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The Human Microbiome Project: Beginning and Future Status

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In 1884 Robert Koch and Friedrich Loeffler published Koch's Postulates defining our historical understanding of the relationship between an organism and infection: one organism: one disease. In the last decade with research on the microbial community living on and in humans, a new concept of microbial diseases has emerged; that is, alterations of the microbial community can lead to disease including an extension beyond traditional "infectious" diseases to include metabolic disorders such as type 2 diabetes and obesity. As we continue to gain knowledge about the functions of the normal microbiome and the effects of alterations of the microbial population on disease pathogenesis, a new era of diagnostics and therapeutics will evolve. **(Ann Clin Microbiol 2013;16: 162-167)**

Key Words: History, Human Microbiome, Metagenome

INTRODUCTION

The Human Microbiome Project (HMP) was a 5 year, multinational study of the microbial population that live in and on healthy adults [1-3]. This program was funded with a $153 million research grant from the National Institutes of Health (NIH) Common Fund, a funding mechanism designed to support high impact, innovative research that encompasses a number of NIH Institutes and Centers. Additional funding for this program was contributed by individual NIH Institutes such as the National Institute of Allergy and Infectious Diseases (NIAID), the National Cancer Institute (NCI), and National Human Genome Research Institute (NHGRI).

It is important to recognize that the origin of HMP was established through the successful completion of the Human Genome Project (HGP), a 13-year international effort initiated in 1990 and coordinated by funding from the NIH, US Department of Energy, UK Wellcome Trust, and additional support from a number of countries including Japan, France, Germany, and China. The goals of the HGP were to determine the sequences of the approximately 3 billion nucleotides that make up human DNA, identify the approximately 21,000 protein-encoding genes in human DNA, store the collected information in accessible databases, improve tools for sequence analysis and transfer these technologies to the scientific community, and address ethical, legal, and social issues that arise from the project.

BEGINNING OF THE HUMAN MICROBIOME PROJECT

Selected milestones of the HGP are summarized in Table 1. Much like creating a blueprint for a house and construction of the structural frame before walls can be erected and finishing work performed, efforts in the first 3 years of the project were devoted to defining the scope and infrastructure of the project. The Genetic Privacy act in 1994, together with the Health Care Portability and Accountability Act in 1996, addressed ethical and social issues related to the use of genomic sequence information. Interestingly, the US Supreme Court has recently returned to these complex issues when they ruled that naturally occurring genes could not be patented. In 1995, genomes of the first sequenced bacteria, *Haemophilus influenzae* and *Mycoplasma genitalium*, were published followed quickly with the publication in 1996 of the first sequence of a fungus, *Saccharomyces*

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Table 1. Selected landmarks associated with the human genome project

Year	Events
1991	Human chromosome mapping data repository established
1992	Low resolution genetic linkage map of human genome published
1993	Consortium established to coordinate efficient mapping and sequencing
1994	Completion of second-generation DNA clone libraries; genetic privacy act proposed
1995	High resolution maps of chromosomes 16 and 19 produced; first bacteria sequenced-Haemophilus influenzae, Mycoplasma genitalium
1996	First yeast (Saccharomyces cerevisiae) sequenced; health care portability and accountability act prohibits use of sequencing data for health insurance decisions
1997	E. coli genome sequenced; high resolution maps of chromosomes X and 7 produced
1998	M. tuberculosis sequenced; Celera Genomics formed to sequence human genome in 3 years
1999	Human chromosome 22 first to be completely sequenced; joint genome institute sequencing facility opened in california
2000	Completion of working draft DNA sequence of human genome announced; chromosome 21 completely sequenced; working drafts of chromosomes 5, 16, 19 announced; fruit fly Drosophila sequenced
2001	Chromosome 20 finished; publication of working draft sequence of human genome by HGP and Celera Genomics
2002	Draft sequence of mouse genome published
2003	Human chromosomes 6, 7, 14, and Y completed; HGP declared finished
2004-06	Remaining human chromosomes completed
2007	Human Microbiome Project (HMP) begins

cerevisiae, the fruit fly *Drosophila* in 1998, and the mouse genome in 2002. In 1998 Celera Genomics was formed as a private venture and announced they would complete the human genome sequencing in 3 years, introducing an era of scientific competition that would stimulate the genome project. In 1999 the first human chromosome was completely sequenced, followed rapidly with publication of the complete sequences of other chromosome. In 2001 HGP and Celera Genomics jointly published the working draft sequence of the human genome [4, 5] and the final complete sequences of all human chromosomes were published over the next 5 years. Much like efforts to send a man to the moon, the greatest legacy of the HGP was the development of technologies and informatic solutions that allowed the generation and analysis of tremendous amounts of sequencing data [6].

