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Nutri(meta)genetics and cardiovascular disease: novel concepts in the interaction of diet and genomic variation

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Abstract

In addition to the interaction of nutrition and genetic variation on the genesis and natural history of cardiovascular disease, recent studies have revealed an entire, new genome that resides in the trillions of microbes that exist in various human habitats, predominantly in the gut that may also contribute to the pathogenesis of cardiovascular disease. This microbial genome and the proteins for which it codes have important functions in homeostatic adaptations to the past and present changes in diet and environment accompanying human civilization. Both preclinical and clinical investigations suggest the role of commensal microbiota in promoting adverse cardiovascular risk. Specifically, microbial metabolism of methylated amines leads to direct pro-atherogenic effects in humans. Further investigations are needed to understand the complex relationships among nutritional status, genetic variation, and the microbial genome, which may explain the recent negative results of clinical trials of nutritional interventions such as B-vitamin therapy to lower plasma homocysteine levels. The results of such contemporary genomic investigations would allow us to utilize personalized nutritional interventions to reduce cardiovascular risk.

Keywords

genetic variation; genome microbiome; metagenome; nutrition; trimethylamine oxide; choline; phosphatidyl choline; betaine; flavin monooxygenase; cardiovascular risk; obesity; diabetes; atherosclerosis; homocysteine; B-vitamins

Introduction

The last decade has shown that the human gene pool associates with that of microbes that live as commensals in the human body, predominantly in the gastrointestinal system [1, 2]. In fact, the genetic repertoire of microbes far exceeds the number of human genes. Using high-throughput sequencing to analyze the microbiome in fecal samples obtained from 124

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European individuals, Qin and colleagues characterized 3.3 million non-redundant microbial gene sequences (approximately 150 times the number of human genes) derived from 576.7 gigabases of DNA sequence [3]. High-throughput sequencing methods have obviated the need for stool culture to decipher the microbial genome, and have allowed identification of the taxonomy and genetics of the human microbiota. The two phyla, *Bacteroides* and *Firmicutes* (which are gram positive bacteria and include both anaerobic bacteria such as *Clostridia* and aerobic bacteria such as *Bacilli*), account for approximately 90% of microbial species inhabiting the human gut. The Human Microbiome Project Consortium recently reported a comprehensive analysis of 4,788 specimens from multiple habitats obtained from 242 healthy Western adults [4]. Similar to a previous small study [5], microbiomes were found to be fairly unique to individuals even in a somewhat homogeneous population. There were significant differences in percentages of bacterial phyla between habitats in an individual; however, metabolic pathways related to the microbiome were more concordant between habitats. Establishment of a stable gut microbial composition may occur over the initial years of life [6]. Although antibiotic treatment disrupts gut microbial ecology, there is a return towards the previous state within a week of discontinuation of antibiotics, although the return is not complete and results in a 'new' microbiome [7]. It is not clear as to what would be the impact of multiple courses on antibiotic treatment on the individual and the population.

Studies as described below clearly demonstrate the importance of these microbial genes in human health, for which reason the combination of human and microbial genomes has been termed the human metagenome. In this review, we will detail current knowledge about the human metagenome as it relates to human health and cardiovascular disease.

Human metagenome: herald of health and harbinger of disease

Through human history and continuing into the present day, the human diet has varied temporally and geographically as food sources have changed. The gut microbiota have evolved in concert with these changes, and is crucial for metabolizing certain nutrients for which human intestinal enzymes do not exist, for harvesting energy from nutrients, and for synthesizing vitamins [8, 9]. It is not clear whether metabolism of various xenobiotics protects humans from diseases or increase susceptibility to disease; however, microbiota do affect the metabolism of common drugs. In addition, metabolism of intestinal contents, such as bile acids, by gut microbiota affects lipid metabolism and modulate the effects of lipid-modifying drugs, such as statins [10, 11]. In addition, the gut is an area of significant antigen exposure, and the human gut microbiota are thought to play crucial roles in innate and adaptive immunity as a result [12–14]. This view suggests an important role for gut microbiota in inflammatory bowel diseases [15–17] and extra-intestinal diseases with an immune component, such as asthma [18]. Recent studies also show an important role for microbiota in regulating neural and behavioral responses [19]. An intriguing relationship between the microbiome and cardiovascular disease was suggested by the observation that germ free mice have lower oxygen consumption, lower cardiac output, and smaller hearts [20–22]. Further studies are needed to determine if the microbiome influences remodeling of the heart.

