Precision/Genomic Medicine for Domestic Cats

Reuben M. Buckley, PhD, Leslie A. Lyons, PhD*

KEYWORDS

• Precision medicine • Genomic medicine • Direct-to-consumer genetic testing

KEY POINTS

• In veterinary health care, precision/genomic medicine should be considered at the level of species, population/breed, and the individual.
• Precision/genomic medicine will develop as a standard-of-care in veterinary medicine.
• Direct-to-consumer genetic testing implements precision/genomic medicine.

PRECISION/GENOMIC MEDICINE

Precision/genomic medicine (P/GM) is the practice of disease treatment and prevention that considers individual variability with regard to genetics, environment, and lifestyle.1 Historically, health care has evolved around the needs of the population with a one-size-fits-all approach. For example, 2 patients displaying the same symptoms would typically be diagnosed with the same disease and therefore receive the same drug for treatment. Ideally, the drug prescribed would have shown strong efficacy across a broad population, rather than an alternative drug that may have had higher levels of efficacy in some but with only small effects in most. A P/GM approach would instead focus on each patient’s individual characteristics for both diagnosis and treatment. In the preceding example, genetic markers may have indicated that one of the patients would have responded well to the alternative drug, potentially leading to a speedier recovery with fewer side effects for that patient.

The practice of P/GM requires a detailed profile of each patient’s molecular characteristics and an understanding of how those characteristics affect disease processes and the treatment options in question. In this context, genomics is potentially the most valuable tool available for PM.2 Each individual’s genome is a unique combination of DNA variants, in which the DNA variants themselves were inherited from the individual’s parents, are often found in others within the population through shared ancestry, and can contribute in various amounts to physiological processes. Moreover, genomics,
or genomic medicine, provides the framework necessary for PM. Because individual DNA variants are distributed across populations, these variants can be correlated with specific medically relevant traits, such as a DNA marker that indicates a positive response to a specific drug or susceptibility to a disease. Also, because each individual has a unique combination of these DNA variants, knowledge generated from such correlations can be translated into a precise individualized molecular profile that can guide diagnosis and treatment. The P/GM approach applies to cats as well.3–5

The entire repertoire of approximately 21,000 genes of a domestic cat influences its overall biology,8 physiology, health, and appearance; however, for many genetic diseases, only a limited number of genes and variants influence the disease, trait, or health problem.7 By considering the DNA variation present in an individual’s disease/traits-causing genes, genetically defined therapies, targeted at specific genes, can then be identified and applied.3,5 The therapies are targeted on a perturbed biological pathway, with the intention of correcting the disturbance, or activating an alternative pathway, which will rectify the adverse health condition. In cancers, the genetic profile of the tumor’s DNA can be used to help select more appropriate cancer treatments, attacking the tumors more directly and efficiently. PM implies individualized treatment that should be more efficient and more effective, which should improve quality and quantity of life for the specific patient. Already becoming an active approach for canine cancers, cats too will benefit from P/GM.8–10

Although P/GM is a new term, it is not necessarily a new concept and has been gradually infiltrating veterinary medicine for years.10 Although the goal of P/GM has been to focus on individual characteristics, throughout history, steps have been taken to refine our understanding at increasingly higher levels of resolution. Discussed in the following sections, are several different levels of interpreting patient characteristics that have been used to inform a P/GM approach within feline veterinary medicine.

Species-Level Precision Medicine

Although a new “buzz word,” P/GM is not really a new concept and is a routine aspect of veterinary medicine. Unlike human medicine, veterinary medicine has the added complication of treatment for widely different species. Trial and error in the use of acetaminophen demonstrated this valuable drug for many species, is toxic in cats.11,12 Glucuronidation is catalyzed by the UDP-glucuronosyltransferases (UGTs), a superfamily of conjugative liver enzymes that transfer glucuronic acid to a nontoxic, more water-soluble, and readily excreted glucuronide metabolite. UDP-glucuronosyltransferase 1A6 (UGT1A6), the major species-conserved phenol detoxification enzyme, is a pseudogene in domestic cats, thus nonfunctional, and cats also are reported to have a less diverse pattern of UGT1A isoform expression compared with other species.13 Such differences most likely reflect the highly carnivorous diet of Felid species and resultant minimal exposure to phytoalexins, which are usually found in plant tissue.14,15 Slow glucuronidation of acetaminophen and other drugs, such as acetylsalicylic acid (aspirin),16 account for the slow clearance and extreme sensitivity of cats to the toxicity of these drugs. Therefore, species-specific factors, such as dietary requirements and genetics, contribute to a cat’s inability to metabolize certain drugs that are effective in most other species.

