Personalisierte Ernährung und Einteilung/ Klassifizierung von metabolischen Typen basierend auf genetischen, epigenetischen und mikrobiologischen Analysen

Personalized nutrition and classification of metabolic types based on genetics, epigenetics and gut microbiota

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Abstracts

Humans vary in their need and response to diet. Genetic dispositions, such as single nucleotide polymorphisms (SNPs) are frequently used for clustering consumers in metabolic types (metabotypes), according to individual characteristics of energy extraction from food or risk for metabolic diseases. Results are then used for individualized concepts for weight management or weight loss. However, SNPs explain only a minor part of metabolic variability whereas epigenetic regulation of metabolic enzymes, GI microbiota and lifestyle are of ample importance. In a pilot study enrolling 37participants under nutritional advice we analyzed results from a panel of SNPs, epigenetic markers and microbiota as well as food frequency questionnaires. The results of this study clearly indicate that epigenetic and microbiota markers need to be integrated in the definition of metabotypes. Such improved metabotypes may then enable an improved guidance for a personalized nutrition.

Keywords: personalized nutrition, metabolic types, SNPs, epigenetic, gut microbiota

Menschen unterscheiden sich in ihren Ernährungsbedürfnissen und deren Stoffwechsel. Genetische Veranlagungen, wie zum Beispiel Einzelnukleotidpolymophismen (engl. Single nucleotide polymorphisms SNPs) werden häufig verwendet um Patienten in verschiedene metabolische Typen (metabotypes) einzuteilen, passierend an den individuellen Eigenschaften wie zum Beispiel Energieextraktion aus verschiedenen Nahrungsmitteln oder das genetische Risiko für metabolische Erkrankungen. Diese Einteilungen können weites für ein individuelles Konzept für Gewichtsmanagement und Gewichtsverlust verwendet werden. Nichtsdestotrotz können SNPs nur einen kleinen Teil der metabolischen Variabilität erklären, weshalb epigenetische Regulation von Enzymen, die gastrointestinale Mikrobiota und der Lebensstil von selber Bedeutung sind. In einer Pilotenstudie mit 37 Teilnehmern und Ernährungsberatung wurden SNPs, epigenetische Marker, gastrointestinale Mikrobiota sowie Ernährungsfragebogen analysiert. Die Ergebnisse der Studie zeigen deutlich, dass epigenetische Marker als auch Mikrobiota zu den Analysen der Metabotypes integriert werden sollte. Diese verbesserten Metabotypes könnten eine bessere personalisierte Ernährungsberatung ermöglichen.

Schlüsselwörter: Personalisierte Ernährung, metabolische Typen, SNPs, Epigenetik, Darmmikrobiota

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INTRODUCTION

Dietary preferences and habits are controlled by socioeconomic, psychological, behavioral and in particular biological determinants such as hunger, satiety and sensory aspects (5). Body weight and composition, as well as metabolic rate are affected by nutrient intake and biochemical pathways regulating nutrient absorption, distribution, metabolism, excretions and other cellular energy processes (4). Genetic and epigenetic mechanisms act as key regulators and predispositions may even forecast the response to a weight loss intervention (3,23). The field nutrigenetics offers a new opportunity to evaluate the role of genes, which determine metabolism, disorders and further use the predisposition of genes for a personalized nutrition. Genome wide association studies (GWAS) indicate that particular gene polymorphisms such as single nucleotide polymorphisms (SNPs), the most common type of genetic variation, are related to obesity. SNPs within fat mass and obesity associated genes (*FTO*) were shown to increase the Body Mass Index (BMI) by 0.4kg/m²/allele, caused by an increased intake of fat (2, 11). For example, variants in *Melanocortin 4 receptor (MC4R)*, a gene activating stress neuropeptides, can be linked with lifestyle, food intake and eating habits and as well stress. Carriers of the risk allele have a significant higher intake of processed food and fruits according to recent studies (12). T*ranscription factor 7-like 2 (TCF7L2)* is a key regulator of glucose homeostasis and has been most consistently associated with Diabetes Mellitus 2 (DM2).SNP rs7903146 has been reported to have the highest effect on development of DM2 (11).