Nutrition, metagenome, and cardiovascular risk factors

Le Chatelier and colleagues demonstrated that low bacterial species diversity is associated with adiposity, insulin resistance, dyslipidemia and inflammatory phenotype in Danish individuals [23]. A small number of bacterial species accounted for the differences between individuals with low and high bacterial diversity. Preclinical studies have also focused on this relationship between obesity and microbial genome. For example, Backhed and colleagues showed that transplantation of normal cecal microbiota from normal mice to adult germ free mice led to a 60% increase in body fat content as well as insulin resistance despite a lack of increase in caloric intake [24]. This study suggested that increased absorption of monosaccharides and consequent hepatic lipogenesis and suppression of production of fasting-induced adipocyte factor, a circulating lipoprotein lipase, may be responsible for this adiposity response. Studies of genetically obese and lean mice have shown that obese gut microbiota are characterized by a decrease in *Bacteroides* and an increase in *Firmicutes* compared to lean mice [25]. The same group also demonstrated that obese microbiota conferred an increase in energy harvested from the diet, and that colonization of germ free mice with gut microbiota from obese mice led to increased adiposity compared to gut microbiota from lean animals [26].

In addition to the association with insulin resistance mentioned above, multiple pre-clinical and human studies demonstrate a role for the microbiome in the pathogenesis of diabetes. The microbiome signature in children with type I diabetes is significantly different from that of healthy children [27]. The interaction between innate immunity and the microbiome has been shown to be crucial for development of type I diabetes [28]. In a study of adult onset diabetes, Karlsson and colleagues examined the microbiome from fecal samples of European women with normal or impaired glucose control and overt diabetes [29]. They observed significant differences in the microbiome composition of women with diabetes, and were able to develop a mathematical model of the microbiome to predict a diabetic metabolic profile in women with impaired glucose tolerance. The authors applied this model to a Chinese cohort and discovered that microbiomic markers for diabetes differed between Chinese and European subjects.

Nutrition, metagenome, and atherosclerosis

A recent meta-analysis of 21 prospective epidemiological studies with a cumulative population of approximately 350,000 subjects and 5–23 years of follow-up data showed that there is no significant association between dietary intake of saturated fat and risk of coronary artery disease, stroke, or cardiovascular disease (CVD) [30]. A likely explanation, as the authors contend in another publication, could be that saturated fat was replaced by other nutrients that increased atherogenic risk [31]. Alternatively, it is possible that genetic or metagenomic variation interacts with nutrients to promote atherogenesis. For example, in a study of 13 controls and 12 subjects with symptomatic carotid atherosclerosis, analysis of the gut microbiome demonstrated a preponderance of the genus *Colinsella (belonging to the phylum Actinobacteria)* in patients with atherosclerosis and of the genuses *Roseburia* (butyrate-producing bacteria that belongs to the phylum *Firmicutes*) and *Eubacterium (Firmicutes phylum)* in healthy controls [32].

One of the more exciting developments in this area has been the demonstration of how the gut microbiome interacts with nutrients to promote atherosclerosis. Wang and colleagues demonstrated that metabolism of choline, a common nutrient, by gut microbiota promotes atherosclerosis ¨[33]. Metabolomic studies were utilized to show that trimethylamine oxide (TMAO), a common metabolite of choline and phosphatidyl choline, was associated with an increased risk of CVD. Utilizing germ-free mice, the investigators proved the necessity of gut microbial flora for production of TMAO from choline. TMAO was found to increase the accumulation of cholesterol in macrophages and consequent foam cell formation. In addition to TMAO, plasma levels of choline as well as betaine, a product of choline oxidation, were associated with an increased risk of atherosclerosis in humans. The same group further examined the clinical implications of these findings in human subjects ¨[34]. In a study of 40 healthy volunteers, they demonstrated that dietary challenge with phosphatidyl choline increased the plasma levels of TMAO. Antibiotic treatment to suppress gut flora markedly decreased plasma levels of TMAO. Analysis of TMAO levels in 4,007 patients undergoing elective coronary angiography showed a significant relationship between an elevated TMAO level and the risk of major cardiovascular events during three years of follow-up.