Population-Level Precision/Genomic Medicine

Regularly, medical interventions are leveraged at the level of groups within a population defined by shared characteristics, such as, age, ethnicity, race, and sex. Such groupings are useful, as certain diseases are known to be more prominent in specific
groups, allowing for enhanced detection or monitoring in susceptible groups. The same principles are also used in veterinary medicine, where domestic animals have the added benefit of breeds or breed structure, which were created by strictly enforcing and documenting mating patterns among individuals that carry traits desired by breeders. Importantly, individuals within the same breed share extremely high degrees of common ancestry and are often easily recognized by the presence of distinctive features, such as coat texture or stature. One example of P/GM leveraged at the population level in cats is the genetics and detection of various blood types. Importantly, blood type incompatibilities leading to transfusion reactions and neonatal isoerythrosis have been recognized in cats for decades and are of particular concern for specific breeds and populations. Genetic variants in the cat control the function and efficiency of the enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH), which converts neurominic acid to acetyl acid, thereby changing a cat’s blood group from Type B to Type A. Serologic, cross-matching and now genetic testing predicts the red blood cell antigens and hence identifies potential incompatibilities. Thus, every time a transfusion donor was decided or a breeding was conducted using blood type information, the veterinarian or breeder was performing a rudimentary form of P/GM for the cat.

**Individual-Level Precision Medicine**

**Individual genetic tests**

To apply P/GM at the individual level, genetic analyses of well-recognized candidate genes can be individually scanned in hopes of finding causal DNA variants for a disease, condition, or novel phenotype. Knowing the gene involved with the presentation may direct the treatment plan for the patient. These specific candidate gene analyses are well proven and are generally conducted in 2 steps, which are reviewed in “Direct-to-Consumer Genetic Testing for Domestic Cats” by Lyons and Buckley, elsewhere in this issue.

**Whole genome and whole exome sequencing**

Although genetic testing and candidate gene screening are aspects of P/GM, whole genome (WGS) and or whole exome sequencing (WES) are the newest techniques to advance the concept. Highly accurate and robust genome assemblies are the backbone of WGS/WES, as high-quality reference genome assemblies facilitate accurate comparisons between patient and healthy control genomes. The new long-read assembly, Felis_catus_9.0, the genome sequence of an Abyssinian named Cinnamon, is one of the strongest assemblies for any species, besides humans and mice. A second requirement for effective P/GM is the establishment of a database that has a robust collection of DNA variants that are normally found in cat genomes. The 99 Lives Cat Genome Sequencing Initiative has collated both published and yet unpublished cat genomic data to establish an extensive dataset of known DNA variants in cats (http://felinegenetics.missouri.edu/99lives). The 99 Lives project has evaluated approximately 300 domestic cat genomes to produce a cat DNA variant dataset with more than 70 million variants. In addition, research support groups, such as the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), Ensembl (www.ensembl.org), and the University of California–Santa Cruz (https://genome.ucsc.edu/) use other information, such as RNA sequencing data, to periodically improve the annotation of the cat genome by improving the positioning and descriptions of genes and their exon-intron boundaries. Hence, armed with a strong reference genome, good annotation, and an expanding variant database, significant advancements in applications of P/GM in cats is technically feasible. Several cat diseases
have already been discovered by using WGS and the required supportive resources.\textsuperscript{5,20–23}

Today, any cat with a suspected genetic disease can have its DNA submitted for WGS/WES. Institutions such as the University of Missouri College of Veterinary Medicine perform this task regularly for both cats and dogs. Once sequencing is complete and data become available, bioinformatics tools can be used to compare the patient’s DNA with the reference genome and variant datasets to detect and prioritize potentially harmful mutations specific to the patient. Here, annotation information is used to determine in which gene the variant resides and the possible impact that variant has on the protein produced by that gene. In summary, the tools are available, the cost is reasonable, and WGS/WES and bioinformatic processing can each be accomplished within a week. Therefore, genetics can support bedside to benchtop and back to bedside diagnosis or treatment within a reasonable timeframe and support the health care of cats in a precise and individually focused manner.

