding variants such SORT1 rs12740374 (high LDL-C and very LDL particle number).[59] Examples of rare LDL-C raising variants are those with genes implicated in familial hypercholesterolemia (FH) and other phenotypically related lipid disorders such as LDLR, APOB, PCSK9, LDLRAP1,[60–62] LIPA,[63] ABCG5, ABCG8[64] and apolipoprotein E.[65 66]

However, there is still no evidence about whether these variants may constitute a contraindication to KD, although some direct-to-consumer genetic reports make these claims. Some studies indicate that the increase in LDL-C observed on KD and other low-carb high-fat diets reflects an increase in LDL-C particle size rather than number, a change that is associated with reduced cardiovascular risk and accompanied by an improvement in other cardiovascular risk factors such as TG and HDL.[67–70] On the other hand, even large LDL-particles can become atherogenic in presence of genetic factors that reduce LDL clearance such in the case of FH and other lipid disorders.

Therefore, we still do not have an answer to the question clinicians care most about: What patients would do better on KD and what patients would do worse? In absence of evidence-based guidelines, clinicians who manage patients who are 'hyper-responders' to KD typically follow a pragmatic approach involving the assessment of additional cardiovascular markers (eg, LDL particle number, TG, HDL, coronary calcium score) as well as family history of cardiovascular disease. If these markers are altered, patients may consider stopping KD or starting a cholesterol lowering medication. With patients who express a desire and willingness to give KD a last chance, a common practice is to have them reduce the amount of red meat[71] and replace saturated fats with unsaturated fats and experiment (Ethan Weiss, personal communication).

(FAODs) (online supplemental table S1). A special case of FAOD is CPT1A deficiency due to the so-called arctic variant, which is the most common CPT1A allele (68%–85%) in indigenous arctic populations but is extremely rare in the general population (box 1). In contrast to the mutations causing primary CPT1A deficiency, which completely abolish enzymatic function, the arctic variant has modulatory effects on CPT1A that decrease its baseline activity but increase its activation in the context of a diet high in omega-3 fatty acids and low in carbohydrates such as that traditionally consumed by Arctic populations. This might explain its positive selection in these populations and the potential risks that this variant may pose in view of the recent shift to a Western diet in the Arctic (box 2).

Other inborn conditions provide an indication for the use of KD as either a first-line therapy or adjunct therapy to improve symptoms and outcomes (online supplemental table S2). These conditions include glucose transporter 1 (GLUT1)-deficiency syndrome, pyruvate dehydrogenase (PDC) deficiency, drug-resistant epilepsy, glycogen storage disease, mitochondrial disorders, urea cycle disorders, purine metabolism disorders and amino acid metabolism disorders. Most of these conditions, with the exception of glycogen storage disease, present with seizures, impaired brain function and neurological symptoms that show significant improvements on KD through complex yet not fully elucidated mechanisms.

TOWARDS EVIDENCE-BASED RECOMMENDATIONS

There is still insufficient evidence for the use of genetic variants for the prediction of KD response. While some variants can have strong effects on KD response, these variants are found in rare congenital disorders and are therefore not relevant for the majority of the population (online supplemental tables S1 and S2). On the other hand, common SNPs that are widely represented in the population have small effects sizes and work together with other gene variants and lifestyle factors to affect complex trait such as KD response (table 1). Future studies with robust design and adequate power should replicate and quantify the associations observed in intervention studies of KD response, test additional promising candidate gene variants (online supplemental table S3), and identify new variants. This will ultimately facilitate the development of prediction models that quantify the contribution of each of these variants on KD outcomes as well as their interaction with dietary intake and other risk factors.

Candidate genes

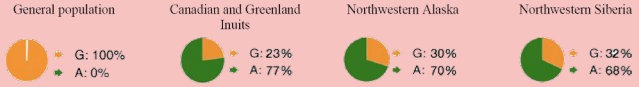
Promising candidate SNPs include those associated with KD response in intervention trials (table 1), as well as additional SNPs presented in online supplemental table S3, which we have selected based on the two following criteria: (1) location within genes that are mutated in rare diseases with strong effects on the metabolism of ketones and fats (online supplemental tables S1 and S2) and (2) associations with metabolic traits in observational studies.

The discovery of new genetic variants in large well-designed GWAS will be critical for the development of more accurate prediction models of KD response for personalised patient profiling. Data sets from large intervention GWAS could be used for predicting an individual's likelihood of successful response before the start of a KD intervention using models that integrate baseline factors (eg, age, height, sex, baseline weight) and outcome-related variables (eg, target outcomes such as macronutrient composition, total energy intake, expected weight loss, seizure reduction, etc). For example, a model of this kind was able to predict with a ~80% accuracy the

Box 2 The 'Arctic variant of CPT1A

One of the strongest selective sweeps in human evolution

The so-called 'arctic variant' of CPT1A (rs80356779 G>A; c.1436C>T or Pro479Leu on the reverse strand) is the most common allele (68% to 85%) in indigenous arctic populations (Alaska, Greenland, Northern Canada, and northeastern Siberia)[72–76] but is absent in other publicly available genomes.[72] A strong positive selection, a 'selective sweep', has driven this variant to high frequency in circum-Arctic populations, possibly as a result of the selective advantage it originally provided to a high-fat/low carbohydrate diet or cold environment.[75]



Effects on enzyme function and dietary context

In contrast to the mutations causing primary CPT1A deficiency, which completely abolish enzymatic function, the arctic variant has two counteracting effects on CPT1A activity that interact with dietary factors. While the variant induces a 25%–50% reduction in CPT1A activity,[73] it also reduces CPT1A sensitivity to inhibition by malonyl-CoA in response to insulin signaling.[77 78] As a result, given that the traditional arctic diet was rich in nutrients that activate CPT1A, the enzyme produced by the arctic variant might have been even more active than the wild-type one.[78] The traditional arctic diet was not only high in fat and low in carbohydrates but also four times higher in omega-3 polyunsaturated fatty acids (PUFAs) compared with a standard Western diet.[79] Omega-3 PUFAs are strong activators of CPT1A and have been shown to increase ketone levels twice as much as saturated fat in humans.[80 81]

How ketogenic diet (KD) might have driven the selective sweep of the arctic variant

What might have driven the selective sweep of the arctic variant? Beneficial health effects have been observed in Greenland Inuits (protection from atherosclerosis)and[76] in Yup'ik Eskimos (lower adiposity with higher high-density lipoprotein)[82] In contrast, homozygous infants eating non-traditional diets have higher mortality rate due to infectious disease and intolerance to fasting (lower ketone production and hypoglycaemia).[83 84] This paradox might be explained by the need to adapt to long-term KD during evolution and the recent shift to a Western diet in the Arctic. One hypothesis, which frames ketosis as a potentially harmful state, is that the advantage of the arctic variant was to decrease ketosis through reduction of CPT1A activity thus preventing the risk of ketoacidosis.[85] Another hypothesis is that the arctic variant would have enabled to maintain ketosis despite the high protein content of traditional Arctic diets[86 87] through lower inhibition of CPT1A by malonyl-CoA.[73] If true, this second hypothesis would support the role of ketosis as essential biochemical state in the context of carbohydrate restriction. We still don't know which, if any, of these hypotheses may be true since most of the available evidence is based on studies in children eating non-traditional diets. In any case, it is likely that the traditional arctic diet might have provided an environment that maximised the beneficial effects of the arctic variant, whereas the shift to a Western diet might have unmasked its potential risks.