The gene *Peroxisome proliferator-activated receptor gamma (PPARG)* encodes a regulator of adipocyte differentiation. Galbete et al. showed that subjects consuming a high amount of carbohydrates and carrying the risk allele had an increased obesity risk (13,14). Fatty acid desaturases (FADS) are enzymes involved in the metabolism of polyunsaturated fatty acids (PUFAs). It has been reported that individuals with a polymorphism in rs174547 within the FADS1 gene have increased triglyceride (TG) levels, decreased high density lipoproteins (HDL), cholesterol and an increased coronary artery disease risk (10). Leptin is an adipocyte-secreted hormone and regulates energy homeostasis, blood pressure and food intake. Polymorphisms at the *leptin receptor (LEPR)* decrease the beneficial effects of leptin, like reducing appetite and food intake, and moreover lead to an increased energy metabolism (19). *Angiotensin-converting enzyme (ACE)* gene variants are associated with endurance performance, like swimming, cycling and running, based on lower *ACE* activity and increased bradykinin. This mutation results in more oxygenated blood delivered to the working muscles (8). The SNP *transcription factor AP-2ß (TFAP2B)* rs987237 has a significant association with waist to hip ratio (3). Martinez et al. showed a higher weight loss in wildtypes with a low fat and low caloric diet (20,21).

Epigenetics. The main epigenetic mechanisms are DNA methylation, histone modifications and non coding RNAs (3). DNA methylations occur mainly in cytosines followed by guanines (CpGs) by the addition of methyl groups to the pyrimidine ring in position 5 of cytosine (1). Influenced by internal and external factors such as diet, lifestyle and environment, DNA methylations at CpGs are specific and vary over time within an individual and further may act transgenerational (1,3,22). CpG methylation can change the activity of a gene and therefore is able to repress or promote its expression. Epigenetic markers such as DNA methylation of specific CPGs are used as predictors for metabolic risks and predictors for the success of a diet related treatment, like weight loss or weight maintenance (7). For instance, an elevated Interleukin 6 (IL6) release in blood is linked with a decreased gene promoter methylation. High *IL6* blood levels are associated with several inflammatory diseases (8). Moreover, studies showed a higher *IL6* methylation in obese individuals. Aumueller et al. reported that low *IL6* methylation is associated with a better weight loss (25).

Another promising epigenetic marker associated with metabolism constitutes the *long interspersed element 1 (LINE1)*. *LINE1* is a retrotransposon, which is widely expressed in the human genome (3) and is associated with genetic instability and chromosomal abnormalities (22). Usually assessed to estimate global DNA methylation, *LINE1* methylation is related to BMI, DM2, insulin resistance, cardiovascular disease, inflammatory response and cancer (3,9) as well as obesity and metabolic syndrome (MetS).

Microbiota. The microorganisms in the gut are a highly metabolic active community and are regarded as a regulator of its host homeostasis. The gut microbiota contains 100 times more genes than human cells. The composition of the microbiota varies over lifetime with diet as strongest impact factor (15). Indigestible complex carbohydrates are a major source for carbon, the main substrate for the gut microbiota. After their fermentation short chain fatty acids (SFAs), like acetate, propionate and butyrate are produced and absorbed via the colon mucosa. SFAs show multiple health promoting activities and have beneficial effects in appetite regulation, lipid and glucose metabolism (15,16). However, an imbalanced gut microbiota affects metabolites like butyrate and lipopolysaccharides (LPS), which interfere with the host's epigenetic mechanism and may trigger pro-inflammatory processes (24,29). GI microbiota have been grouped in enterotypes according to main bacterial groups with relevance for metabolic characteristics and discussed critically (30-32) .

OBJECTIVES

The Metabotype-Study was initiated to evaluate a clustering of participants into four different metabotypes based on differences in genetics as well as epigenetics and GI – microbiota. Analysis of cluster of SNPs as described scientifically and already used commercially should be complemented with analysis of epigenetic CpG methylation of metabolic relevant genes and analysis of gut microbiota composition. The hypothesis that a solely SNP based categorisation of metabotypes misses important aspects was supported by the outcome of a comparison of a SNPs analysis and an integrated analysis of SNPs including epigenetic and microbiota marker.

METHODS

The study population included 37 healthy men and women from 30 to 60 years of age. Exclusion criteria were chronic diseases, colitis ulcerosa, supplementation of pre- or probiotics, antibiotic intake and BMI over 30. Blood spots were used for sample collection of capillary blood. DNA extraction was conducted with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). SNP analysis was performed with the StepOne Plus (Thermo Fisher, Massachusetts, USA) using TaqMan Mastermix and TaqMan SNP Genotyping Assays

from Thermo Fisher (Massachusetts, USA). For epigenetic analyses DNA was bisulfite converted, using the EpiTec bisulfite kit (Qiagen, Hilden, Germany). High resolution melting curve analysis was conducted to assess *LINE1* and *IL6* methylation. For quantification analysis of microbiota real-time polymerase chain reaction (PCR) was applied using TaqMan qPCR and SYBER Green qPCR in a Rotorgene 3000 after DNA extraction of stool samples using QIAamp Fast DNA Mini Stool Kit.