With the successful completion of the Human Genome Project, efforts turned to the Human Microbiome Project. To put the complexity of this program into perspective, consider the fact that bacterial cells outnumber human cells in the host by 10 : 1 [7]. It is estimated at a 200 lb (90 kg) adult carries 2 to 6 lbs (1-3 kg) of bacteria. The human genome contains approximately 21,000 protein-coding genes while the bacterial population contributes at least 100-fold more unique protein genes [8]. Sequencing the human genome generated 23 Gb of data [1], while the microbiome generated 150-fold more sequence data [9].

In 2007 the Human Microbiome Project was launched with goals very similar to those defined for HGP: preliminary characterization of the human microbiome, development of a reference set of microbial genome sequences and establishment of resource repositories (the structural framework for this project), development of new sequencing tools and technologies for computational analysis, catalogue the microbial population and their metabolic activities at discrete body sites in healthy adults, define the relationship between the microbiome and health and disease, and address ethical, legal and social implications of HMP research. The central efforts of this project consisted of collecting samples for 242 healthy adult volunteers, including 129 males and 113 females, with participants recruited at two sites and screened comprehensively for evidence of disease and recent drug usage that would potentially influence the microbiome. A total of one specimen was collected from the nares, nine oral specimens, one stool specimen, four skin specimens, and three vaginal specimens. Multiple specimens were collected over a 22 month period from each volunteer so sequential changes in the microbiome could be analyzed. A total of 11,174 samples were collected; 5,177 collected samples were analyzed by sequencing targeted regions of the 16S rRNA gene and a subset of 681 specimens was also analyzed by whole genome shotgun sequencing. This dual sequencing approach provided very different information. The 16S rRNA gene sequencing defined the quantitative membership of a population; that is, the individual species of bacteria in a specimen and their relative proportion could be determined. In contrast, whole genome shotgun sequencing defined the composite genetic potential or metabolic activity of the population.

GENETIC ELEMENTS USED IN THE HUMAN MICROBIOME PROJECT

The 16S rRNA gene and shotgun sequencing revealed that there was substantial variation in the number of species and gene composition at different body sites [2,7]. The body sites with the greatest richness in species were stool specimens, followed by mouth, skin and nasal, and vaginal was the least complex. Although assessment of the number of species recognized at a site were influenced by the sequencing target (richness assessed by sequencing the V1-V3 region of 16S rRNA gene was consistently higher than sequencing the V3-V5 region) and depth of sequencing (greater diversity revealed with more targeted sequences), the relative richness remained constant [10]. Not surprising, the genetic diversity paralleled the taxonomic richness with greatest diversity in stool specimens and lowest in vaginal specimens. Taxonomic and genetic diversity was further revealed when microenvironments were examined, such as different regions of the mouth, gastrointestinal track, skin surface, and vagina [11]. For example, Grice and Segre [12] elegantly demonstrated the microbial populations of different topological regions of the skin surface had characteristically distinct populations that were reproducibly observed among adult volunteers.

In contrast with the taxonomic diversity that exists in and on the human body, relatively few organisms were persistently found in all individuals (the core microbiome is defined as the species shared by 95% or more of individuals at a specific site). The highest number was present in the mouth, followed by the nose, stool and skin, and again the fewest found in the vagina. It was observed that the core microbiome was typically highly abundant representing 75% or more of the total population, while the large number of other species was represented in the minority population. In most samples, the core microbiome was accompanied by low-abundant species of the same genus. There was tremendous variation of species among individuals; however, there was less variation in functional redundancy in the genetic composition at each site [13]. That is, the taxonomic diversity of a population is great but the functional properties are highly conserved in microbiomes associated with health. This is not surprising if we consider that the microbiome is a community that exists in a mutualistic beneficial relationship with its host, providing needed metabolic functions, stimulating innate immunity, and prevention of colonization with unwanted pathogens. Thus interpersonal variations of the microbiome exist in healthy individuals as long as the needed functions are satisfied [12].

If the microbiome characterizes health, alterations in the microbiome can signify disease. For example, shifts in the skin microbiome are associated with episodic exacerbations of atopic dermatitis and progression to chronic wound infections [12], shifts in the vaginal microbiome from relatively few predominant organisms to a heterogenous mixed population is associated with the progression to disease [14,15], and characteristic changes of the intestinal microbiota are associated with diseases such as Clostridium difficile infections (CDIs), ulcerative colitis, obesity, Crohn's disease, inflammatory bowel disease and colorectal cancer [16].

APPLICATION OF THE HUMAN MICROBIOME PROJECT FOR INTERPRETATION OF DISEASE

There are physiological explanations for the observed pathology associated a disruption of the microflora (commonly referred to as dysbiosis or the state of imbalance in the microbial ecosystem). For example, *C. difficile* is able to proliferated and express enterotoxins when the intestinal microbiome bacteria are suppressed with antibiotics. Ulcerative colitis is associated with an increased level of mucin-degrading sulphatases, leading to degradation of the protective mucosal lining of the intestinal wall. The microbiotia of obese patients are able to more effectively extract nutrients from the patient's diet than organisms in lean patients [17].