Toward evidence-based recommendations for homozygous infants

Rather than being a cause of infant death, the arctic variant is likely to be only one of a complex set of contributing factors due to the shift to a Western diet rich in refined carbohydrates and low in omega-3 PUFAs.

Continued

Box 2 Continued

A prospective cohort study is currently testing the hypothesis that pre- and postnatal exposure to n-3 PUFAs may reduce the adverse effects of the arctic variant in Alaska Native children. The results of this study may shed some light on the gene-diet interactions that might have driven the selective sweep of the arctic variant and facilitate the development of evidence-based recommendations for homozygous infants identified by newborn screening.[88] Public recommendations to increase feeding with nontraditional carbohydrate-rich diets are not based on evidence and may have unintended harmful consequences.[78]

probability of losing >5% of body weight after following for 1 year, one of four hypocaloric diets with carbohydrate composition ranging from 35% to 65% using data from the first 3 months of weight loss in the POUND-LOST trial.[33]

Polygenic scores

The most relevant GWAS of KD response could also be used to develop polygenic scores, which enable greater power than individual SNPs for detection of interactions with environmental factors, including diet. Polygenic scores provide a more quantitative metric of an individual's genetic likelihood to express a given trait, such as a certain KD response, based on the cumulative impact of many common SNPs. Weights are generally assigned to each SNP according to the strength of its association with a given trait (effect estimate). Individuals are scored based on how many risk alleles they have for each variant (eg, zero, one, two copies) included in the polygenic score. Recent studies have shown that polygenic scores can greatly enhance the ability to identify clinically meaningful variations in the predisposition to common lifestyle-related diseases such as coronary artery disease, type 2 diabetes and obesity.[34 35] These advances have been made possible thanks to the conduction of GWAS with large sample size enabling more precise effect estimates, the development of algorithms that combine genome-wide sets of variants, and the availability of large biobanks for validation and testing. Using the same tools, it is possible to develop polygenic scores for the prediction of diet response that would complement those for disease risk prediction by facilitating the design of personalised diet interventions for individuals at higher polygenic risk for certain lifestyle-related diseases.

Dynamic biomarkers

Another promising approach is to apply a systems genetics framework to integrate polygenic gene prediction with dynamic biomarkers such as metabolic, epigenetic, transcriptomic profile and the microbiome to identify 'hub genes' that regulate gene networks interacting with environmental factors. These dynamic biomarkers rapidly respond to dietary manipulation and interact with both physiological factors (eg, sex, age) and lifestyle-related factors (IR, activity level, stress, etc). Once implemented, ketogenic therapies require oversight, especially in

vulnerable patient populations. Therefore, biomarkers of compliance and efficacy that can be monitored by patients and their medical teams are important to develop. One such proposed tool is the Glucose:Ketone Index (GKI), a value used to describe the ratio of glucose to ketones (in mmol/L) in the blood of individuals on ketogenic metabolic therapies. Preliminary data from animals and small human trials suggest that GKI may successfully predict therapeutic efficacy of the KD in brain cancer. Similarly, subjective qualitative markers such as self-reported appetite, energy levels and sleep quality may also be used to predict candidacy, compliance and efficacy of ketogenic therapies. Impairments in one or more of such areas may support initiation, alteration, or discontinuance of the KD, depending on the individual patient and their specific lifestyle and medical needs.

Since people vary widely in their response to a given amount of carbohydrate, biomarkers may be useful in determining who is most appropriate for a KD, or what level of carbohydrate restriction is required to enable weight loss, disease reversal or other desired outcomes. One such marker is the monounsaturated fatty acid palmitoleic acid (cis-16:1n7). Palmitoleic acid is the primary fatty acid product of de novo lipogenesis (DNL). It is produced from palmitic acid by stearoyl CoA desaturase-1, increasingly proportionally more than any other fatty acid when carbohydrate is provided in excess driving up DNL.[36] Since palmitoleic acid is relatively low in the diet, it's abundance can be useful as proxy of the metabolic pathway that converts carbohydrate to fat, with the caveat that very high intakes of specific palmitoleic acid-rich foods (eg, macadamia nuts and avocado) may confound this association. Beyond its role as a surrogate for DNL, greater abundance of palmitoleic acid in the circulation or in tissue membranes is strongly linked to a host of metabolic derangements including obesity,[37] MetS,[38 39] type 2 diabetes,[40–42] heart failure[43 44] and CVD mortality.[45 46] Thus, higher proportion of plasma palmitoleic acid is an early indication of carbohydrate intolerance (ie, more carbohydrate directed toward DNL), and an independent risk factor for diabetes and CVD.

We have reported in multiple studies that there is a remarkable stepwise uniformity in the response of circulating palmitoleic acid to varying level so of carbohydrate. Palmitoleic acid consistently decreases when carbohydrates are restricted, especially KDs, and increases when more carbohydrate is consumed.[47–49] This effect is not significantly altered by weight loss or sex. Notable, is the fact that at any given level of carbohydrate intake palmitoleic acid levels vary, likely reflecting hereditary factors manifesting in an IR (ie, carbohydrate intolerant) phenotype. Whereas the direction of palmitoleic acid change is consistent as people add more carbohydrate to their diet, the magnitude varies. As such, if a person is able to maintain a relatively low level of palmitoleic acid it suggests they are 'appropriately' disposing of carbohydrate through oxidative pathways as opposed to DNL. In contrast, if palmitoleic acid levels are high or are rising it suggests that the current level of carbohydrate intake is not being managed in a healthy manner. In this way, palmitoleic acid could be used to titrate a personalised level of carbohydrate consumption.

Establishing common standards of study design and variant interpretation

While this review focuses on genetic variants of KD response, it is also a call to action for researchers, clinicians and funding institutions to establish common standards for study design and variant interpretation for the field of nutrigenomics as a whole. This will enable the building of a nutrigenomic knowledge base and accelerate the implementation of nutrigenomics in precision medicine.

