



Nutrient-gene interactions: application to pet nutrition and health



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KEY POINTS

- ▶ The canine genome sequence is complete and the feline genome project is underway
- ▶ Finding single nucleotide polymorphisms (SNPs) affecting response to nutrients or drugs will allow both researchers and veterinarians to formulate diets based on genotype, identify populations susceptible to disease, and effectively treat diseased pets
- ▶ Epigenetic inheritance persists over generations and may contribute to an animal's ability to respond to specific nutrients
- ▶ It is now established that nutrients may influence gene expression, including mRNA production (transcription), mRNA processing, protein production (translation) and post-translational modifications, thereby influencing the metabolic status of an animal
- ▶ Future research will allow for the formulation of genotype-based diets, the use of biomarkers for early detection of disease, and new therapies for companion animal diseases

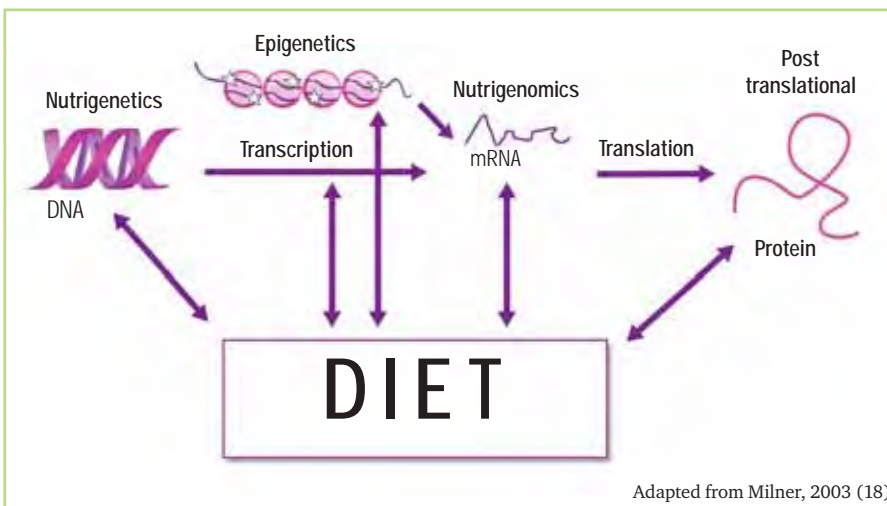
Definition of terms

Term	Definition
Nutrigenetics	The effect of genotype on nutrient absorption, metabolism, transport, or excretion
Nutrigenomics	The effect of nutrient(s) on gene expression
SNP (Single Nucleotide Polymorphism)	A single nucleotide change in the DNA sequence of a gene
Epigenetics	A stable and heritable change in gene expression that is independent of changes in DNA sequence

Introduction

Companion animals, namely dogs and cats, present unique challenges to the fields of veterinary medicine and nutritional science. In addition to their metabolic idiosyncrasies, nutrition and care are based on promoting lifelong health, not on production traits (e.g. average daily gain, feed efficiency) as it often is in livestock species. Furthermore, pet owners continue to anthropomorphize cats and dogs and are willing to spend large sums of money for what is deemed necessary to provide for optimal health and happiness of their pets. Knowing this, it may not be surprising that companion animal nutrition and medicine often mirrors that of humans. For example, many current research programs include the testing of “functional” ingredients, identifying the effects of maternal nutrition, maintaining health in geriatrics, or studying nutrition’s role in disease prevention and treatment. Now that the “omics” era is upon us, the challenge of identifying nutrient requirements not only based on life stage, but also according to genotype exists.

Figure 1. Diet-Gene interactions.



Given its importance as a biomedical model for humans, the dog was the first non-rodent mammal to have a completed genome sequence. Led by the efforts of the National Institutes of Health (NIH), the first draft of the canine genome was completed in July of 2004. Since then, a high-quality draft genome sequence and a map of single nucleotide polymorphisms across breeds has been completed (1). Although it is a couple of steps behind that of the dog, significant progress has been made with the Feline Genome Project, which is now underway. Both genome sequences will serve as valuable tools in companion animal research and veterinary medicine.

