The marriage of nutrigenomics with the microbiome: the case of infant-associated bifidobacteria and milk[1–5]

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ABSTRACT

Broadly, nutrigenomics examines the association of exogenous nutrients and molecular responses to maintain homeostasis in an individual. Phenotypic expression profiling, often transcriptomics, has been applied to identify markers and metabolic consequences of suboptimal diet, lifestyle, or both. The decade after the Human Genome Project has been marked with advances in high-throughput analysis of biological polymers and metabolites, prompting a rapid increase in characterization of the profound nature by which our symbiotic microbiota influences human physiology. Although the technology is widely accessible to assess microbiome composition, genetic potential, and global function, nutrigenomics studies often exclude the microbial contribution to host responses to ingested nutritive molecules. Perhaps a hallmark of coevolution, milk provides a dramatic example of a diet that promotes a particular microbial community structure, because the lower infant gastrointestinal tract is often dominated by bifidobacteria that flourish on milk glycans. Systems-level approaches should continue to be applied to examine the microbial communities in the context of their host's dietary habits and metabolic status. In addition, studies of isolated microbiota species should be encouraged to inform clinical studies and interventions as well as community studies. Whereas nutrigenomics research is beginning to account for resident microbiota, the need remains to consistently consider our microscopic partners in the human holobiont. *Am J Clin Nutr* 2014;99(suppl):697S–703S.

INTRODUCTION

As a cross-disciplinary subfield, nutrigenomics seeks to explicate causality between digestion and absorption of exogenous nutrients and the corresponding molecular responses to maintain homeostasis in an individual. In addition, transcriptional profiles have been used to identify antecedent events and metabolic consequences in disease states rooted in a deleterious diet and/or lifestyle. As the term implies, nutrigenomics inquiry is enabled by the various "omics" technologies (eg, genomics, transcriptomics, proteomics, and metabolomics) to disentangle the relation between nutrients, genotype/haplotype, and host physiologic responses to dietary molecules with gene sets and their encoded metabolic operations (1, 2). Whereas nutrigenomics often examines interactions of mixed bioactive molecules with a large suite of potential cellular targets, pharmacology seeks to match specific receptors with small molecule ligands (3). Currently, these investigations are primarily conducted by quantifying phenotype through profiling global gene expression and, to a lesser extent, through circulatory metabolites and tissue proteomes that result from experimentally defined dietary/lifestyle interventions (4, 5). Until recently, the host microbiota was an excluded variable, despite clearly functioning as an active microbial sieve that repurposes incompletely digested dietary molecules, and is now known to be intimately integrated in host metabolism and immune maturation (6). Massively parallel DNA sequencing, along with advanced analytic methodology, are currently at the leading edge of incorporating community-level microbial functions into preexisting metabolic models.

Ordinarily, the infant gastrointestinal tract (GIT)[6] is understood to be sterile in utero and to be rapidly colonized amid events inherent to parturition (7). Accordingly, there are multiple routes by which microbial inoculation may proceed, including early exposure to vaginal and fecal microbiota, ingestion of epidermal microbes and potentially breast milk, as well as the inherently close interactions between the neonate and mother (8). In addition, there is considerable evidence that vaginal delivery promotes colonization by a subset of bacteria distinct from caesarian-delivered infants. Unique to early development, relatively higher oxygen tension of the GIT is reflected in the overrepresentation of facultative anaerobes. Eventually these founding colonizers reduce the environment to favor anaerobic genera, most notably *Bifidobacterium*, which often dominate before weaning (9). In contrast, the adult distal GIT character-

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[6] Abbreviations used: GIT, gastrointestinal tract; HMP, Human Microbiome Project; MOM, milk-oriented microbiota; MWAS, metagenome-wide association study; NGS, next-generation sequencing; T2D, type 2 diabetes.

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istically supports species from the bacterial phyla *Bacteroidetes*, *Firmicutes*, and, to a lesser extent, *Actinobacteria* in temporally stable consortia. After an antibiotic challenge, distal microbiota have proven remarkably resilient as they reassemble into their pretreatment composition functionally, if not completely compositionally (10). Moreover, interindividual heterogeneity within lower-order taxa is uniformly observed, although metagenomic studies have indicated a general conservation of function regardless of phylotype presence and relative abundance (11, 12).