The scientific validation of nutrigenetic variants will require the use of large sample size, common definitions for dietary protocols, and patient stratification to evaluate heterogeneity of response in different patient subgroups. Large sample sizes will minimise both false positive and false negative associations and enable the replication of the most significant associations across different studies. Reproducibility of results will also require the establishment of common standards to define what constitute a 'high-fat', 'low-carb' or 'ketogenic' diet—definitions that are highly variable from study to study—as the effects of high fat intake are heavily dependent on the quantity and quality of dietary carbohydrates.[13] A valuable method of defining a KD may be one that elevates ketones above approximately 0.5 mM. Future studies of KD response should also evaluate modified KD protocols tailored to different subgroup of people with the goal of both personalising and expanding the applications of KD in clinical settings.

The clinical implementation of genetic variants of KD response, and nutrigenetic variants in general, will also require the development of a rigorous framework for variant interpretation. A model for such a framework is the partnership between ClinGen and ClinVar, two efforts of the National Institutes of Health to support genomic interpretation and implementation. The ClinGen programme employs Expert Panels to assess the clinical validity and actionability of disease-related gene variants, which are then shared with the public through the ClinVar database. Building a similar framework for the interpretation of nutrigenetics variants will require several steps including: (1) the development of common guidelines such as those proposed by Grimaldi *et al*[15] and establishment of Expert Panels for the identification of scientifically valid and clinically useful variants; (2) public sharing and crowdsourcing of variant submission from clinical testing labs, research institutions, public databases and professional societies; (3) the creation of working groups to refine and update guidelines as they are tested and deployed by the community. Taking these steps will greatly accelerate the implementation of nutrigenetics in clinical practice.

Twitter Lucia Aronica @LuciaAronica and Dominic P D'agostino @DominicDAgosti2

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ORCID iD

Lucia Aronica http://orcid.org/0000-0002-8991-4858

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Table S1. Rare genetic conditions for which the KD is contraindicated

Clinical Snapshot

Why KD is contraindicated?

These rare mutations lead to defects in ketone production (ketogenesis) or breakdown (ketolysis) that impair the body's ability to use ketones as fuel. **Clinical signs and symptoms** These disorders manifest with acute episodes of metabolic decompensation during any condition that causes hypoglycemia and subsequent increase in increase the body's demand for ketone bodies and fatty acids (e.g. carbohydrate restriction or prolonged fasting, exercise, and times of physiologic stress, such as illness, sleep deprivation, or extreme weather). These episodes present with abnormal levels of ketones and/or glucose in the blood (hypoketotic hypoglycemia or ketoacidosis), metabolic acidosis, and toxic effects on the brain leading to vomiting, dehydration, difficulty breathing, lethargy, seizures and coma.

Therapeutic considerations

Carbohydrates

While affected individuals should consume carbohydrates as part of their diets, especially before and during exercise, those who over-consume carbohydrates in hope of preventing acute attacks may be at higher risk of developing obesity and associated disorders with no proven benefit for the underling metabolic issue.

Dietary fatty acids and MCT supplementation for FAODs

Long-chain FAODs (i.e. deficiency of CPT2, CACT, VLCAD, and LCHAD) may benefit from reduction of dietary long-chain fatty acids and supplementation with MCT (15%– 18% of total calories) — which can bypass the block in long-chain FAO and improve exercise tolerance when administered before exercise (0.5 g/kg lean body weight) [PMID: 21763168, PMID: 22030098]. For LCHAD patients, MCT preparations with a higher ratio of decanoate to octanoate may be most effective to reduce the accumulation of potentially toxic long-chain 3-hydroxy-fatty acid [PMID: 12621125]. Infant formula should have reduced content of long-chain fatty acids plus MCT with continuous feeds for CACT neonates or every 2–3 hours during the day and continuous at night. In patients with primary carnitine deficiency, carnitine supplementation may be provided at a dose of 200 to 300 mg per kilogram of body weight divided throughout the day. In patients with pyruvate carboxylase deficiency, supplementation with thiamine has been shown to ameliorate symptoms likely by facilitating an alternative mechanism for pyruvate oxidation. Thiamine pyrophosphate is the coenzyme for pyruvate dehydrogenase, a key enzyme for an alternate route of pyruvate

Inborn errors of ketogenesis and ketolysis

Condition	Prevalence	Gene & best characterized mutations	Enzyme function & clinical signs	BHB levels	Blood Glucose
Mitochondrial HMG-CoA synthase 2 deficiency	< 20 patients reported worldwide	HMGCS2 rs137852636 rs137852637 rs137852638 rs137852639 rs28937320 rs137852640	Mitochondrial HMG-CoA synthase 2 catalyzes the condensation of acetyl-CoA and acetoacetyl-CoA to form HMG-CoA in the first steps of ketogenesis in the liver. Patients are generally asymptomatic unless during fasting or infection, which makes the diagnosis very difficult. Clinical manifestations include severe hypoketotic hypoglycemia, encephalopathy, and hepatomegaly. Genetic testing is required to confirm the diagnosis.	Low	Low
HMG-CoA lyase deficiency	About 100 cases reported in Saudi Arabia, Portugal, Spain	HMGCL rs752137615 rs121964996 rs121964997 rs121964998 rs786205431	HMG-CoA lyase catalyzes the formation of acetoacetate from HMG-CoA within the mitochondria in the liver and is required for the catabolism of the amino acid leucine in dietary proteins. Clinical acute symptoms usually appear within the first year of life often triggered by fasting, infection, or other types of stress. However, some patients can develop hypoglycemic crises and neurological symptoms even in adolescence or adulthood. They include: hypoketotic hypoglycemia due to	Low	Low

			impaired ketone production, organic acid buildup and metabolic acidosis due to defective breakdown of leucine, vomiting, seizures, and lethargy. If untreated, the disorder can lead to breathing problems, convulsions, coma, and death.		
SCOT deficiency	< 35 cases reported	OXCT1 rs121909299 rs121909300 rs267606930 rs75134564 rs121909301 rs121909302 rs121909303	SCOT/OXCT1 plays a central role in extrahepatic ketone body catabolism by catalyzing the reversible transfer of coenzyme A (CoA) from succinyl-CoA to acetoacetate. Impaired function of SCOT/OXCT1 results in inability to break down ketones outside the liver. This can result in permanent ketosis and attacks of ketoacidosis during illness or stress. While patients with "mild" SCOT mutations may be asymptomatic and have non-ketotic periods, those with more severe enzymatic defects can present persistent ketonemia and ketonuria.	High/ very high	High or low
Beta-ketothiolase deficiency	1 in 1 million	ACAT1 rs120074140 rs120074141 rs727503796 rs145229472 rs1131691567 rs1280110907 rs120074142 rs120074143 rs120074144 rs387906282 rs387906283 rs120074145 rs120074146 rs120074147 rs120074148 rs779565865	Mitochondrial ACAT1, also known as beta-ketothiolase or T2, carries out the last step of ketolysis in extra-hepatic tissues. In this reversible reaction acetoacetyl-CoA is broken down into two molecules of acetyl-CoAa, which can be used to produce energy. In the liver, ACAT1 carries out this chemical reaction in reverse, which is the first step in ketogenesis, and helps break down the amino acid isoleucine. Acute symptoms manifest during ketoacidotic attacks between the ages of 6 months and 24 months, which are often triggered by fasting, infection, or increased intake of protein-rich foods. Affected children present hyperketotic hypoglycemia and metabolic acidosis due to buildup of organic acids in the blood, which can damage the body's tissues and organs, particularly the nervous system. The resulting toxic encephalopathy can lead to vomiting, dehydration, difficulty breathing, lethargy leading to coma, and seizures. The clinical outcome greatly improves if the disease is diagnosed in the first ten days of life.	High/ very high	High or low
Fatty acid oxidation disorders (FAODs)					
Condition	**Prevalence**	**Gene & best characterized mutations**	**Enzyme function & clinical signs**	**BHB levels**	**Blood Glucose**
Primary carnitine deficiency	(1:200,000)	SLC22A5 rs72552727 rs121908887 rs386134217 rs72552735 rs121908888 rs121908889	Solute Carrier Family 22, Member 5 (SLC22A5) is a transmembrane protein that transports carnitine into cells in a sodium-dependent manner. Cells need carnitine to bring fatty acids into mitochondria to produce energy in form of ATP. Mutations in the SLC22A5	Low	Low

