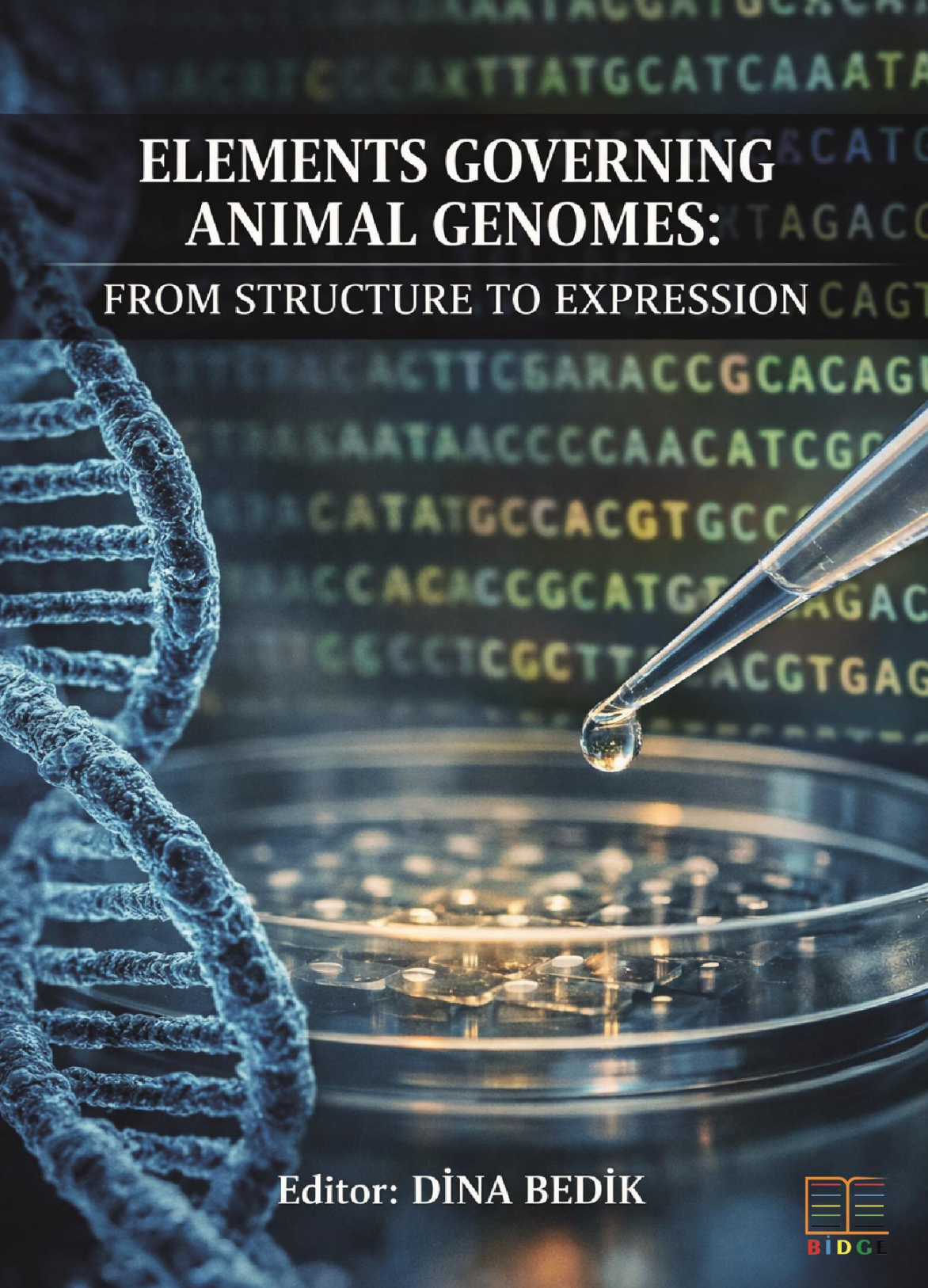
ELEMENTS GOVERNING ANIMAL GENOMES:

FROM STRUCTURE TO EXPRESSION



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CONTENTS

STRUCTURAL ORGANIZATION AND FUNCTIONAL EFFECTS OF TRANSPOSONS IN ANIMAL GENOMES

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Introduction

Transposons (transposable elements, TEs) are mobile DNA sequences that have the ability to move from one location to another within the genome and, in some cases, increase their copy number (Muñoz-López & García-Pérez, 2010). These genetic elements were first discovered by the American cytogeneticist Barbara McClintock during studies conducted on the maize (*Zea mays*) genome and were introduced to the scientific community through her research published in 1950 (McClintock, 1950). While examining pigmentation changes in maize kernels, McClintock demonstrated that certain genetic elements could change their positions on chromosomes, and that this movement could affect gene expression and lead to phenotypic variations. For this reason, these genetic

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elements were initially referred to as “jumping genes.” McClintock’s discovery challenged the classical genetic understanding that genes occupy fixed positions on chromosomes and led to an important paradigm shift regarding the dynamic nature of genome organization.

The importance of transposons in genome biology was not fully understood for a long time, and these elements were often regarded merely as “selfish DNA” that maintains its own propagation or as nonfunctional genomic sequences (Kidwell & Lisch, 2000). Advances in molecular biology, genomics, and bioinformatics, particularly with the widespread use of next-generation sequencing technologies, have enabled a better understanding of the roles of transposons in genome organization, the regulation of gene expression, and genome evolution (Kazazian, 2004; Feschotte & Pritham, 2007). Today, transposons are recognized not only as DNA fragments capable of moving within the genome, but also as important contributors to the shaping of genome architecture and the formation of gene regulatory networks.

Transposons occupy a substantial portion of the genomes of eukaryotic organisms. Genomic analyses have revealed that a significant fraction of the genome in many organisms consists of sequences derived from transposons (Bourque et al., 2018). For example, approximately 45–50% of the human genome is known to consist of sequences originating from transposable elements (Lander et al., 2001; Bourque et al., 2018). Similarly, in many animal genomes, transposons constitute a considerable part of the genomic content and exert important influences on genome size, gene organization, and chromosomal architecture (Chalopin et al., 2015; Platt et al., 2018; Nishihara, 2019). The proliferation of transposons within the genome can lead to the spread of multiple copies of the same sequence across different chromosomal regions, and it is

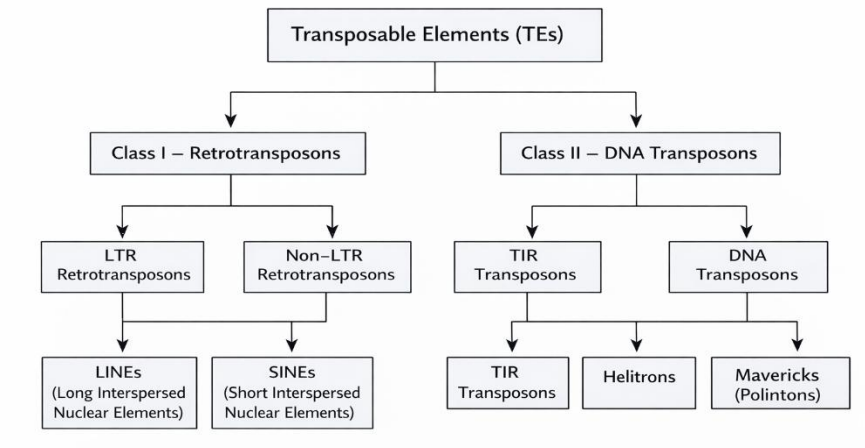
known that some transposon families can reach thousands of copies within the genome (Muñoz-López & García-Pérez, 2010).

Transposons are not merely passive components of genomic structure; they can also play active roles in many biological processes. The insertion of transposons near gene regions or regulatory elements can lead to changes in gene expression, create new promoter or enhancer regions, and thereby influence gene regulatory networks (Feschotte, 2008). Recombination between transposon sequences can also lead to genomic rearrangements, gene duplications, and the formation of new genes. For these reasons, transposons are considered to play an important role in the generation of genomic and epigenomic diversity, in adaptive processes, and in the evolutionary diversification of species (Kidwell & Lisch, 2001; Bourque et al., 2018).

Classification of Transposons

Transposons are classified according to their mechanisms of movement within the genome and the molecular processes they utilize. According to the widely accepted classification system, transposons are divided into two main groups: Class I (retrotransposons) and Class II (DNA transposons). This classification provides an important framework for explaining how transposons proliferate within the genome and through which mechanisms they integrate into new genomic locations (Finnegan, 1989; Capy, 2005; Wicker et al., 2007).