Throughout one's life, diet is a primary determinant of the population structure of the GIT microbiota and now is regarded a thematic feature of microbiome theory (13–15). Evidence supporting this hypothesis is partially, albeit powerfully, furnished by experiments conducted with "humanized" gnotobiotic mice that are colonized with human intestinal microbiota. In one particularly incisive experiment, humanized mice were monitored during a low-fat, plant polysaccharide–rich diet through a transition to a high-fat and sugar feed representing the modern Western diet (13). Interestingly, the composition and metabolic potential of the microbial community shifted very rapidly in response. This modulation corresponded with a decreased representation of *Bacteroidetes* and a significant *Firmicutes* enrichment, specifically of the class *Erysipelotrichi*. Compositional remodeling of the community altered the efficiency by which energy was extracted from digesta encountered in the colon. Additional studies have correlated microbiome diversity or aberrant composition with disease states including metabolic disorders (16). Well-established clinical practices to prevent or manage metabolic diseases necessarily require drastic dietary and lifestyle modification, ostensibly to promote a beneficial physiologic trajectory (eg, insulin sensitivity) within the individual (17). However, this treatment regimen may be incomplete because purposeful dietary influences on the composition, and thus function, of the microbota may achieve further reductions in deleterious metabolites and/or signaling molecules (eg, elevated postprandial glucose). As such, the use of targeted dietary interventions to deploy cryptic functions nested within the endogenous microbiota is tantalizing in its potential to manage chronic conditions and, ideally, provide prophylaxis in at-risk individuals.

Nature provides a dramatic example of diet directing the establishment of a defined microbial community. Developing infants have universally ingested a single nutritive source through the course of evolutionary history, irrespective of geography, genotypic variation, or era. Apart from breast milk, human dietary habits do not involve universal consumption of a specific food source at any developmental phase. Human milk, like that in all mammals, supplies many bioactive components that benefit the infant beyond nutrition (18, 19). Microbially active milk constituents may positively or negatively influence populations found at various locales along the GIT. Accordingly, soluble or conjugated milk glycans simultaneously affect the fitness of certain microbial subpopulations. Both milk oligosaccharides and other glycoconjugates are recalcitrant to digestion in proximal GIT sites and are thus encountered by the colonic microbiota. As an example of positive enrichment, infant-harbored bifidobacteria are capable of using milk glycans consistent with their frequent overrepresentation in breastfed infants (20–23). Similarly, the *Bacteroides* species prevalent in nursing infants are also capable of consuming milk oligosaccharides as a sole carbon source in vitro (24). In an elegant experiment, *Bifidobacterium longum* subsp. *infantis* was shown to be numerically dominant over *Bacteroides thetaiotamicron* while successfully competing for a purified milk oligosaccharide (ie, lacto-*N*-neotetraose) in biassociated gnotobiotic mice (25). Although conducted in a simplified system, this is important in vivo evidence for the enrichment of bifidobacteria via milk glycans at the expense of taxa favored by a postweaning diet.

In a complementary role, milk also negatively regulates the composition of the infant microbiome, most notably through glycan receptor mimicry. Accordingly, certain pathogen adhesins possess an affinity toward milk glycan structures that resemble otherwise targeted host epithelial ligands. Thus, would-be pathogens are neutralized and expelled from the host via normal bulk transit through the GIT (26, 27). In addition to carbohydrates, bactericidal peptides and lactoferrin, among other milk molecules, contribute to mold the microbial communities of the infant gut (28, 29). Therefore, milk harbors both the information and catalytic function to dictate, in part, the character of this microbial landscape. Establishment and maintenance of this protective milk-oriented microbiota (MOM) may prove to be critical in fulfilling the distinct physiologic needs during the early stages of development (30). Although there is some measure of experimental support for this, it remains an intriguing hypothesis that is currently under investigation. Nevertheless, MOM is a useful guide for targeted manipulations in infants afflicted with diseases stemming from microbial imbalances, including necrotizing enterocolitis as well as late-onset sepsis in preterm infants (31, 32).

ARE GIT MICROBIOTA AN EXAMPLE OF COEVOLUTION WITH THE HOST AND FOOD?

Coevolution of a mutualistic relation is defined as reciprocal adaptation between the host and its associated symbiont, or community, that delivers a net benefit to both entities (33). Despite distinct genomic signatures of coevolution, adaptive traits are often identified qualitatively or in the absence of evidence altogether. The host may manifest phenotypic consequences of coevolution through dependence on capture of microbial products (eg, vitamins). Moreover, there are often corresponding alterations to the host genome, or its expression, to enhance sequestration of the target molecule or molecules and deemphasize host-side biosynthesis if the potential existed. Of course, the converse phenomenon provides multiple examples of microbial symbionts that have reduced their genome to degrade superfluous biosynthetic capabilities in lieu of molecules provided by the host in abundance (34). In cases in which complete codependence exists, symbionts are often vertically transmitted from mother to progeny, most notably in low-diversity insect bacteriomes (35). An obligate microbial composition remains to be determined for any stage of human development within the environmental context experienced by the holobiont (ie, union of host and microbial cells). Although there is compelling evidence that germ-free laboratory animals exhibit a multitude of developmental and physiologic defects that are manageable in controlled conditions, this would be devastatingly deleterious to fitness in nature (6, 36).