		rs121908890 rs68018207 rs121908891 rs121908892 rs121908893 rs267607054 rs267607053 rs267607052	gene result in an absent or dysfunctional SLC22A5 transporter leading to low intracellular and blood levels and urinary loss of carnitine. This results in reduced energy production within mitochondria, muscle weakness and hypoglycemia. Fatty acids may also build up in cells and damage the liver, heart, and muscles leading to hypertrophic cardiomyopathy, congestive heart failure, arrhythmias, sudden death, hypotonia, muscle weakness. Symptoms typically appear during infancy or early childhood but some people are asymptomatic.		
CPT1A deficiency	(1:50–100,000)	CPT1A rs80356778 rs80356787 rs80356774 rs80356790 rs80356791 rs80356798 rs1169875761 rs28936374 rs80356800 rs80356780 rs80356779	The CPT1A enzyme attaches carnitine to long-chain fatty acids to form acylcarnitines that can cross the inner membrane of mitochondria. Once these fatty acids are inside the mitochondria, they can be metabolized to produce energy after the remotion of carnitine. Mutations in the CPT1A gene reduce or eliminate the activity of the CPT1A enzyme (residual enzyme activity between 0 and 10%). As a result, carnitine is not attached to long-chain fatty acids, which cannot enter mitochondria and be converted into energy. This leads to low levels of ketones and glucose in blood (hypoketotic hypoglycemia). Fatty acids may also build up in cells and damage the liver, heart, and brain leading to cardiomyopathy (infantile form), congestive heart failure, muscle weakness, rhabdomyolysis, and exercise intolerance.	Low	Low
CPT2 deficiency	Myopathic form: >300 cases; severe infantile form (hepatocardiomuscular): ~30 families; lethal neonatal form: ~18 families.	CPT2 rs74315293 rs74315294 rs28936375 rs28936376 rs28936673 rs28936674 rs74315295 rs74315296 rs397509431 rs74315297 rs74315298 rs74315299 rs74315300 rs121918528 rs2229291 rs1799821	The CPT2 enzyme removes carnitine from fatty acids that have entered the mitochondria (acylcarnitines) and adds coenzyme A to form acyl-CoA esters that can be broken down to produce energy. Mutations in the CPT2 gene reduce the activity of the CPT2 enzyme. As a result, long-chain fatty acids remain attached to carnitine as acylcarnitines and cannot be metabolized to produce energy. This leads to low levels of ketones and glucose in blood (hypoketotic hypoglycemia). Fatty acids and long-chain acylcarnitines may also build up in cells and damage the liver, heart, and muscles causing the other signs and symptoms of the disorder. Mutations that lead to extremely reduced enzyme activity typically cause the more severe forms of CPT II deficiency (a lethal neonatal form and a severe infantile hepatocardiomuscular form), while those that result in partially reduced enzyme	Low	Low

			activity usually lead to a less severe myopathic form of the disorder. [1]		
CACT deficiency	~30 cases	SLC25A20[1] rs587776759 [1]rs1553686314 rs151340616 rs587776760 rs541208710 rs28934589 rs587777286 rs587777287	CACT transports long-chain fatty acids attached to carnitine (long-chain acylcarnitines) across the inner mitochondrial membrane as part of the carnitine shuttle system. Once acylcarnitines are inside the mitochondria, CACT removes carnitine, and transports it back out of mitochondria.[1][2]Mutations in the SLC25A20 gene reduce the activity of the CACT protein. As a result, long-chain fatty acids cannot be transported into mitochondria and converted to energy. This leads to low levels of ketones and glucose in blood (hypoketotic hypoglycemia). Fatty acids and long-chain acylcarnitines may also build up in cells and damage the liver, heart, and muscles leading to hypertrophic cardiomyopathy, congestive heart failure, arrhythmias, and muscle damage. Because neonates depend largely on metabolism of long-chain fatty acids for energy, children with severe CACT deficiency have a poor prognosis, with most dying before 1 year of age. Some affected individuals have a less severe form of the condition and do not develop signs and symptoms until early childhood. These individuals are at risk for liver failure, nervous system damage, coma, and sudden death.	Low (neonatal, severe)	Low (neonatal, severe)
MCAD deficiency	(1:10–15,000)	ACADM[1] rs77931234[1]rs1225471006 rs121434274 rs121434275 rs121434276 rs121434277 rs387906297 rs864621963 rs121434278 rs121434279 rs121434280 rs121434281 rs121434282 rs121434283 rs74090726	MCAD is required to metabolize a group of fats called medium-chain fatty acids (MCTs). These fatty acids are found in foods and body fat and are produced when longer fatty acids are metabolized. MCAD catalyzes the initial reaction in the beta-oxidation of C4 to C12 straight-chain acyl-CoA esters. [1][2]Mutations in the ACADM gene reduce the activity of the MCAD protein. The resulting defect in the oxidation of MCTs to acetyl-CoA, which is used to produce ketones, can lead to hypoketotic hypoglycemia and lack of energy (lethargy), particularly during periods of fasting, although some individuals remain completely asymptomatic in absence of significant metabolic stress. MCTs or partially metabolized fatty acids may build up in tissues, damage the liver and brain, inhibit gluconeogenesis, and produce metabolic acidosis. This abnormal buildup causes the other signs and symptoms of MCAD deficiency. These include muscle weakness, exercise intolerance, rhabdomyolysis. Symptoms typically appear during infancy or early childhood.	Lack or only trace of urinary ketones	Low