In addition to genome sequence data, new technologies developed in the areas of molecular biology, computer science and bio-informatics, and nanotechnology have provided researchers with a powerful toolkit to complement existing techniques. These tools will enable great research strides in companion animal nutrition and health in coming years.

Diet is arguably the most important environmental factor affecting the phenotype (physical characteristics) of an animal. Not only does the genetics (DNA) of an animal affect how the body responds to diet, but diet influences gene expression, including transcription (mRNA synthesis), mRNA processing and transport, translation (protein synthesis), and post-translational modifications (**Figure 1**). Moreover, new findings in the field of epigenetics have demonstrated that nutrient status *in utero* and/or during the early postnatal period may have vast effects on lifelong metabolism and disease risk.

Although the field of nutrigenomics is still in its infancy, its importance has already been demonstrated. The primary means by which nutrients and genes may interact, a few examples, and its application to the future of companion animal health and disease will be reviewed.



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Figure 2.
Bedlington Terrier.

■ Nutrigenetics

Although the absorption, metabolism, excretion, etc. of most nutrients is now well described, the factors contributing to the large inter-animal variation present in these processes in the canine and feline population are poorly defined. The genetic background of the population studied may be one of the primary factors responsible for these differences. The term “nutrigenetics” may be used to describe the effect of genotype (genetic background) on nutrient absorption, metabolism, transport, and excretion. An animal’s response to a specific nutrient, for example, may be dependent on the DNA sequence and consequent amino acid sequence and protein structure of the genes involved with its absorption and metabolism.

While variation in DNA sequence may be altered in several ways, the simplest change is known as a single nucleotide polymorphism (SNP), in which one nucleotide is replaced with another. Single nucleotide polymorphisms may be thought of as “variations in a recipe” (2). Each gene is a recipe for a specific protein or group of proteins that perform a given biological function. Depending on the location and type of SNP, the phenotypic effects may be severe, mild, or undetectable. While many SNP may not have a biological effect, others may change the recipe, resulting in a different quantity or type of protein being produced. Even if the SNP effects are not detectable alone, they may have additive effects and significantly alter protein functionality, potentially contributing to an animal’s response to diet or susceptibility to disease. Although this area is in its infancy, the importance of SNP profiles has been recognized and is an area that is being heavily researched in humans, pets, and livestock species.

Human genome research has already identified numerous SNP believed to be associated with increased risk of disease, many of which are applicable to companion animals. One example is the connection between interleukin-1 (IL-1) and the incidence of inflammatory diseases. In humans, individuals having a specific variation of the IL-1 gene have shown an increased risk of several diseases, including Alzheimer’s disease, periodontal disease, and increased risk of heart attack. Inflammation is emerging as an important component of many chronic diseases, including those prevalent in the companion animal population such as obesity, diabetes, periodontal disease, arthritis, and inflammatory bowel disorders. Studying genotype variation, including SNP profiles, is a prime target for the development of disease prevention and treatment strategies in humans as well as in companion animals. Although significant time and funding is required to perform these studies, the rewards heavily outweigh the costs involved and must be done in companion animals.

Even though the identification and/or significance of most SNP are unknown or poorly described at present, several examples are available that may demonstrate the implications of their use in the future. One of the best examples is that of copper toxicosis present in the Bedlington Terrier breed (**Figure 2**). Although this disease has been studied for decades, it has only been recently found to be caused by a mutation in the MURR1 gene, with several genetic variants (*i.e.* SNPs) associated with the disease identified (3). A MURR1 mutation results in defective copper metabolism, thereby allowing copper to accumulate in hepatic tissue, ultimately leading to toxicity. Before the genetic mutation of this disease was known, the best method for diagnosis was measuring hepatic copper concentrations at 1 year of age. However, hepatic copper concentrations may already be dangerously high by that time. Because hepatic copper concentrations are not elevated in animals carrying only 1 mutated allele, this measurement is unable to identify carriers of the disease.

Given our current knowledge, a genetic test for such a disease enables screening for MURR1 mutations, identifying affected animals and carriers of the mutation. Genetic testing may be performed soon after birth, allowing the owner and veterinarian to

modify the diet accordingly. This is only one example of a nutrient-gene interaction and how it may impact the health and longevity of an animal. As more of these interactions are identified, more complex diets may be devised according to the genotype of an animal.