To identify coevolutionary relations with mammals and their resident microbes, codivergence inference is a simple yet

insightful method predicated on detecting phylogenetic similarities in both the host and symbiont evolutionary lineages (37, 38). During the course of speciation, the entirety of the microbial community phenotype may be subjected to strong selection, leading to microbiome differentiation mirroring host trait innovation as well as environmental conditions (eg, diet). An example is found in koalas that prepare their joeys for weaning from milk to eucalyptus (38). The mother koala produces and excretes what is referred to as pap, which contains a higher concentration and different composition of bacteria than her feces, to prime the joey for leaf and branch digestion (39, 40).

MICROBIAL COMMUNITY STRUCTURE DEFINES FUNCTION

The method de rigueur for defining microbial community diversity involves sequencing a segment of the small subunit ribosomal DNA phylogenetic marker (ie, 16s amplicons) (41, 42). High-throughput multiplexing of samples with so-called next-generation sequencing (NGS) platforms has enabled questions aimed at resolving the interface of food, microbiota, and human health (43, 44). Informing these nutrition-focused studies, the Human Microbiome Project (HMP), completed in 2012, was a multicenter investigation that used NGS technology on a large scale. The HMP densely sampled microbial communities of a large number of subjects at various body sites to describe the healthy adult microbiome in a Western population. Consistent with previous studies, the HMP found that a particular phylotype was not universally present throughout a subject's body or between individuals (45). Instead, metagenomic reconstruction of community metabolism indicated that similar metabolic networks were common between individuals. This finding further confirmed that functional redundancy exists within heterologous consortia (46, 47). Furthermore, each body habitat was characterized by signature taxa that contributed heavily to the composition of the community as previously reported.

The link between obesity and metabolic syndrome and microbiota has been described in pioneering work on the topic (16, 48, 49). There are several potential contributing factors including both bacterial and social antecedents, such as endotoxemia and Western dietary norms, respectively (50, 51). The HMP, in addition to concurrent research initiatives, sought to link microbial population structure with genetic functional potential through metagenomics (45, 52). Not surprisingly, the HMP study indicated that metabolic pathways were evenly distributed between individuals including ribosomal and translational processes, ATP synthesis, and glycolysis among other basal microbial activities. Additional features were consistently present in fecal extracts, albeit at low abundance, including spermidine biosynthesis, methionine degradation, and hydrogen sulfide production. Interestingly, a significant fraction of the GIT metagenome cannot be assigned a function and is postulated to be responsible for metabolic plasticity, responding to environmental conditions and signals, dietary deviations, or introduction of xenobiotics (45, 53).

Of tremendous interest to the field of nutrigenomics would be a reliably predictive coupling of microbial composition with quantitatively assessed at-risk individuals and those managing chronic metabolic diseases. To this end, a large-cohort clinical study examined the fecal metagenome donated from 345 Chinese subjects to ascertain almost 60,000 markers linked with type 2

TABLE 1
Glycoside hydrolases present in representative bifidobacterial genomes

Glycoside hydrolase	*B. adolescentis* ATCC 15703	*B. angulatum* DSM 20098	*B. bifidum* PRL2010	*B. breve* UCC2003	*B. catenulatum* DSM 16992	*B. dentium* Bd1	*B. longum* DJO10A	*B. longum infantis* ATCC 15697	*B. minimum* DSM 20102	*B. pseudocatenulatum* DSM 20438	*B. pseudolongum* AGR2145	*B. subtile* DSM 20096	*B. thermacidophilum* DSM 15837
α-Mannosidase	1	0	0	3	1	0	3	2	0	0	0	0	0
β-Galactosidase (or similar)	10	5	6	10	7	12	4	8	0	7	5	1	4
α-Galactosidase	3	2	1	2	3	6	3	1	2	2	4	2	2
β-Xylosidase	6	5	1	0	7	8	9	1	0	11	3	0	2
N-acetyl-β-hexosaminidase	0	0	4	1	0	0	1	3	0	1	0	0	0
α-L-Arabinofuranosidase	2	1	1	0	3	2	5	1	0	3	1	0	1
Arabinogalactan endo-1, 4-β-galactosidase	0	0	0	1	0	1	1	1	0	0	0	0	0
α-Sialidase	0	0	2	1	0	0	0	2	0	0	0	0	0
α-L-Fucosidase	0	0	2	1	0	1	0	5	0	1	1	0	0

