The potential of translational research in dogs in human medicine

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Abstract. Dog is an important animal model for translational research to study human diseases. This translational research includes identification of causative genes for shared diseases with humans, identification of new drug targets for the treatment of patients with monogenic diseases, canine clinical trials conducted before and parallel with human clinical trials, and intervention research in healthy dogs. For future research, I propose two important considerations. First, knowledge of the genetic epidemiology of diseases in each breed of dog used is indispensable to precisely identify dogs with the best potential in translational research. Second, research in dogs has a significant potential to expand actionable disease treatment to patients with genetic risk of disease. Translational research in dogs can help enhance human medical sciences in addition to veterinary medicine.

Key words: animal model, clinical trial, dog, spontaneous disease model, translational research

Introduction

Dogs are recognized as an important animal model for human diseases for several reasons [1, 2]. First, dogs share ~80% of diseases with humans [3]. The use of cutting-edge diagnostic techniques has revealed comparable clinical manifestations of several diseases in dogs and humans [1]. Second, since veterinary medicine is divided into several specialties similar to those of human medicine, dogs can be investigated with higher precision. Veterinary and human medical researchers are collaborating and using comparative approaches, such as the Canine Comparative Oncology Genomics Consortium within the United States National Cancer Institute [1]. Third, dogs share the same environment as humans and are also exposed to bacteria, pollutants, and toxins. By contrast, mice are generally studied using temperature and diet-controlled systems (Fig. 1). Fourth, dogs have a shorter lifespan of about 10 years, depending on the breed type [4], enabling researchers to test a treatment in a shorter period. For instance, the disease-free intervals involving canine osteosarcoma and lymphoma were assessed within approximately 18 months, whereas the same study in humans was completed in more than 7 years [5]. This advantage also makes dogs a useful animal model in geroscience studies due to their similarities in several age-related changes and diseases compared to those in humans [6]. Fifth, dogs exhibit breed-specific diseases, some of which are rare in humans, such as sarcomas [7]. Myxomatous mitral valve disease accounts for up to 37% of the mortality of Cavalier King Charles Spaniels [8]. Lastly, most diseases develop spontaneously in dogs, while diseases in mouse models are induced with genetic modification [9] (Fig. 1C) and exogenous methods, such as toxins [10] or viruses [11], that are carried out in controlled environments (Fig. 1E).

Most of these reasons are already considered to be advantages, with some being more advantageous in the context of the particular translational research. The spontaneous model needs more consideration. The aforementioned approaches in the establishment of mouse models are considered artificial. The diseases that develop spontaneously in dogs feature similarities to the development of some human diseases. Two significant similarities are the involvement of genetic and environmental factors similar to those in humans and high disease heterogeneity. In humans, several diseases are caused by various genetic and environmental factors. Common diseases, such as cancer and diabetes, are primarily caused by genetic variants with small effects (polygenic, Fig. 1A) and environmental factors (Fig. 1D). Several hundred genetic variants have been identified with the help of genome wide association studies, however, their effect is small (approximately 1.1-fold) [12]. Analyses using large sample sizes (e.g. 100,000) can explain a small proportion of heritability. Therefore, numerous genetic variants that each have a minimal effect are likely to contribute to disease development [13]. A spontaneous model is required to mimic these conditions. Subsequently, each individual has different levels of genetic risk and contributions from environmental factors. High heterogeneity is another likely outcome of a spontaneous model. In an artificial animal model, such as a mouse model, subjects may have a similar risk when subjected to genetic modification and exogenous procedures in a controlled...
environment. Therefore, dogs not treated in this manner are likely to exhibit high heterogeneity. However, we further demonstrate and recommend treating such an advantage with care.

It is unlikely that dogs will replace mice and other traditional model animals because of some inherent disadvantages. As stated by Krogh: “for a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied” [14]. Therefore, dogs should be used as required to fill the critical shortcomings of traditional animal models. Here, I consider four examples of translational research, and two important considerations for future research.