Many other known canine or feline SNPs are not necessarily associated with nutrition, but may play roles in health and disease or in selection criteria. A few examples are listed below. The Siamese cat has a distinguishing phenotype, a light-colored torso and darkened mask on the face, paws, and tail. This coloring pattern, commonly referred to as a “pointed” phenotype, is a type of albinism. A recent study evaluated tyrosinase mutations associated with coat coloring of Siamese and Burmese cats (**Figure 3**), the latter producing more pigment through the torso (4). Evaluation of the “pointed” phenotype determined that the mutation present in the Siamese breed is very sensitive to temperature, resulting in pigment only at the cooler extremities of the body. On the contrary, mutations present in Burmese cats were found to be less sensitive to temperature, explaining the pigmentation found throughout the entire body of this breed.

Several studies in dogs have focused on changes in immune function due to variants in the genome. Such studies may identify individuals or groups more susceptible to disease or those with a limited ability to fight infection. The Canine Major Histocompatibility Complex genes (DLA) are

considered highly polymorphic in dogs. Evaluation of the DLA class II variants found high variation between dog breeds (5). Due to comparisons of dogs to wolves, it is believed that these genes were selected for by evolutionary fitness. Furthermore, human intervention in dog breeding has created severe genetic bottlenecks, which may have caused changes in these regions and made some breeds more susceptible to certain diseases (5).

Extensive research is also underway in the area of pharmacogenetics, which evaluates the response of a patient to a particular drug. It is well established that genetic variation affects the means by which drugs are metabolized. In humans, individuals may be placed into various groups (e.g. poor, intermediate, extensive, or ultra metabolizers) based on their genetic profile (6). For some drugs already on the market, the dose and most effective form of drug may be aided by first identifying the genetics of the patient. This approach to medicine will become more widespread in the future not only in humans, but also for companion and production animals.

Several genes associated with drug-metabolism (e.g. cytochrome P450 enzymes) have been evaluated in dogs. The function of one such gene, CYP1A2, has been evaluated in dogs and was shown to have a SNP variant in the coding region of the gene (7). This gene is expressed in the liver and is important in determining the metabolic capacity in both dogs and humans. The particular variants

of this gene identified so far cause the gene to become non-functional (7). Numerous other variations likely exist in the canine CYP1A2 and other CYP genes, likely having great implications in canine pharmacogenetics.

A canine SNP chip is now commercially available, but reports of its use have yet to be published. As research using this chip and other mapping strategies identify more SNP of importance, genome screening



Figure 3. Siamese cat

Burmese cat

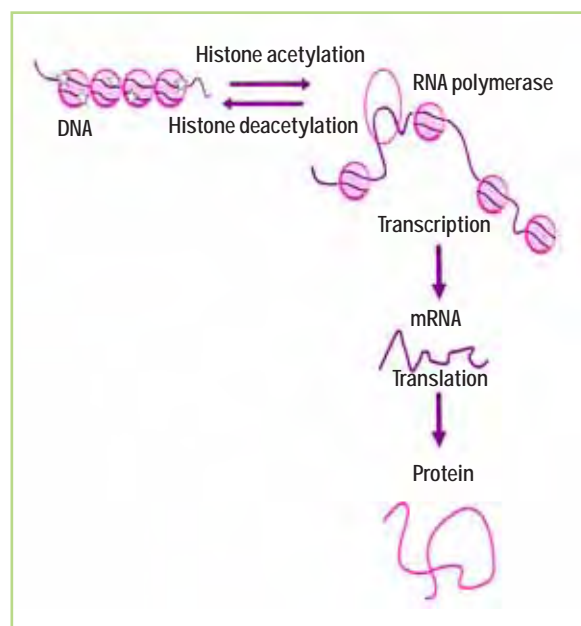
techniques will become more important when recommending diets and/or prescribing drugs. Although ethical complications may exist, their use in companion animals could serve as an important tool for veterinarians and pet owners alike. Genetic testing that enables informed breeding decisions may some day allow the prevention of severe genetic defects and decrease the frequency of disease genes, while maintaining genetically diverse pure-bred populations (8). Similar methods may also be used to prevent or effectively manage animals known to be susceptible to disease through dietary or pharmaceutical intervention. Each discovery will take us one step closer to personalized diets and medicines as is being done in human medicine.