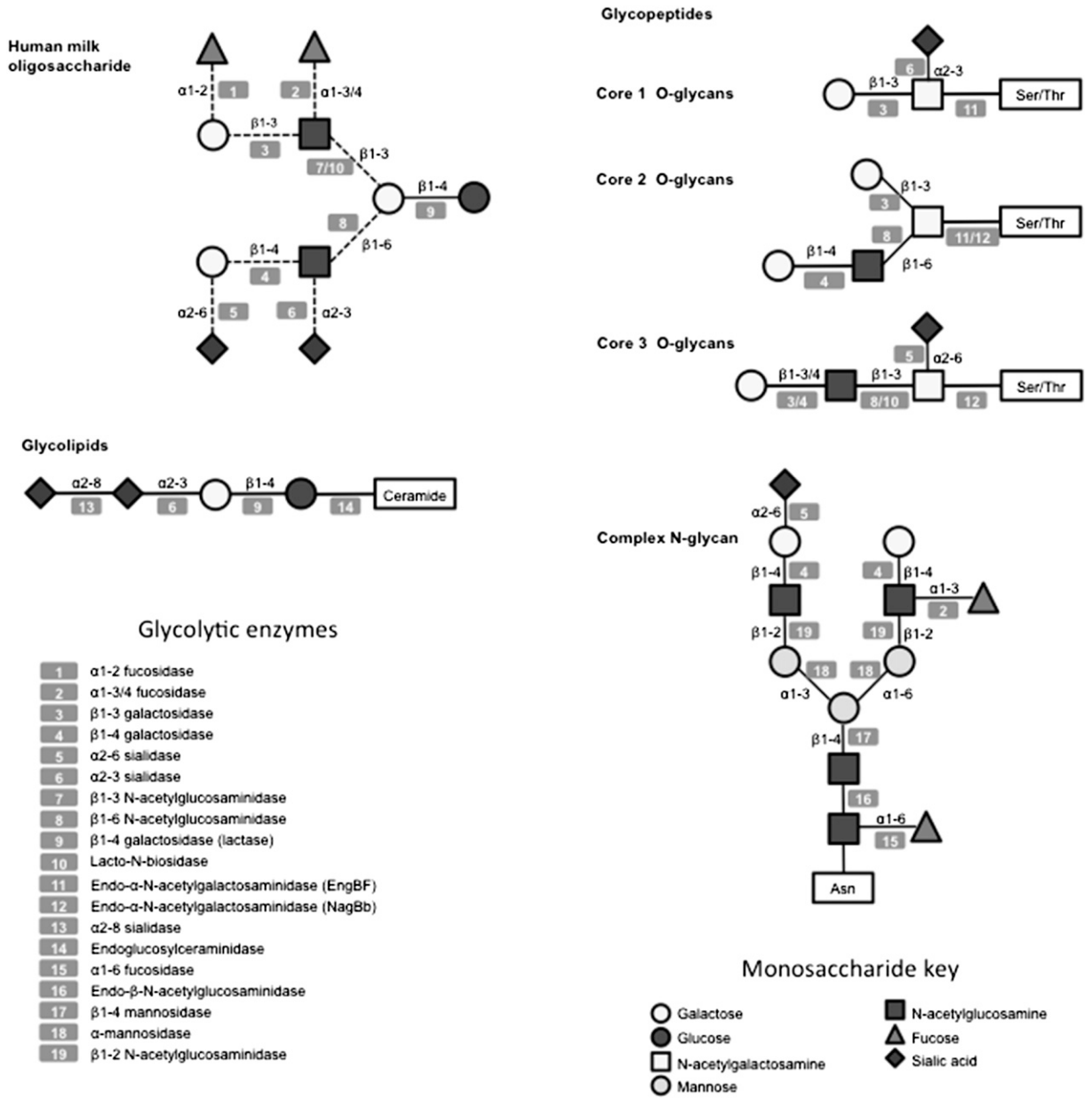

FIGURE 1. Various soluble and conjugated milk glycans and the glycosyl hydrolases used to cleave the connecting linkages.

diabetes (T2D) (54). Qin et al (54) termed this approach a metagenome-wide association study (MWAS), which is the logical extension of single nucleotide polymorphism linkage with holobiont phenotypes rather than solely considering the host genome. Additional observations of the T2D subjects included microbial metabolism changes commensurate with dysbiosis, which in turn diminished the butyrate-producing population. Importantly, this study is proof-of-principle of a microbe-inclusive nutrigenomics MWAS and potential diagnostic markers that may be clinically relevant once explored further. A subsequent study characterized the fecal metagenome of 145 Western women exhibiting various states of glucose control and described community and functional differences that were linked to T2D status. Interestingly, the biomarkers identified differed between the Chinese and the European studies, further accentuating the importance of predictive tools that account for geography, phenotype, age, and sex (55).