In a separate study, Dr. Hazen's group also examined whether gut microbial metabolism of L-carnitine, a trimethylamine found abundantly in red meat and essential to fatty acid transport, promotes atherosclerosis ¨[35]. In this study, human vegans given a carnitine load (8 oz. steak) did not have an elevation of plasma TMAO levels while omnivorous human volunteers demonstrated a significant elevation in TMAO levels. Antibiotic treatment to suppress microbiota attenuated TMAO production after carnitine challenge. The composition of gut microbiota varied between omnivores/individuals with higher plasma TMAO levels vs. vegans/individuals with lower TMAO levels. In experiments performed in mice, supplementation of L-carnitine was found to alter gut microbiota, increase synthesis of TMAO, and promote atherogenesis by inhibiting reverse cholesterol transport *in vivo*. A novel Rieske-type protein with oxygenase/reductase function has been shown to effect the conversion of carnitine to trimethylamine (TMA), the precursor of TMAO, by human gut microbiota [36]. This discovery suggests the potential utility of specific genes in the microbiome as markers of atherosclerotic risk or as therapeutic targets. Another potential target is flavin monooxygenase (FMO). Since an FMO3 mutation and decreased function is responsible for the fish odor syndrome or trimethylaminuria due to impaired metabolism and accumulation of trimethylamine (TMA) [37], it is likely that FMOs are responsible for oxidation of gut microbiota-derived trimethylamine (TMA) to TMAO. A study by Bennett and colleagues showed that FMO3 was the key enzyme responsible for TMAO production in mice [38]. In a study of French-Canadian adults, 3 polymorphisms of FMO3 were identified that were associated with variations in TMAO production [39].

These studies clearly demonstrate that gut microbiota promote atherosclerosis by modification of dietary components that segregate with known dietary risk factors such as fat [40]. Gut microbiota composition is dynamic, with enrichment of microbial species that metabolize specific dietary components. These studies clearly establish gut microbiota as a target for cardiovascular prevention and treatment using nutritional manipulation. Genetic

variation in enzymes that modify microbiota-derived metabolites would be crucial in determining the effects of nutritional modulation.

Microbiome, methionine-homocysteine cycle, and methylation

Interaction of nutritional status with genetic variation in the methionine-homocysteine cycle has been investigated as a pathogenic factor in atherosclerosis since the initial reports by Dr. McCully linking homocystinuria to arterial vascular disease [1]. Although multiple observational studies have shown a relationship between plasma homocysteine levels and atherothrombotic risk, nutritional intervention utilizing combinations of B-vitamins to decrease the plasma homocysteine level has not been demonstrated to improve cardiovascular outcomes (reviewed in detail in [42, 43]). One potential reason for lack of effect of B-vitamins could be genetic variability. For example, a study of folate supplementation in healthy female identical and non-identical twins showed that changes in plasma homocysteine level in response to folate was highly heritable [44]. The variability was not based on polymorphisms of methylene tetrahydrofolate, a well-known heritable factor affecting homocysteine metabolism, underscoring the presence of unidentified genetic factors affecting methionine-homocysteine cycle in humans.

Changes in plasma homocysteine occur due to a variety of genetic and environmental factors, suggesting that the methionine-homocysteine cycle is crucial to health and perturbed in various pathological conditions [42, 43]. The major function of the methionine-homocysteine cycle is the regulation of methylation reactions, with S-adenosylmethionine donating methyl groups for all methylation reactions of the body except remethylation of homocysteine to methionine. Choline and its oxidation product, betaine trimethylglycine, provide exogenous methyl groups, are crucial for methyl supply, and interact closely with the methionine-homocysteine cycle. In fact, betaine is the methyl donor for remethylation of homocysteine by the enzyme betaine-homocysteine methyl transferase. Plasma levels of homocysteine, choline, and betaine have been linked to cardiovascular risk. A recent report examined the associations of plasma choline, betaine, and TMAO with the risk of major adverse cardiac events in over 3,000 subjects undergoing coronary angiography [45]. Choline and betaine were associated with TMAO levels and with increased risk of cardiac events; however, the relationships of choline and betaine with cardiovascular risk were rendered non-significant when TMAO was added as a covariate, indicating that the predictive value of choline and betaine were due to microbiota-derived TMAO production.

In the RISTOMED study, a European study that aimed to use dietary modifications to reduce aging-induced diseases in older healthy adults, a personalized diet with or without probiotics was administered and the effects on inflammatory markers, oxidant stress, B-vitamins, and homocysteine were measured [46]. Administration of probiotics increased the prevalence of bifidobacteria in the gut microbiota, as well as folate, and B_{12} levels, and decreased plasma homocysteine levels, suggesting a close relationship between gut microbiome composition and the methionine-homocysteine cycle. These studies suggest that the gut microbiome interacts closely with the methionine-homocysteine cycle and methyl balance *in vivo*, and highlights the need to study the gut microbiome in investigations of the relationship between hyperhomocysteinemia and CVD.

Human gut microbial genome: a modifiable risk factor?