			Possible complications include seizures, breathing difficulties, liver problems, brain damage, coma, and sudden death.		
VLCAD deficiency	(1:40-80,000)	ACADVL rs113690956 rs118204014 rs387906249 rs387906251 rs387906252 rs118204015 rs2309689 rs118204016 rs387906253 rs28934585 rs118204017 rs118204018 rs118204016	VLCAD is bound to the inner mitochondrial membrane, where it catalyzes the first intramitochondrial step of the oxidation of long-chain fatty acids to acetyl-coA for the production of ketones and energy.Mutations in the ACADVL gene severely reduce or abolish the activity of the VLCAD enzyme. Like with other FAO disorders, this leads to hypoketotic hypoglycemia and lethargy, particularly during periods of fasting. Very long-chain fatty acids or partially metabolized fatty acids may build up in tissues and damage the heart, liver, and muscles leading to the other signs and symptoms of VLCAD deficiency. These include hypertrophic ardiomyopathy, arrhythmias, sudden death, muscle weakness, exercise intolerance, recurrent rhabdomyolysis, hypoketotic hypoglycemia, and “Reye-like” hepatic syndrome.	Lack or only trace of urinary ketones	Low or normal
SCAD deficiency	(1:35,000-50,000)	ACADS rs121908003 rs61732144 rs121908004 rs57443665 rs28940872 rs1800556 rs1799958 rs121908005 rs387906308 rs28940874 rs121908006 rs28941773 rs28940875 rs147442301 rs387906950 rs387906951	Acyl-CoA dehydrogenase short chain (ACADS) or SCAD catalyzes the first steps in the oxidation of short-chain fatty acids (SCFA) to acetyl-CoA, which is used to produce ketone bodies that can supply the energy needs to compensate for the lack of adequate glucose in presence of hypoglycemia.SCAD deficiency is viewed as a biochemical phenotype rather than a disease, and some people never develop any symptoms. When SCAD activity is reduced, short-chain fatty acids are not converted into energy, whereas some ketone formation can still occur. This can lead to hypoglycemia with normal or elevated ketones, lethargy, and muscle weakness. Metabolic decompensation is typically triggered by low blood sugar (e.g. fasting or increased energy expenditure due to a catabolic state such as infection, surgery, fever, etc.), which mobilizes FFAs for oxidation to acetyl-CoA and production of ketone bodies. The accumulation of fatty acid intermediates can also inhibit gluconeogenesis leading to metabolic acidosis with elevated ketone levels and toxic effect on the liver. Two distinct clinical phenotypes have been identified. One type has been observed in infants with acute acidosis and muscle weakness (generalized); the other has been observed in middle-aged patients with chronic myopathy (skeletal muscles).	High	Low

LCHAD	1: 62,000 (Finnish population, probably much lower in US)	HADHA rs137852769 rs137852770 rs786205088 rs781222705 rs137852771 rs137852772 rs137852773 rs137852774 rs137852775	Hydroxyacyl-Coa Dehydrogenase, Alpha Subunit (HADHA) is part of a protein complex called mitochondrial trifunctional protein, which is required to break down long-chain fatty acids. Four alpha subunits are produced from the HADHA gene, and four beta subunits are produced from the HADHB gene. Like other FAO disorders, LCHAD deficiency due to HADHA mutations leads to hypoketotic hypoglycemia and lethargy, particularly during periods of physiological stress such as fasting, illnesses, or weather extremes. Long-chain fatty acids or partially metabolized fatty acids may also build up in the liver, heart, muscles, and retina, inhibit gluconeogenesis, and produce metabolic (including lactic) acidosis. Affected infants and children usually present by 2 years of age. Muscle, particularly myocardium, requires a lot of energy and, therefore, becomes functionally impaired resulting in lethargy, hypotonia, cardiomyopathy, and risk of sudden death. Early-onset symptoms include cardiomyopathy, hypoglycemia, neuropathy, and pigmentary retinopathy. Later in childhood, people may experience muscle pain, breakdown of muscle tissue, and a loss of sensation in their arms and legs (peripheral neuropathy).	Lack or only trace of urinary ketones	Low
Pyruvate carboxylase deficiency	1 in 250,000	PC rs28940589 rs28940590 rs28940591 rs113994143 rs119103241 rs119103242	Pyruvate carboxylase is active in mitochondria, where it is involved in gluconeogenesis in kidneys, liver, and pancreas, where it helps regulate insulin secretion, lipogenesis in adipose tissue, and synthesis of neurotransmitters and myelin in the brain. In newborns, acetyl-CoA derived from pyruvate metabolism is an important source of energy. Pyruvate carboxylase deficiency leads to defective production of glucose through gluconeogenesis and accumulation of lactic acid and ammonia, which damages organs and tissues. Ketone levels are increased, especially during any condition leading to hypoglycemia such as carbohydrate restriction and prolonged fasting. Myelin formation and neurotransmitter production are also impaired, contributing to the neurologic features of pyruvate carboxylase deficiency.	High	Low
ALAD deficiency porphyria	Only ~10 cases, all males, have been reported worldwide. This is in contrast to	ALAD rs121912980 rs121912981 rs1800435 rs121912982	ALAD combines two molecules of delta-aminolevulinic acid (ALA) to form porphobilinogen (PBG) for the production of heme. Heme is vital for all of the body's organs, although it is found	Not affected	Not affected

	the other acute porphyries, in which more women are symptomatic.	rs121912983 rs121912984 rs749066913	mostly in the blood, bone marrow, and liver. [1] [2] Hereditary ALAD deficiency is extremely rare whereas acquired forms due to enzymatic inhibition through heavy metal (e.g. lead) poisoning, are more common. ALAD deficiency results in build-up of toxic levels of ALA in the body leading to acute attacks characterized by abdominal pain, vomiting, muscle weakness, seizures, fever, and neurological symptoms such as anxiety and hallucinations. These signs and symptoms can be life-threatening, especially if the muscles that control breathing become paralyzed. Any condition leading to hypoglycemia such as prolonged fasting and very low carbohydrate diets, can trigger acute attacks in some undiagnosed and non-symptomatic individuals by increasing excretion of heme precursors.

Abbreviations. SNP identification numbers (noted as "rs...") are the unique SNP identifiers from the NCBI dbSNP database. HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; SCOT: Succinyl-CoA:3-ketoacid CoA transferase; OXCT1: 3-oxoacid CoA-transferase 1; ACAT1: Acetyl-CoA acetyltransferase 1; FAOD: fatty acid oxidation disorders; FFAs free fatty acids; CPT1A: Carnitine palmitoyltransferase 1A; CPT2: carnitine palmitoyltransferase 2; CACT: carnitine-acylcarnitine translocase; MCAD: medium-chain acyl dehydrogenase; VLCAD: very long-chain acyl dehydrogenase deficiency; SCAD: short-chain acyl dehydrogenase; LCHAD: long-chain 3-hydroxyacyl-CoA deficiency; MCTs: medium-chain fatty acids; ALAD: Delta-aminolevulinic acid dehydratase; BHB: beta-hydroxybutyrate. Blood levels of BHB and glucose refer to those observed during metabolic decompensation unless otherwise stated.