Although promising, systems-level approaches to determine microbial community function have generally lagged behind the relatively mature methods to profile diversity. As such, RNA-seq metatransciptomics has been applied to ex vivo fecal communities in both animal models and in human studies alike. A pertinent study that examined both approaches entailed providing a probiotic cocktail to human subjects and to gnotobiotic mice with a humanized GIT microbial community (56). Surprisingly, the probiotics did not greatly alter the microbiome composition relative to the pretreatment communities. However, the mouse microbiota metatranscriptome exhibited discernible metabolic

changes, most notably in plant oligosaccharide utilization function. The human metatranscriptome showed a similar response, largely during the feeding trial itself. There are a few additional examples of gut metatranscriptomes, including a comparative study of sow- and formula-fed piglets (57).

In addition to broad surveys of community diversity and functional potential, the need remains, perhaps somewhat more urgently, to characterize the biology of individual commensals as it pertains to GIT persistence and host interactions. Whereas this has traditionally been the purview of microbiologists focused on the genetics and physiology of a preferred organism, this expertise is invaluable to fully integrated nutrigenomics investigations performed by cross-disciplinary teams. To illustrate this, mechanistic studies of infant-associated bifidobacteria functional genomics and carbohydrate metabolism have informed clinical trials and potential novel diagnostics and therapeutics (58–60). Moreover, this long-term effort has yielded a rich depth of understanding of the genetic underpinnings (61, 62), biochemical function (63, 64), and physiologic state (65) of the microbiota during milk oligosaccharide consumption. This is in addition to describing the specific milk glycan substrates preferred and consumed (22, 23, 66). As a direct result, it is now possible to test the hypothesis that a discrete subset of milk glycans could be delivered with a predictable effect on the subject's microbiome. Furthermore, *B. longum* subsp. *infantis*, as well as other GIT-borne bifidobacteria, are now characterized to the point that matching specific genome-encoded enzymes (**Table 1**) with substrate glycan linkages on which they hydrolyze is now possible (**Figure 1**). This further elevates the functional annotation of subsequent nutrigenomic approaches that harness metagenome sequencing (eg, MWAS) to predict the microbial potential to use dietary glycans, milk or otherwise.

CONCLUSIONS

In this age of democratized access to high-throughput sequencing (67), it is clear that nutrigenomics models of the human holobiont should seek to not only profile host gene expression but also to account for the high level of interconnectedness with their microbiota both in form and function. Microbial phylogenetic diversity overlaid with metatranscriptomics is reminiscent of traditional nutrigenomics expression profiling in response to an exogenous perturbation, although this approach has yet to enter mainstream use. Looking ahead, holistic examination of subject metabolism would be considerably enhanced by viable quantitative metatranscriptomics, although this may require further optimization of sample preparation and throughput to realize its potential to describe community-level phenotypes, in addition to individual isolates. Similarly, metabolomics is emerging as a powerful tool when trained on host tissue or GIT luminal fluid, but it is currently underused in microbiome studies (68–71). As a consequence of the current ease by which DNA is sequenced, there has been a proliferation in facile, open-source NGS analysis software oriented to both entry-level users as well as experienced bioinformaticians (72, 73). As additional omics technology matures, it would be tremendously useful for similar metabolomics and proteomic programs to be developed and, importantly, supported.

There are several outstanding questions that present opportune targets for microbially integrated nutrigenomics research. Functional redundancy between microbiomes has been consistently reported, particularly in descriptions of metagenomic potential. However, the expression of this potential redundancy has yet to be observed over the course of human development. Clearly, an infant's trophic requirements vary as he or she grows, perhaps reflected in the metabolic appropriateness of a given microbiome-expressed phenotype. Regardless of phylogenetic composition, do deviations from ideal phenotypic expression have acute and/or chronic consequences? Does the expression program of a bifidobacterial-dominated MOM change in response to signals secreted in breast milk, the infant host, or both? Moreover, it is unclear to what degree host gene expression responds to fluctuation in the microbial metatranscriptome, although there are early indications of this (74). In any event, there are limitless questions pertaining both to the host and microbe side of the human holobiont. One may argue, however, that the most intriguing nutrigenomics inquiry is centered at the confluence of both domains.

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