Differences in gut microbiota in response to dietary variation and interventions suggest that the microbiome is modifiable. A study of children from Africa (Burkina Faso) and Europe (Italy) showed the impact of diet on gut microbiota across populations [47]. Children from Burkina Faso who eat a fiber-rich diet had over-representation of *Bacteroides* and less *Firmicutes* compared to European children on a western diet. In another study, weight loss induced by caloric restriction and high fiber intake was shown concomitantly to increase gut microbial diversity and improve lipid and glucose metabolism in obese individuals [48]. In studies using germ-free mice, Backhed and colleagues demonstrated that germ-free mice are resistant to obesity induced by a western diet while colonization of germ-free mice with gut microbiota of normal mice led to increased fat deposition [49]. Gut microbiota have also been proposed to possess specific anti-atherogenic effects – metabolism of an anthocyanin (pigments derived from grapes and responsible for the color of red wine) by microbes and generation of protocatechuic acid has been shown to promote reverse cholesterol transport in mice [50]. Overall, these studies suggest the close relationship between diet and the microbiome, and the potential for modifying the gut microbiome by diet to induce a favorable diet-metabolism interaction.

Nutrition, human microbial genome and genetics: an integrated target for cardiovascular prevention?

Since metagenomic variation among humans includes not only human genomic variation, but also variation in the microbial genome, it is likely that the human genome, microbiome, and nutritional status interact to influence health and disease. For example, mice with genetic deficiency of Toll-like receptor 5, an important component of the innate immune response in the gut, develop characteristic gut microbiota that confer susceptibility to metabolic syndrome [51]. In humans, bile acids derived from gut microbiota metabolism have been shown to affect host response to simvastatin [11]. This pharmacogenetic study demonstrated that three secondary bile acids derived from microbiota could be used to predict a good response to simvastatin, and that levels of simvastatin and bile acids were correlated. Interestingly, a single nucleotide polymorphism in a gene coding for an organic anion transporter was associated with levels of bile acids. In terms of atherogenic TMAO levels, a human genome-wide association study did not reveal a definitive relationship of genetic variation with plasma TMAO levels [52], which, in light of earlier experimental work reviewed here, suggests that other metagenomic and/or environmental/nutritional factors must be considered to define disease risk optimally.

Twins who are discordant for the obesity phenotype provide a unique opportunity to investigate the relationship between the gut microbiome and nutritional status independent of genetic variation. Gut microbial composition is similar between monozygotic and dizygotic twins and was also similar in mothers indicating the effect of familial environment in establishing the microbiota signature [53]. Ridaura and colleagues identified four twin pairs who had sustained differences in body mass index, and collected fecal samples from them [54]. When fecal microbiota from obese and lean twin pairs were introduced by gavage into germ free mice, the mice that received gut microbiota from obese individuals developed an obesity phenotype. Housing mice that had received obese and lean microbiota in the same

cage led to reestablishment of the lean phenotype in these coprophagic mice, indicating that the gut microbiome is a rapidly modifiable factor that modifies phenotype.

Another clinically relevant situation in which nutritional status and the microbiome interact longitudinally is that following gastric bypass surgery in obese patients. Furet and colleagues analyzed the gut microbiota from 13 lean individuals and from 30 obese individuals before and after gastric bypass surgery [55]. In addition to changes in the microbiota profile at baseline between lean and obese subjects, the investigators noticed significant changes in microbiota composition at three months after surgery that was correlated with fat mass independent of food intake.

Similar to genome-wide association studies, microbiome-wide association studies have been attempted to identify linkage between microbiome variation and disease. Qin and coworkers performed shotgun sequencing of the gut microbiome in a Chinese population and identified approximately 60,000 microbiome markers associated with type-2 diabetes mellitus [56]. Gut microbiome markers were found to be useful in differentiating healthy and diabetic subjects with a high level of specificity.

Systems biology or network medicine approaches hold the potential for improved understanding of biological determinants of phenotype owing to the great power of these approaches in holistically evaluating the complexity of the multiple genomic and environmental determinants of phenotype 57, 58]. Greenblum and coworkers obtained microbiome data from 124 unrelated European individuals [59]. A metabolic network was derived by identifying genes coding for enzymes and pooling data from all individual microbiomes studied. In this network, nodes represented enzymes and connections between nodes (or directed edges) represented enzymes that catalyzed sequential reactions. Differences in organization of the network and of gene distribution within the network varied between lean and obese individuals, indicating the potential of network approaches to identify differences in microbiomes between health and disease.

Conclusions

The human microbiome is a complex ecosystem that co-exists with the human genome and is subject to rapid change depending on individual and population variations in dietary intake. In addition to diet, genetic variation, especially in immune mechanisms, may also affect gut microbiota. Perpetuation of major cardiovascular risk factors such as obesity and diabetes may be dependent on the close interaction between diet and microbial augmentation of energy harvesting from foodstuffs. Further studies are needed to understand the many potential interrelationships among nutritional status, genetic variation, and the microbiome. Modulation of these interactions may lead to great strides in the prevention and treatment of CVD.

Acknowledgements

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