■ Epigenetic inheritance and metabolic programming

Nutrient-gene interactions may also occur through epigenetic mechanisms. Epigenetics is one of two primary mechanisms by which the expression of genes is regulated. Epigenetic regulation refers to control of gene expression that is relatively stable and is heritable over generations. Despite the ability of these modifications to persist after replication, these changes in gene expression and regulation are independent of changes in DNA sequence. Epigenetic inheritance is influenced by numerous factors, including diet and hormone concentrations during early development and throughout life. The epigenetic modification most often studied involves DNA methylation patterns. In general, methylation near gene promoter regions is associated with the silencing of genes (*i.e.* transcription is repressed). Histone acetylation patterns may also affect transcription rate (**Figure 4**).

Recent studies have linked epigenetic inheritance with lifelong metabolic status, a process termed “metabolic programming”. Given its link to chronic diseases such as obesity, diabetes, heart disease, and behavioral disorders, the nutritional effects on epigenetic inheritance continue to be heavily tested. Initial experiments focused solely on nutrient status *in utero*, demonstrating the importance of maternal nutrition. However, it is now well established that both prenatal and postnatal nutrition contribute to metabolic programming.

Figure 4. Epigenetic mechanisms of gene expression.



A convincing study on survivors of the Dutch famine during World War II has not only demonstrated the importance of prenatal and postnatal nutrition, but also shown that these effects may be passed on for several generations (9). Low birth weight offspring of women who had restricted access to food during gestation were reported to have an increased incidence of obesity, glucose intolerance, and hypertension as adults (10,11). The most remarkable finding from these studies was that an increased risk of disease was also detected in the third generation (*i.e.* the grandchildren of the women who survived the famine), demonstrating that maternal diet may affect not only a woman’s offspring, but future generations. Numerous rodent experiments have reported similar findings and have begun to identify the mechanisms involved in these processes.

Although good maternal nutrition of dogs and cats has been appreciated for decades, very little is understood in this area. This lack of knowledge has translated into poorly defined feeding recommendations and commercially available products for gestating and lactating mothers. Well-defined nutrient requirements for young, developing puppies and kittens, and the impact of nutrient status during this time on long-term health are also nonexistent. Given the recent advances in epigenetics, including our ability to measure DNA methylation status, great advances in metabolic programming should be expected in the future.

■ Nutritional regulation of gene expression- Nutrigenomics

In contrast to epigenetic modification, gene expression may also be regulated through unstable processes controlled by activators and repressors of DNA transcription. Genes associated with lipid metabolism, for example, are often regulated in this manner, being affected by such factors as energy source (fat *vs.* carbohydrate) and metabolic state (fasting *vs.* fed state) (12). Thus, nutritional status may affect the amount of enzymes present by causing large changes in the gene transcription rate.

Until recently, changes in gene expression attributed to diet were thought to be mediated predominantly through hormonal action or the nervous system. However, recent research has demonstrated that macronutrients (*e.g.* glucose, fatty acids, and amino acids) and micronutrients (*e.g.* iron, zinc, and vitamins) can regulate gene expression. There are also several bioactive food components that are thought to act as transcription factors directly affecting gene expression, including carotenoids, flavonoids, monoterpenes, and phenolic acids (13).

Nutrients may affect gene expression via direct or indirect mechanisms. Most of the examples provided above occur through direct regulation. However, nutrients such as dietary fiber, which is fermented in the gut by bacteria, lead to the production of compounds such as short-chain fatty acids. These by-products may then act as secondary messengers to affect gene expression. Limited research has been conducted in this area, but has increased the level of interest pertaining to fermentable fibers and their effects on the body. These initial reports remind researchers and veterinarians that we must not only consider what direct effects a diet may have on an animal, but what effects it will have on intestinal bacteria and the indirect effects coming from such changes.