Metabolic types. For our study we chose 12 SNPs in total. *MC4R* rs17782313, *TCF7L2* rs7903146, *IL6* rs1800795, *SLC6A14* rs2011198, *FTO* rs9939609, *PPARG2* rs1801282 have been associated either with BMI and obesity or DM2 (2). Moreover *MC4R* rs17782313 and *LEPR* rs9436740 are linked with satiety, *IL6* rs1800795 with weight regain and *SLC6A14* rs2011198 with eating disorder development. Others like *TFAP2B* rs987237, *FADS1* rs174547, and *ADRB3* rs4994 as well as *FTO* rs993609 and *TCF7L2* rs7903146 are reported to correlate to different metabolic types. The *ACE* gene is associated with different sport types (19,26). For the classification of the different metabotypes we focused solely towards SNPs linked with nutrition and metabolism (18). As already described by Martinez et al. we gave points from zero to two for each SNP (2).

Verarbeitete Fälle

	Fälle					
	Gültig		Fehlend		Gesamt	
	N	Prozent	N	Prozent	N	Prozent
ACE * Körperliche_Bewegung_t0	37	100,0%	0	0,0%	37	100,0%

ACE * Körperliche_Bewegung_t0 Kreuztabelle

Anzahl

		Körperliche_Bewegung_t0					
		mehrmals täglich	täglich	4-6x/W	1-3x/W	1-3x/M	Gesamt
ACE	strenght	6	6	2	2	0	16
	balanced	4	3	2	2	2	13
	endurance	0	3	0	5	0	8
Gesamt		10	12	4	9	2	37

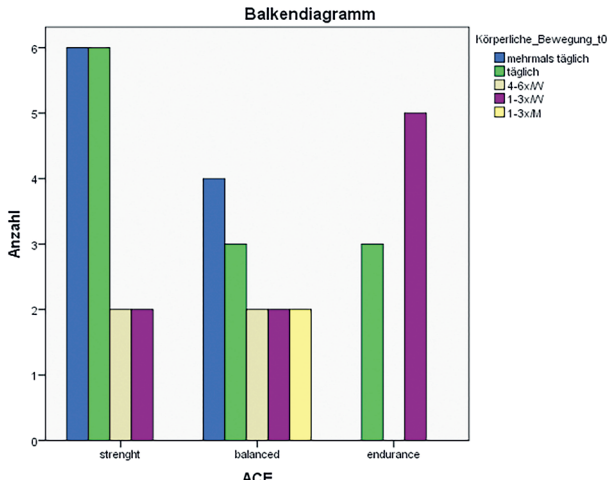

Figure 2: Sport types and physical activity

RESULTS

Figure 1 shows the contribution of the different metabotypes using SNP analysis.
In total we found 23 balanced types, 7 glyco, 2 protein and 5 fat types. In **figure 2** the distribution of different sport types and physical activity in our study population is shown. **Figure 3** shows the connection between IL-6-methylation and the amount of Cluster IV. The higher the methylation of IL-6, the higher the amount of Cluster IV bacteria. **Figure 4** shows the correlation between the different forms of the TCF7L2-SNP and the amount of Bacteriodetes. The wild type shows the highest quantity of Bacteriodetes.