Table S2. Rare genetic conditions for which KD may be indicated

Clinical Snapshot

Why KD is indicated?
KD is a first-line therapy in children with GLUT1-DS and PDCD, which impair the production of energy from glucose thus leading to alterations in brain development and function. In both conditions, KD provides ketones as an alternative fuel for the brain and the body thus producing significant improvements in neurological symptoms (motor function, seizures, cognitive performance).

KD can also ameliorate symptoms and laboratory parameters in other rare conditions such glycogen storage disease, disorders of mitochondrial energy supply, urea cycle, purine metabolism and amino acid metabolism, and drug resistant epilepsy. In glycogen storage disease, the reduction of blood insulin levels and use of ketones as an alternative energy source likely underlies the reduction in glycogen storage and improvements in exercise tolerance observed in patients treated with KD. In the other conditions, KD leads to reduction or elimination of seizures and improvement of brain function and neurological symptoms through complex yet not fully elucidated mechanisms [PMID: 18266755].

Clinical signs and symptoms
These conditions have different etiology and clinical presentation. Please see the "Enzyme function and clinical signs" column for further details.

Therapeutic considerations
Therapeutic ranges of BHB and utility of using exogenous ketone supplementation differ among these conditions. Please refer to the "KD benefits and case reports" column for further details.

Genetic conditions in which KD directly targets the underlying metabolic defect

Condition	Prevalence	Gene & best characterized mutations	Enzyme function & clinical signs	Benefits of KD/exogenous ketones and case reports
GLUT1 deficiency syndrome	1:90000 (Australia)	SLC2A1 rs80359829 rs80359828 rs80359822 rs80359816 rs80359814 rs80359816 rs121909739 rs121909740 rs80359812 rs267607060 rs267607061 rs80359818 rs202060209 rs267607059 rs387907312 rs387907313 rs397514564 rs13306758 rs398123069 rs864309514 rs864309522	GLUT1 transports glucose into cells for use as fuel. In the brain, the GLUT1 protein is involved in moving glucose across the blood-brain barrier and between glia cells, which protect and support neurons. Impaired function of GLUT1 leads to reduced glucose available to brain cells and defects in brain development and function. In addition to mutations in the SLC2A1 gene, other gene mutations can lead to GLUT1 deficiency syndrome and explain a favorable response to KD.	KD is the first line therapy for GLUT1 deficiency. Ketone bodies bypass the GLUT1 defect and enter the brain by a monocarboxylic acid transporter (MCT1). Therapeutic ranges of blood ketones are 2-4 mM in presence of very low blood glucose levels (<40 mg/dL). Exogenous ketones have not been shown to provide additional benefits as adjunct therapy, possibly because MCT1 is already saturated at physiological levels of blood ketones typically induced by a ketogenic diet (1-3 mM) [PMID: 28510035, PMID: 25415176, PMID; 12555938; PMID 16217704; PMID 15622525; PMID: 25914049. Review: PMID 19304421].

PDC deficiency (PDCD)	Rare, ~500 reported cases, likely under-diagnosed	PDHA1 (80% of cases) rs606231184 rs606231185 rs137853250 rs137853251 rs606231186 rs606231187 rs606231188 rs137853259 rs137853252 rs606231189 rs137853253 rs137853254 rs137853255 rs137853256 rs2229137 rs606231190 rs137853257 rs606231191 rs137853258 rs1555935690 rs121917898 PDHB rs28935769 rs28933391 DLAT rs119103240 rs797044957 PDHX rs1554989996 rs724159828 rs724159829 rs724159830 rs724159979 rs113309941 rs387906998 rs1135402725 PDP1 rs1554572756 rs267606938	PDC converts pyruvate, which is formed from the breakdown of carbohydrates to acetyl-CoA. PDC is made up of several enzymes including pyruvate dehydrogenase or E1 (PDHA1 and PDHB genes), E2 (DLAT gene), E3, as well as the PDHX, PDP1 enzymes, which regulate the activity of the complex. Defects in any of these enzymes impair the function of PDHC resulting in decreased conversion of glucose-derived pyruvate into acetyl-CoA, buildup of lactate, and severe defects in brain development and function.	KD is the first line therapy for PDC deficiency [PMID: 30407699; PMID: 824610; PMID: 12621116]. Due to its low-carbohydrate content, KD lowers the production of lactate from pyruvate while providing ketones as an alternative fuel for energy production. Ketone bodies bypass the oxidation of pyruvate in mitochondria and provide an alternative route for the production of acetyl-CoA. Therapeutic ranges of BHB are 0.2-2 mM in presence of blood glucose levels <85 mg/dL. Exogenous ketones have been shown to have some efficacy as adjunct treatment to KD in PDCD children [PMID: 28510035].
Genetic conditions in which KD ameliorates clinical symptoms and laboratory parameters				
Condition	**Prevalence**	**Gene & best characterized mutations**	**Enzyme function & clinical signs**	**Benefits of KD/exogenous ketones and case reports**
Disorders of carbohydrate metabolism				

GSD type III (Forbes or Cori disease)	1 in 5,400 (North African Jewish population); 1 in 100,000 (United States)	AGL rs387906244 rs113994126 rs113994129 rs113994127 rs113994134 rs113994133 rs369973784 rs199922945 rs118203964 rs113994132 rs387906246 rs780504025 rs113994128 rs267606639 rs267606640	The glycogen debranching enzyme breaks down the side chains of glycogen, which stores energy from carbohydrates in muscle and liver. Different mutations in the AGL gene can affect different isoforms of this enzyme producing two clinical phenotypes: GSD IIIa, which involves liver and muscle, and GSD IIIb, which involves only the liver (Dagli et al 2010).	KD may reduce glycogen storage in muscle and liver through the reduction of blood insulin levels and provide an alternative fuel for energy through the induction of ketone bodies production. A few case studies reported that KD (classical KD or Modified Atkins Diet), with or without supplementation of exogenous ketones, significantly improved cardiomyopathy in GSD IIIa [PMID: 25308556 (n=2, cKD); PMID: 25431232 (n=2, MAD); PMID: 21857385 (n=1, 2:1 KD)].
GSD type V (McArdle's disease)	1:100,000	PYGM rs116987552 rs119103251 rs119103252 rs267606993 rs119103253 rs119103254 rs144081869 rs119103255 rs119103256 rs786200874 rs267606993 rs119103257 rs119103258 rs119103259 rs119103260 rs764313717 rs397514631	Myophosphorylase breaks down glycogen in muscle cells. Enzymatic defects can cause exercise intolerance with muscular pain and myoglobinuria.	KD may reduce glycogen storage in muscle and liver through the reduction of blood insulin levels and provide an alternative fuel for energy through the induction of ketone bodies production. A few case studies reported that KD induced a marked improvement in exercise tolerance and quality of life in both children and elderly patient [PMID: 18425888, PMID: 16049943, PMID: 17915573]
Disorders of mitochondrial energy supply				
mtDNA depletion syndromes (MDS)	Unknown. Together, mitochondrial diseases occur in about 1 in 4,000 people.	POLG rs113994099 rs113994095 rs121918044 rs121918045 rs121918046 rs113994098 rs113994094 rs121918047 rs121918048 rs121918049 rs113994096 rs121918050 rs113994097 rs121918051 rs41549716 rs121918052 rs1567185775 rs121918053 rs121918054 rs121918055 rs121918056 rs267606959	Polymerase gamma is one of the enzymes catalyzing mtDNA replication. Enzymatic defects can cause intractable epilepsy with variable associated clinical symptoms.	KD may reduce seizures. In a case study in 6 patients with POLG mutations, 5 of them experienced a substantial seizure reduction [PMID: 26109259].