Unlike testing epigenetic inheritance that may require long-term experiments involving several generations, identifying nutrient-related changes on transcription or translation may be done over a fairly short time period. Differences may be noted following the consumption of a single meal or after only a few days or weeks of a particular dietary regimen. Past experiments of this kind were

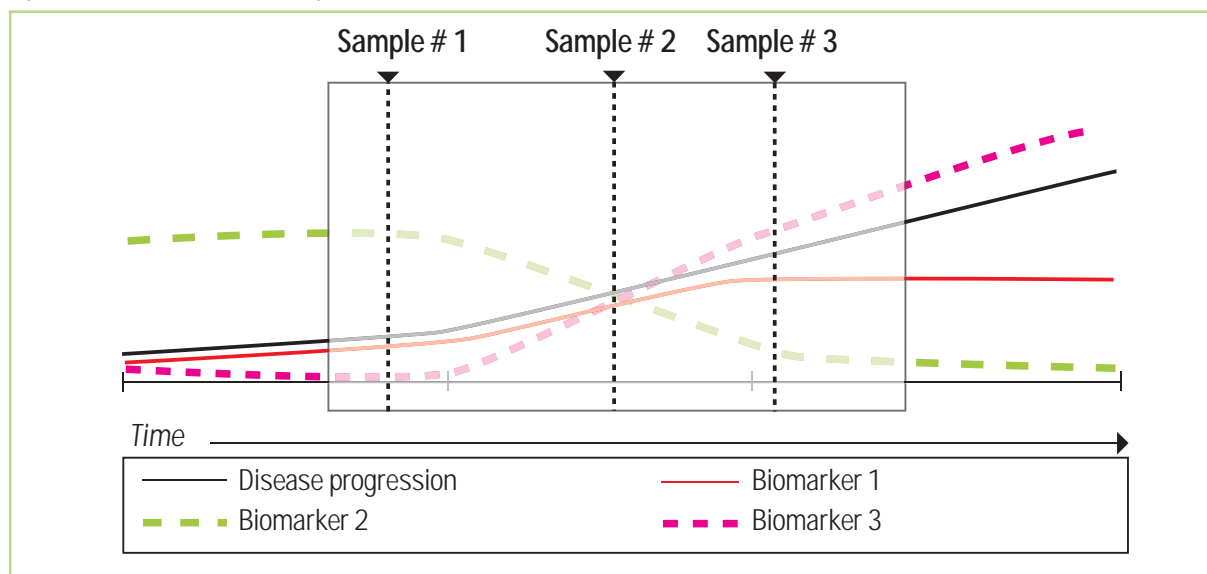
restricted in dogs and cats due to the limited gene sequence data in these animals. Most studies also focused on the response of a single gene or small number of genes relevant to the disease or metabolic pathway studied.

The recent emergence of the canine genome sequence has allowed researchers to develop commercial microarray chips capable of measuring thousands of gene transcripts simultaneously, providing a global view of gene expression. Microarrays allow researchers to identify an animal's "metabolic signature" or "disease signature" based on the gene expression profile. Given the recent availability of commercial arrays, a limited number of experiments have been conducted in canines, especially those pertaining to nutrition or disease. Of those published so far, many have compared gene expression profiles of healthy *versus* diseased dogs, including studies on osteoarthritis (OA) (14), cancer (15), and dilated cardiomyopathy (DCM) (16).

Canine OA leads to cartilage degeneration and presents problems similar to hip dysplasia. Following microarray analysis of articular cartilage, a gene responsive to mechanical impact, MIG-6, was associated with OA (14). These researchers further evaluated gene expression concentrations of MIG-6 using real time PCR to validate the array data. The study utilized Labrador Retrievers and Labrador Retriever/ Greyhound crosses determined to be high or low risk for OA based on dorsolateral subluxation scores. The MIG-6 gene was found to be elevated in dogs in the high risk OA group and it is hypothesized that this gene may regulate cartilage degradation and production of cartilage in the dog (14).