### Research Examples

**Identification of causative gene(s) for diseases shared with humans**

Dog species have undergone two bottle necks. The first occurred more than 10,000 years ago when the species diverged from the wolf ancestor. The second occurred approximately 200 years ago when each individual breed developed from the dog species [15]. Specific dogs are frequently used for breeding. As a consequence, each breed has breed-specific diseases. For instance, Pembroke Welsh corgi often suffer from degenerative myelopathy [16]. Cancer-related death largely varies between 14.5 and 55.8% among breeds [17]. The identification of causal pathogenic variant(s) leads to the genetic test before mating to reduce the disease rate. In the context of translational science, this facilitates the identification of novel causal genes for corresponding human diseases and the discovery of new mechanisms for treatment and drug development. Dogs with a pathogenic variant could be useful as a disease model. Projects that have been initiated include the LUPA project in Europe [18] and dog disease mapping project (DogDNA) in the United States (https://www.broadinstitute.org/project-spotlight/dog-disease-mapping-project-dogdna).

If a breed-specific disease is found to be monogenic, the genetic analysis strategy is straightforward. A successful example comes from Liege in Belgium [19]. Five old English sheepdogs suffered from chronic airway inflammation and one dog from situs inversus. Their phenotypes were similar to human primary ciliary dyskinesia (PCD). Nasal and tracheal biopsies and epithelial cell cultures confirmed the findings. Pedigree analysis strongly suggested autosomal recessive inheritance. A 15-Mb segment on chromosome 34 was identified by homozygote mapping with a single nucleotide polymorphism array in only 5 cases and 10 controls. Although there are 151 genes in this locus,
researchers focused on ciliary-related genes to identify a truncate variant in CCDC39, because PCD results from a ciliary abnormality. Furthermore, genotyping of this variant in 10 cases and 102 controls, and expression analysis suggested that CCDC39 was a causal gene for canine PCD. Other truncated variants were identified with additional sequencing of CCDC39 in human PCD patients. The findings from functional assays have suggested that CCDC39 is important for the assembly of the inner dynein arms, dynein regulatory complex, and for normal ciliary motility. A similar strategy was undertaken in various other diseases, including psychiatric disease [20]. The process is being hastened by the development of various genomic resources [21].

Identification of new drug targets to rescue patients with rare diseases

Despite researchers’ efforts, monogenic diseases are still prevalent. This presents a new opportunity of identifying novel drug targets. In monogenic diseases, not all dogs with a causal pathogenic variant develop the disease. A modifier gene/variant is recognized to reduce the possibility of disease development. A new drug mimics the function of a modifier when it works to reduce disease development. The Jagged1 gene for Duchenne muscular dystrophy (DMD) is an example of such a gene/variant [22]. A golden retriever was infected with a pathogenic variant of DMD. However, two healthy dogs (escaper) with the same pathogenic variant were identified in Brazil. In the myogenin binding site consensus sequence, one variant was identified using a combination of linkage, whole genome sequencing, and transcriptome analyses, which increased Jagged1 gene expression. This potentially new mechanism was supported by Jagged1 overexpression analysis to rescue dystrophin deficient zebrafish. The observations implicate Jagged1 is a novel target for dystrophin-independent DMD therapy. Another study reported an example of canine degenerative myelopathy [23]. The hypothesis-free genome wide scan may be an effective method to identify new drug targets and such escaper dogs would be useful to check adverse effects potentially caused by a developed drug. Further efforts to identify escaper dogs for other monogenic diseases are required.

Canine clinical trials before and parallel with human clinical trials

Pilot studies for a new drug before and parallel with human clinical trials have been conducted with dogs. In the development of a new drug, rodent and non-rodent animals have often been used in preclinical research prior to human clinical trials. However, they may often be poor predictors for human clinical trials because of methodological flaws [24]. The average number of new drugs approved by the U.S. Food and Drug Administration (FDA) has decreased since the 1990s [25]. To resolve the challenges in preclinical research, canine clinical trials conducted before or parallel with human clinical trials may present a good opportunity. Canine clinical trials are useful to check drug safety and efficacy while treating dogs. Several canine clinical trials have been run before or parallel with human clinical trials to determine safety/tolerability and pharmacokinetics, and to demonstrate proof-of-concept [26]. Canine clinical trials for neuropsychiatric diseases may be also a reasonable target. Promising results of novel anxiolytic agent discoveries in laboratory rodent studies have rarely translated into effective outcomes in humans in the past 50 years [27]. However, dogs display psychiatric disorders, such as anxiety disorders, and the same medications (i.e. clomipramine and fluoxetine) approved by the FDA have been used and have proven efficient for dogs with such disorders [28]. Furthermore, a genetic study on fear in German Shepherds suggested a shared molecular etiology of anxiety across species [29].