MELAS	Unknown. Together, mitochondrial diseases occur in about 1 in 4,000 people.	MTTL1 rs199474657 rs199474658 rs199474659 rs199474660 rs199474661 rs199474662 rs199474663 rs199474664 rs199474665 rs199474666 rs199474667 rs199474668	Mitochondrial transfer-RNA, leucine, 1 (MTTL1) incorporates the amino acid leucine into mitochondrial proteins. MTTL1 mutations impair the ability of mitochondria to make proteins, use oxygen, and produce energy leading to neurological problems and other specific symptoms of MELAS.	KD may reduce seizures. The use of KD and magnesium citrate as add-on therapy to anti-epileptic drugs lead to complete remission of seizures in a 22-year old patient carrying the rs199474663 MTTL1 mutation [n=1; PMID: 24656211].
Disorders of the mitochondrial respiratory chain (MRC)				
Isolated complex I deficiency (NADH ubiquinone oxidoreductase deficiency)	1 in 8500	NDUFV1 rs121913659 rs768050261 rs121913660 rs121913661 rs199683937 ACAD9 rs387906242 rs387907041 rs387907042 rs368949613 rs115532916 rs377022708 rs762521317 rs1057518752 FOXRED1 rs267606829 rs267606830 rs387907087 Other genes involved: MTND1-6 MTFMT NDUFA1-2, 9-13 NDUFAF1-6 NDUFB3,9-11 NDUFS1-8 NDUFV1-2 NUBPL ELAC2 PPA2 TIMMDC1 TMEM126B MTTL1	Complex I is the first of five mitochondrial complexes that carry out a multi-step process called oxidative phosphorylation, through which cells derive much of their energy. Mutations in any of the components or regulators of Complex I can cause a wide variety of symptoms affecting many organs and systems of the body, particularly the nervous system, the heart, and skeletal muscle. They can also cause Leigh syndrome and Leber hereditary optic neuropathy.	MRC defects are one of the most common causes of childhood epilepsy PMID: 18266755. KD may reduce seizures. Compared to carbohydrate oxidation, beta-oxidation of fatty acids provides more FADH2, thereby bypassing complex I of the mitochondrial respiratory chain. A few case studies reported that KD may reduce seizure frequency (4:1 KD) (n=24; Lee et al 2008); n=9, Kang et al 2007; n=1, Seo et al 2010; n=1, Yoon et al 2014).), normalize cognitive function (n=1; Kang et al 2006), and improve ophthalmoplegia [n=1, PMID: 17162199]

Isolated complex II and IV deficiency and complex I/IV deficiency	Unknown	TMEM70 rs183973249 rs387907070 ATP5F1A rs587776960 rs587777788 ATP5F1E rs387906929 ATPAF2 rs104894554 MT-ATP6 rs199476133 rs199476135 MT-ATP8 rs267606881	Mutations in in mtDNA genes as well as in the nuclear gene TMEM70 can cause complex V deficiency. The resulting reduction in oxidative phosphorylation can lead to cell death by reducing the amount of energy available in the cell. Energy demanding organs, such as the nervous system, heart, liver, kidneys, and skeletal muscles, are most affected.	KD reduced or eliminated seizures in a few case studies of patients with different types of MRC deficiencies including isolated complex II, complex IV and complex I/IV deficiency [n=5, PMID: 17241212; n=24, PMID: 18266755]
Urea cycle disorders				
ASL deficiency	1 in 70,000 to 218,000	ASL rs28940585 rs28941472 rs28940287 rs28940286 rs28941473 rs28940287	ASL is an enzyme of the urea cycle, which processes excess nitrogen into urea. Urea is excreted by the kidneys preventing the buildup of nitrogen in the form of ammonia. ASL deficiency results in a buildup of ammonia, which damages the brain and other tissues causing frequent epilepsy, neurological problems, and other signs and symptoms of argininosuccinic aciduria.	KD with ongoing protein restriction may reduce seizure without aggravating hyperammonemia [n=2; PMID: 23430928].
Disorders of purine metabolism				
ADSL deficiency	Fewer than 100 cases reported	ADSL rs119450941 rs119450942 rs119450943 rs28941471 rs119450944	ADSL is a component of the purinosome, a protein complex involved in purine synthesis. ADSL deficiency impairs purine metabolism causing an accumulation of succinylaminoimidazole carboxamide riboside (SAICAr) and succinyladenosine (S-Ado), which are toxic to the brain and cause neurological problems. Approximately 50% of such patients present with epilepsy, which is often resistant to drugs.	KD reduced or eliminated seizures in two case studies of ADSL patients [n=1, PMID: 22140128; n=1, PMID: 23504561].

Disorders of aminoacid metabolism				
Non ketotic hyperglycinemia (NKH)	1 in 55-76000	GLDC rs121964974 rs121964975 rs121964976 rs386833549 rs121964977 rs121964978 rs121964979 rs121964980 AMT rs121964981 rs121964982 rs121964983 rs121964984 rs121964985 rs121964986 rs181134220 rs769468125	The glycine cleavage system (GCS) degrades the neurotransmitter glycine in the mitochondria. Mutations in the components of the GCS (GLDC, AMT, GCSH) cause accumulation of glycine in body fluids leading to severe neurological symptoms, including seizures, myoclonic jerks, and encephalopathy in the first days of life (neonatal form).	In a few reports, classical KD (4:1) in combination with antiepileptic drugs reduced seizures and glycine concentrations in cerebrospinal fluid and plasma, and improved quality of life. [n=3; PMID: 22261077, PMID: 26962342, PMID: 30108280].
Drug-Resistant Epilepsy				
Tuberous sclerosis complex (TSC)	1 in 6000	TSC1 rs118203447 rs118203597 rs118203557 rs118203426 rs118203396 rs137854251 rs137854083 TSC2 rs45512692 rs137854337 rs45517179 rs28934872 rs45517214 rs121964862 rs45483392 rs45516293 rs45517349 rs137854218 rs45517259 rs45517258 rs45517258 rs137854250 rs45515894	Hamartin (TSC1) and tuberin (TSC2) are tumor suppressors proteins that down regulate protein synthesis and cell growth in presence of cellular stress or DNA damage. Mutations in TSC1-2 can cause the formation of benign tumors in many parts of the body as well as brain problems such as seizures, hyperactivity and aggression, and intellectual disability.	KD should be considered as a therapeutic option for seizure reduction, along with other modalities such as surgical resection of one or more tubers, corpus callosotomy, and vagal nerve stimulation [PMID: 16996395: study population: 12 children aged 8 months to 18 years with drug-resistant epilepsy].