An understanding to the influence of dysbiosis on disease pathology can lead to both advanced diagnostic tests and paths for therapeutic intervention. For example, patients with chronic relapsing CDIs have been successfully treated with stool transplants from a spouse or close relative, as well as with artificially created stool specimens consisting of a complex mixture of aerobic and anaerobic fecal organisms [18,19]. Similar mixtures of organisms have been used as prophylaxis for patients at high risk for infection. More limited intervention studies have been reported for other inflammatory bowel diseases [20].

Vincent et al. [21] demonstrated that despite substantial inter-individual variation in the composition of the fecal microbiota, changes in the diversity and composition of the fecal microflora could predict susceptibility and onset of disease. Fecal swabs were analyzed from 599 hospitalized patients, including 25 patients who developed CDI after the initial specimens were collected. When compared with matched controls, a decrease in microbial diversity was associated with *C. difficile* disease.

Specifically, at the family level, Clostridiales Incertae Sedis XI and Bacteroidaceae were depleted and Enterococcaceae were enriched. When controlled for antibiotic usage, the depletion of Clostridiales remained significant.

Morrow et al. [22] also demonstrated that alterations in the gut microbiome could predict development of disease. Necrotizing enterocolitis (NEC) is a devastating intestinal disease that afflicts 10% of extremely preterm infants. Morrow et al. prospectively collected stool samples (to assess the taxonomic complexity of the microbiome) and urine (to assess the metabolomic profiles) from infants $<$29 weeks gestational age, including 11 infants who developed NEC and 21 matched controls. Prior to the develop of disease, a distinct dysbiosis was note with infants with early onset disease with a dominance of Firmicutes (predominantly *Staphylococcus*) and infants with late onset NEC with a dominance of Enterobacteriaceae. Interestingly, alanine in urine was associated with Firmicutes dysbiosis and histidine was inversely present in the urines of infants with Enterobacteriaceae dysbiosis. A high ratio of alanine to histidine was a good predictor of NEC in all infants.

The effect of microbiome alterations have also been described for the pathogenesis of inflammatory bowel disease and colorectal cancer. Proliferation of bacteria such as *Akkermnsia mucinphila* that produce mucin-degrading sulphatases are responsible for degradation of the intestinal wall lining [23]. Additionally, an increase in members of the anaerobic family Prevotellaceae leads to upregulation of chemokine expression mediating inflammation [24]. Enterotoxigenic *Bacteroides fragilis* can also induce T-helper cell mediated inflammatory responses that are associated with colitis and are a precursor to colonic hyperplasia and colorectal tumors [25]. Finally, *Methanobrevibacter smithii*, a minor member of the gut microbiome, enhances digestion of dietary glycans by *Bacteroides thetaiotaomicron* and other core intestinal bacteria leading to accumulation of fat [26].

SUMMARY

We are only beginning to understand the relationship between the microbiome and disease and to realize the benefits of the microbiome project. In 1884 Robert Koch and Friedrich Loeffler defined the relationship between an organism and infection. Koch's Postulates were based on the concept of one organism: one disease. The microbiome research has introduced a new concept of disease caused by a community of organisms, extending beyond traditional "infectious" diseases to include metabolic disorders such as type 2 diabetes and obesity. We are now at the forefront of a new era of "microbial" diseases.

We do not yet know how to utilize the technological skills developed with the Human Genome and Microbiome projects for diagnostic or therapeutic purposes [13]. Likewise, a number of questions remain to be resolve including-can we predict disease in an individual by monitoring changes in the microbiome; which changes are most important-taxonomic or genetic function; can we prevent disease or treat disease by reestablishing a healthy microbiome; does treatment consist of use of individualized taxonomic replacement (e.g., fecal transplant) or universal metabolic supplementation; what is the role of the host genome and environmental factors in shaping the microbiome; what will be the informatic requirements for guiding diagnostics or therapeutic. Regardless of the answer to these and other questions, it is certain that the future will reveal exciting innovations in the management of disease.

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인간 전유전체 사업의 시작과 향후 전망

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1884년, Robert Koch와 Friedrich Loeffler는 미생물 감염을 정의하기 위한 Koch's Postulates를 발표하였다. 그러나, 최근 10년간 인체 미생물 연구 조사(Human Microbiome Project)에서 미생물과 질병의 관련에 대한 새로운 개념이 제시되고 있다. 이들 미생물 군집에 대한 연구는 고전적인 미생물-감염병의 관계를 미생물-대사 질환(제2형 당뇨병이나 비만) 등으로 범위를 확대할 수 있음을 시사하고 있다. 정상 미생물 군집의 기능과 질병의 병태생리학, 그리고 미생물의 조절 역할에 대 지식 축적이 계속 진행됨에 따라 진단과 치료의 영역은 새롭게 발전할 것이다. [Ann Clin Microbiol 2013;16: 162-167]

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