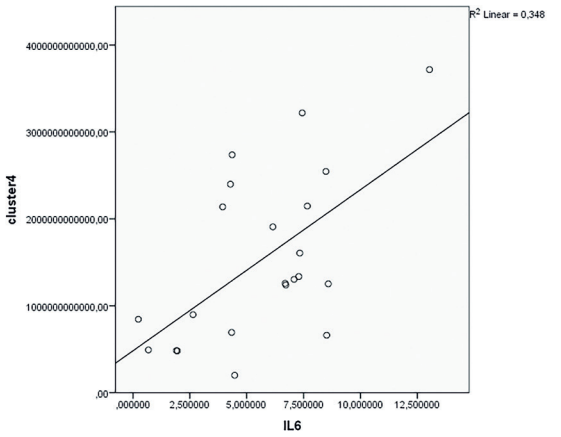

Figure 3: *IL6* Methylation and *Cluster IV*

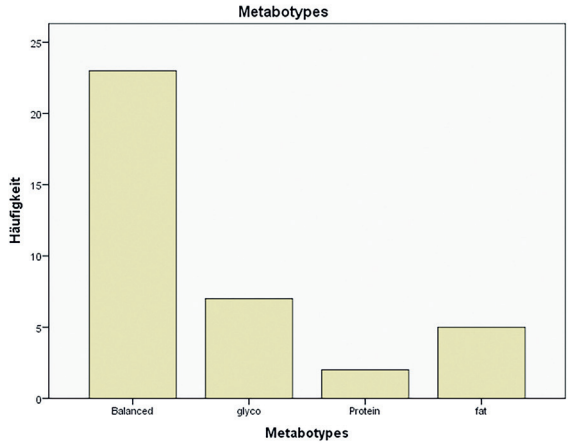

Figure 1: Different Metabotypes

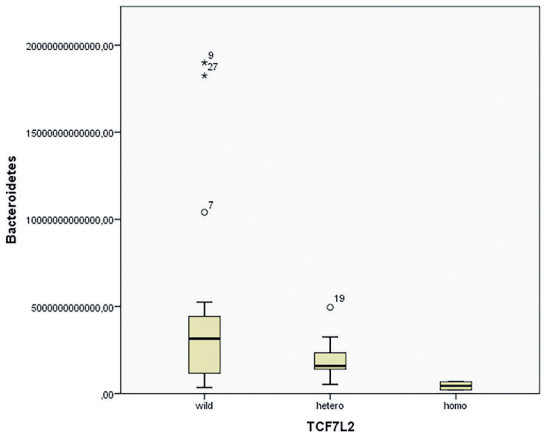

Figure 4: *TCF7L2* and *Bacteroidetes*

DISCUSSION

Our main findings identify an association between genetics, epigenetics and gut microbiota variations. Considering the outcome for the strength sport type, individuals in this group have a higher amount of *Firmicutes* in general as well as *Cluster IV* and a higher methylation of *IL6*. A high abundance of *Cluster IV* or *Firmicutes* which is mostly seen in humans with high BMI (27) is therefore suggested to result in a low weight loss. Latter was previously reported to be correlated with a high methylation of *IL6*. Further, *Firmicutes* and *Cluster IV* are associated with increased inflammatory and stress levels as reported in FFQ. Remely et al. showed that *Cluster IV* and *Cluster XIVa* decreased in people with DM2 after weight loss suggesting that people with higher BMI exhibit a higher distribution of these bacterial groups. Our results could additionally demonstrate this outcome with *Cluster IV* (27,28). Furthermore, we observed that the wildtype forms of *TCF7L2* and *LEPR* were correlated to higher amounts of *Bacteroidetes*, which were shown to be higher abundant in lean individuals. Both SNPs can be associated with obesity as well as diabetes: Carrying no risk allele portends persons are more likely lean, thus have a lower risk for obesity and DM2. This could be underlined by showing that a high amount of *Bacteroidetes* tends to correlate with a low risk for obesity, which has also been reported by previous studies (17). *LINE1* is considered to be highly methylated in participants with high BMI (33), which also emerges in our study. Moreover we observed a possible interaction of *LINE1* methylation with *PPARG2*, where heterozygotes were higher methylated, and consequently had a higher BMI. Controversially, the wildtype for *PPARG2* showed a positive correlation with methylation of IL6, which could indicate that wildtype carriers have a less efficiency to lose weight but a lower risk to develop DM2. This study demonstrates that interactions between genetic as well as epigenetic variations, the gut microbial composition and their influences through diet and lifestyle but also physical activity are relevant for genotype based interventions bearing an enormous potential in developing personalized diets based on the genotype (20).

Humans differ in height, weight, activity, cognition, strength, endurance and their preference for food, due to a wide range of biological variables. These variables include allelic polymorphisms and changes in the epigenomic and also metabolomic landscape due to environmental influences (6). DNA methylation changes are directly correlated to dietary interventions, weight loss and regain and further are associated with the development of diseases e.g. metabolic disorders (3). With increasing knowledge of gene-diet interactions for macro-and micronutrients it will be possible to give recommendations based on the (epi) genetic make-up (11,23).

CONCLUSION

Metabolic diseases are a central burden for public health and heath care. There is increasing evidence that genetic, epigenetic and microbiota aspects contribute to individual mechanisms, which result in individual pathways for metabolism and energy extraction from food. Genetic dispositions, such as SNPs are under scientific investigation but already in commercial use for defining metabolic types (metabotypes). These metabotypes define the risk for metabolic diseases, preferences for energy extraction from food and individualized concepts for weight management or weight loss. To our knowledge there is no other study focusing on SNPs and their classification into metabotypes. Furthermore, other nutritional recommendations based on genetic disposition do not consider environmental and nutritional effects on gene regulation. Results show that SNPs can be clearly attributed to metabotypes. Analysis of DNA methylation strengthens the outcome. Furthermore gut microbiota composition shows significant correlation with SNP and methylation according to metabotype clustering.

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