Developmental and epileptic encephalopathy (DEE)	Unknown; 60-65% of cases are undiagnosed due to genetic heterogeneity	SCN1A rs121918624 rs121918625 rs121918629 rs121918630 rs397514458 rs397514459 SCN2A rs387906683 rs387906684 rs387906685 rs387906686 KCNQ2 rs397514581 rs397515420 rs397514582 rs587777219 STXBP1 rs121918317 rs121918318 rs121918319 rs121918320 rs121918321 rs587776641	Developmental and epileptic encephalopathies (DEE) are a group of genetically heterogeneous disorders characterized by early-onset drug-resistant seizures, electroencephalographic abnormalities, and developmental delay. Dravet syndrome (DS) is one of the most genetically homogeneous DEEs, with more than than 80% of DS cases are attributable to variants in SCN1A.	The use of KD has been shown to produce a ≥90% seizure reduction in 77-100% of patients carrying mutations in SCN1A, SCN2A, KCNQ2, or STXBP1. [PMID: 30061856]
ATP1A3-Related Neurologic Disorders	1 in 1 million people	ATP1A3 rs80356537 rs387907281 rs387907282 rs398122887 rs398122887 rs587777771 rs267606670 rs80356532 rs606231441	The ATP1A3 gene encodes the alpha-3 catalytic subunit of the Na+/K(+)-ATPase transmembrane ion pump. It plays a key role in the regulation of electrical activity and neurotransmitter re-uptake in neurons. Mutations in ATP1A3 can cause rare neurological conditions such as alternating hemiplegia of childhood (AHC).	In a case report, KD reduced epileptic seizures and episodes of hemiplegia or uncontrolled movements, and produced long-term improvement of neurological development [n=1, PMID: 29395663].
MED23-associated refractory epilepsy	Unknown	MED23 rs370667926	Med23 is a component of the Mediator complex, a key regulator of protein-coding gene expression. Mutations in MED23 can cause neurological problems characterized by developmental delay and refractory epilepsy such as mental retardation, autosomal recessive 18 (MRT18).	KD eliminated seizures on the first day of administration in a case report in a 2.5 year old child with MED23 refractory epilepsy [PMID: 27311965].

Abbreviations. SNP identification numbers (noted as "rs...") are the unique SNP identifiers from the NCBI dbSNP database. GLUT1: Glucose transporter protein type; PDC: pyruvate dehydrogenase; GSD: glycogen storage disease type III; mtDNA: mitochondrial DNA; MELAS: mitochondrial encephalopathy with lactic acidosis and strokelike episodes syndrome;

ASL: argininosuccinate lyase; ADSL: adenylosuccinate lyase; AGL: amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase; TMEM70: transmembrane protein 70; GLDC: glycine decarboxylase; AMT: aminomethyltransferase; GCSH: glycine cleavage system, H protein; SCN1A: sodium channel, voltage-gated, type i, alpha subunit; SCN2A: sodium channel, voltage-gated, type ii, alpha subunit; KCNQ2: potassium channel, voltage-gated, kqt-like subfamily, member 2; STXBP1: syntaxin-binding protein 1. ATP1A3: ATPase, Na+/K+, Alpha 3. BHB: beta-hydroxybutyrate. Blood levels of BHB and glucose refer to those observed during metabolic decompensation.

Table S3: Candidate SNPs for the selection of KD as therapeutic option with no evidence from intervention studies of KD.

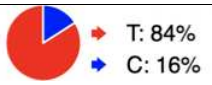
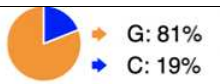
Clinical Snapshot

Are these SNPs ready for clinical implementation?
No. The strength of scientific evidence for the use of this SNPs for the prediction of KD response is "not demonstrated" using a scoring system based on recent guidelines for the interpretation of nutrigenetic variants[1]. These SNPs should be considered as candidate gene variants to evaluate and validate in research studies employing KD or exogenous ketone sources.

How can clinicians use these SNPs?
Clinicians can test the below associations as exploratory outcomes in clinic-based research of KD response. Please refer to the "Trait" column for a list of possible associations to test.

How can clinicians help accelerate the clinical implementation of these SNPs?
Clinicians can contribute to the building of a nutrigenomics knowledge base and accelerate the clinical implementation of these SNPs by testing them in clinic-based research, keeping records of their research data, and promoting the establishment of curated databases of nutrigenetic SNPs where they can submit their research data.

SNPs within ketone/fat metabolism genes associated with metabolic traits in observational studies

Effect allele	Allele frequency	Trait	Effect on trait in observational studies
HMGCS2 rs9943291-G		Blood glucose Hypertension	Increased[2]
SLC22A5 rs10060615-C		DBP	Increased[3]
SLC22A5 rs274555-C		Lean body mass	Increased[4]
CPT1A rs2924679-A		Fat oxidation	Increased[5]
CPT1A rs7938117-A		TC LDL-C HDL-C	Decreased Decreased Decreased[6]
CPT1A rs597539-G		CAC	Decreased[7]
ACADM: rs11161521-T		Carnitine metabolites	Increased[8]
ACADVL rs2286963-T		Carnitine metabolites	Increased[9 10]
ACADS rs1799958-C		Kidney disease serum metabolites (butyrylcarnitine)	Increased[11]
ACADS rs3916-C		Disease serum metabolites (cancer, coronary heart disease)	Increased[12]

Abbreviations: SNP identification numbers (noted as "rs...") are the unique SNP identifiers from the NCBI dbSNP database; HF: high fat diet (fat 40%; carbohydrate 35% or 40%); LF: low fat diet (fat 20%; carbohydrate 60% or 65%); HDL-C: HDL cholesterol; RQ: respiratory quotient; TC: Total cholesterol, LDL-C: LDL cholesterol, TG: triglycerides; MetS: metabolic syndrome; WC: waist circumference; CAC: coronary artery calcification; DBP: diastolic blood pressure.

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