**GENOME ORGANIZATION**

Among higher eukaryotes, very little of the genome codes for proteins,\textsuperscript{24} indicating the vast majority of DNA variants, have an unknown biological impact. To use genomic technologies efficiently in PM, a basic understanding of genome organization is required. The domestic cat haploid genome, which is typical of most mammalian genomes, consists of approximately 2.6 billion base pairs (2.6 gigabases or Gb) spread across 18 autosomes and 1 pair of X and Y sex chromosomes.\textsuperscript{4} The genes themselves are distributed across these chromosomes. Organization also takes place on the level of individual genes, which are composed of exons, introns, and 5' and 3' untranslated regions (UTRs) (Fig. 1). Exons contain the genetic information that is translated into proteins, the molecules that perform most tasks within a cell. Together, all exons combined account for less than 50 Mb of DNA or less than 2% of DNA in the entire genome.\textsuperscript{25} Exons within the same gene are separated by introns. Introns vary widely in size and are usually much larger than exons. Together, all introns combined account for approximately 40% of the entire genome. After transcription, introns are spliced out of the messenger RNA transcript, leaving the remaining transcribed exons to essentially be stitched together before undergoing translation. The remaining DNA found outside of genes is referred to as “intergenic” and comprises less than 60% of the entire genome. Often found within intergenic regions and introns, usually within the immediate vicinity of genes, are DNA elements known as regulatory regions, these elements help determine how much product of any given gene is produced. Although regulatory regions can be an important factor in some traits, such as a retroelement insertion into one of the introns of \textit{KIT} that causes white spotting in coats,\textsuperscript{26} they are difficult to define and remain poorly annotated in the cat.

The final component to consider for mammalian genome organization is the mitochondrial DNA. Discussions of the cat genome generally omit the mitochondrial DNA (mtDNA), as it consists only of a small number of genes involved in controlling cellular energy. In total, the mitochondrial genome is approximately 17 kb in length and is only inherited through the maternal lineage.\textsuperscript{27} No mtDNA disease causing variants have been identified in cats but many are associated with diseases in humans, indicating the mtDNA should also be considered in health studies.

**CHOOSING BETWEEN WHOLE GENOME AND WHOLE EXOME SEQUENCING**

Two sequencing approaches assist the identification of new DNA variants: WGS that captures DNA variants across the vast majority of the genome, and WES that captures
DNA variants only within exons and their immediate flanking regions. The most important questions to ask when considering each approach are (1) how likely will the causative variant be sequenced and identified, (2) which individuals are available for sequencing, (3) what is the cost of each approach, and (4) how useful will the results be for future research and analysis? For sequencing and identifying candidate causative variants, although WGS will most likely sequence the variant, identifying a single candidate from all other variants is challenging and depends on the quality of genome annotation. Because regulatory regions in the cat remain poorly annotated, most meaningful variants will be in exons, and therefore also captured by WES. However, WES is usually not able to detect copy number changes or large structural variants, such as the deletion in \( \text{UGDH} \) that causes feline dwarfism or the \( \text{KIT} \) variants that cause white spotting.\(^{4,26}\) Independent of the expanded range of sequences WGS captures, a variety of analyses demonstrate a number of metrics showing WGS generally outperforms WES,\(^{28,29}\) including diagnostic successes, which is approximately 36% for WES and approximately 41% for WGS.\(^{30}\) In terms of cost, WES can be performed...
for less than $250 per cat and WGS can be performed for approximately $1000 per cat. Also, because WES captures only approximately 2% of the genome, WES file sizes are much smaller than WGS file sizes, requiring significantly less computational resources and time to process and analyze. The final question regards the future usefulness of the data. Because many P/GM approaches require a data set of known variation, this means after an individual’s genome is sequenced, their genome data can be added to a variant dataset and used to influence future cases. Here WGS provides far more utility than WES, in addition to capturing all of the exons, WGS captures the intergenic and intronic regions that require significantly more investigation to understand their influences on health.

Ultimately, when deciding on a sequencing strategy, individuals must also consider their available budget, available computational resources, and be aware there is a strong possibility they may not find a candidate variant.

FUTURE OF PRECISION/GENOMIC MEDICINE

P/GM is also known as P4 Medicine, implying health care that is predictive, personalized, preventive, and participatory.31–36 Lower technological costs, improved bioinformatics, and improved genomic resources, such as genome assemblies, annotations, and variant databases, all afford veterinary health care to encompass P/GM. Besides simply knowing the gene causing a health problem and then treating the symptoms, the ultimate hope for P/GM is to apply drugs that specifically target the pathway of the gene, leading to remediation of the defect and “cure” of the disease. Besides more personalized treatments, the DNA variants of the individual are also important during clinical trials and drug development. Many drugs have failed clinical trials because of poor effects or adverse reactions in patients. As the role of DNA variation becomes clearer, some clinical trials may have failed because of the specific genetics of the patient; that was not the right drug for that individual but may be a very effective drug for others. Thus, many drugs may have been shelved before the understanding of the specific reactions of patients due to their background DNA profiles. By linking standardized, electronic health care records across clinics and to genomic datasets, cohorts of patients can be well defined and ascertained to investigate common and more complex health problems that also involve the environment and lifestyle. P/GM is feasible in companion animals; however, efficiency and robust datasets, including genetic and clinical phenotypes, need to grow and improve to move P/GM to a standard-of-care.

REFERENCES