Cancer is a very complex disease involving numerous biological pathways and is an excellent use of microarray technology. Arrays are currently being used to study cancer in dogs, with preliminary studies evaluating tumor tissue *versus* healthy tissue (15). Another complex disease, DCM, was recently studied using canine microarrays. A preliminary study found the expression of genes involved in energy production (cellular), cell signaling and communication, and cell structure to be decreased in dogs suffering from DCM *versus* healthy controls (16). In contrast, genes associated with cellular

Figure 5. Disease biomarker discovery.



defense and stress response were up-regulated in the diseased population. These initial studies have identified numerous genes and pathways involved in the development of cancer, OA, and DCM and will hopefully be used to design future studies that further enhance our understanding of the disease process and highlight therapeutic targets.

Few studies have used microarrays to evaluate the effects of diet in healthy populations. However, to completely understand the effects of nutrition on health and disease processes, the global effects of changes in nutrient status are needed in healthy populations. Thus, our laboratory is currently investigating the effects of age and diet on gene expression profiles of numerous tissues important metabolically and/or in the aging process, including liver, colon, skeletal muscle, cerebral cortex, and adipose tissue. The preliminary results from this experiment are in press and have been instrumental in the design of ongoing and future canine and feline nutrigenomic experiments. Given the vast amount of canine genome sequence data and research tools now available, great research strides should be expected in nutrigenomics in the next decade. Although canine microarray chips are currently marketed, a feline microarray is yet to become commercially available. As feline genome sequence data becomes more available and the demand for these research tools increases, feline arrays and similar products will reach the marketplace.

Future of nutrigenetics and nutrigenomics in veterinary clinics

The impact of nutrigenomics on the future of companion animal care and nutrition is difficult to predict because it is heavily dependent on technological advances in the field. Economical, ethical, and regulatory issues will also dictate the speed at which these advances are made and the technologies and strategies are adopted. Because optimal health and longevity are primary goals in dogs and cats, strategies to prevent, minimize, or treat disease will likely get the most attention. The future will likely include the use of: 1) genome-wide genetic testing to avoid or limit the prevalence of genetic diseases, identify susceptible populations, identify optimal diets for a given genotype, and identify the most effective pharmaceutical forms and dosages according to genotype; 2) biomarkers for early disease detection and targeted therapeutics; 3) nanotools for analysis of minute tissue biopsies or blood samples.

While genome-wide genetic testing in the veterinary clinic may be many years away, its use will have great health implications in coming years. Currently, over 400 canine and 250 feline genetic diseases have been identified, many of which may be greatly impacted by genome mining experiments (17; <http://omia.angis.org.au/>). Screening genomes and mating individuals according to the presence of genetic mutations known to cause disease, may eliminate many of these diseases.

However, given the bottlenecks that have occurred with many breeds, this method of avoiding disease may not be possible or desirable. For such cases, early intervention using specific dietary regimens, as with the case of copper toxicosis, may be used to manage or limit disease risk. Genetic screening may also provide critical information pertaining to appropriate pharmaceutical dosages and forms.

While genetic diseases are a considerable problem in many dog and cat breeds, the vast majority of diseases affecting the health and longevity of pets are complex in nature. While these diseases often have a genetic component, age, gender, and environment factors such as diet also play prominent roles in their development. For diseases that are not easily identified with genetic testing alone, the use of biomarkers for early disease detection and characterization will be of utmost importance. Genomic biomarkers may occur prior to the appearance of clinical signs currently used to diagnose disease, enabling earlier detection and identifying specific biological targets. Our laboratory is currently searching for disease biomarkers, that either increase or decrease with the progression of a disease (**Figure 5**), with aims of developing such diagnostic tools.

Advances in nanotechnology will complement the aforementioned disease prevention or treatment strategies. It is already possible to use small tissue biopsy or blood samples for DNA testing or gene expression analyses. Our ability to use small biological samples for genetic testing or diagnostic purposes will only improve in the future. The limited invasiveness of these procedures will open new avenues for research and promote the use of these tools in the clinic.

In a time in which dogs and cats are continually becoming more like a family member than pet, it is not surprising that the nutrition and medical care rivals that of humans. The identification of SNP profiles, DNA methylation patterns, and gene expression patterns or signatures affecting or responding to nutritional status and/or disease will require significant time and financial resources, but come with great reward. As new and innovative technologies are developed, and costs for such tools decrease, the use of genomic biology will greatly increase in research and the clinical setting.

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