Intervention research in healthy pet dogs

Intervention research for healthy individuals has the potential to restrict the development of diseases. However, it poses an ethical challenge. For instance, rapamycin has beneficial effects against neurodegeneration and aging, including Alzheimer’s disease [30]. No human clinical trials with rapamycin have been conducted in Alzheimer’s diseases due to off-patent and other reasons, although such trials have been recommended by researchers. The recommendations may be driven by the possibility of investigating drug efficacy in dogs. This trial started in the Dog Aging Project [6]. Using 24 middle-aged companion dogs, the first randomized controlled (clinical) trial with 10 week treatment of rapamycin was reported [31]. No clinical side effects were reported, while the mean corpuscular volume decreased in rapamycin-treated dogs. Further clinical trials with larger numbers of dogs conducted for a longer times are planned. The findings may provide more evidence concerning drug efficacy and further prompt the initiation of human clinical trials.

Important Considerations for Future Research

Genetic epidemiology of diseases in each breed

As discussed above, one of the advantages of dogs as an animal model is breed-specific diseases. However, further study is required to identify the precise cause of breed-specific diseases. This is linked with the spontaneous model. A comparable genetic architecture (polygenic or monogenic) and shared environment with corresponding human disease is essential. For cancer, molecular target drugs for patients with germline and somatic genetic variants are used. For instance, a poly ADP ribose polymerase (PARP) inhibitor works well for patients with pathogenic variants in BRCA1/2 [32] and approximately 5% of breast cancer patients harbor such pathogenic variants [33, 34]. Furthermore, applications related to other cancers have been considered [35]. Testing of this drug in specific breeds with higher prevalence of these cancers before and parallel with clinical trials could be proposed. However, estimation of the genetic architecture may be difficult using only phenotype similarity, since the mechanism of the development of breed-specific cancer in dogs is not well known [36]. Therefore, it is impossible to select suitable dogs for PARP inhibitor until researchers identify subjects infected with a pathogenic variant in...
BRCA1/2.

Limited information on genetic epidemiology of diseases deeply depends on a number of breeds because information on genetic epidemiology would be required for each breed. Furthermore, acquiring country-wise consistency in information may be required. For instance, breed-associated risks for developing canine lymphoma [37] and patellar luxation [38–41] differ among countries. Systematic genetic epidemiological research is possible, however it requires considerable efforts. Therefore, it might be realistic to perform genetic epidemiological research when a dog model is proposed.

Animal model as an effective expansion of “actionable” diseases

Dogs as animal models can help bridge several gaps. Recently, a shift from traditional medicine (Fig. 2A) to personalized medicine (Fig. 2B) has occurred in human medicine, especially for some diseases for which molecularly targeted drugs exist [42]. However, in the future, genetic testing at birth and subsequent care and treatment may protect subjects against high-risk diseases determined by genetic testing (Fig. 2C) [43]. This is technically possible due to the current cost of whole genome sequencing and interpretation of the results [44], although ethical, insurance, and education considerations need to be done carefully. A pilot randomized clinical trial named “the BabySeq Project” explored the medical, behavioral, and economic impacts of newborn genomic sequencing in healthy newborns and those admitted to a neonatal intensive care unit. The results suggested that newborn genomic sequencing can effectively detect risk and carrier status for a wide range of diseases that are undetectable by current newborn-screening assays or predicted using information on infant’s clinical or family history [45]. In this approach, only “actionable” diseases, wherein patients with genetic risk can be treated, are considered. An important direction is how the scope of actionable diseases is expanded. Dogs may be effective (Fig. 2D) due to their aforementioned advantages, which may allow researchers identify dogs with a genetic architecture similar to humans with the corresponding disease.

To close, this review has reflected an animal model
perspective towards human medicine. However, this direction will also attract diverse researchers and other resources in dog research. The result will be also the improvement of veterinary medicine.

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References


4. Selman, C., Nussey, D. H. and Monaghan, P. 2013. Ageing: it’s improvement of veterinary medicine. The result will be also the evolution of dog research. The result will be also the improvement of veterinary medicine.