Figure 1 Basic classification of eukaryotic transposable elements



Reference: Wicker et al., 2007

Class I – Retrotransposons

Retrotransposons are mobile genetic elements that proliferate within the genome through the use of an RNA intermediate (Wicker et al., 2007; Goodier & Kazazian, 2008; Muñoz-López & García-Pérez, 2010). These elements are first transcribed into RNA and are then converted back into DNA through the action of the reverse transcriptase enzyme, after which they are integrated into another region of the genome (Kazazian, 2004; Wicker et al., 2007; Goodier & Kazazian, 2008). This process is referred to as the "copy-and-paste" mechanism or retrotransposition, in which the original copy of the transposon remains in the genome while a new copy inserts into a different genomic location (Finnegan, 1989; Kazazian, 2004; Wicker et al., 2007). Because of this property, retrotransposons can reach high copy numbers within the genome and may contribute substantially to the overall size of eukaryotic genomes (Kazazian, 2004; Wicker et al., 2007; Muñoz-López & García-Pérez, 2010).

Retrotransposons are generally classified into three main groups: Long interspersed nuclear elements (LINE), short interspersed nuclear elements (SINE), and long terminal repeat (LTR) retrotransposons (Finnegan, 1989; Wicker et al., 2007). These elements are particularly widespread in mammalian genomes and constitute a significant proportion of genomic transposons; approximately 45% of the human genome and about 37% of the mouse genome consist of these sequences (Prak & Kazazian, 2000; Lander et al., 2001; Waterston et al., 2002).

LINE Elements

LINE elements are long retrotransposon sequences that are widely found in eukaryotic genomes. These elements are typically several thousand base pairs in length (approximately 4–7 kb) and are capable of encoding the proteins required for their own mobilization (Wicker et al., 2007; Kapitonov & Jurka, 2008). For example, LINE-1 (L1) elements present in the human genome can carry out their own transposition by encoding proteins with reverse transcriptase and endonuclease (EN) activities (Kazazian, 2004; Muotri et al., 2007; Muñoz-López & García-Pérez, 2010; Bhat et al., 2022). While the formation of new copies of LINE elements within the genome contributes to increases in genome size and to the shaping of three-dimensional genome architecture, it can also disrupt genome stability by causing genetic mutations, gene inactivation, and homology-based genomic rearrangements (Muotri et al., 2007; Muñoz-López & García-Pérez, 2010; Bhat et al., 2022; Lawson et al., 2023).

SINE Elements

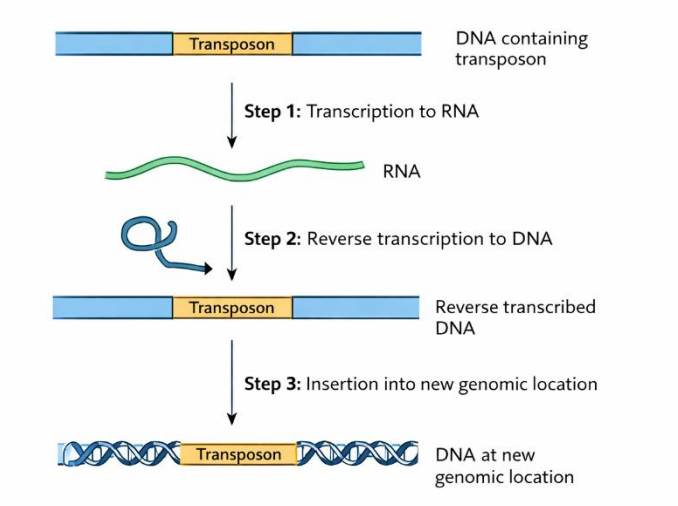
SINE elements are retrotransposons composed of short DNA sequences and generally do not encode the proteins required for their own mobilization. Therefore, SINE elements utilize the enzymatic mechanisms provided by LINE elements in order to move within the

genome. One of the most common SINE elements in the human genome is the Alu element; these sequences are approximately 300 base pairs in length and have more than one million copies in the genome. Alu elements are widely distributed throughout the genome and, in some cases, may contribute to the formation of gene regulatory regions (Cordaux & Batzer, 2009).

LTR Retrotransposons

LTR retrotransposons are mobile genetic elements that contain long repeat sequences at their terminal regions (Wicker et al., 2007; Kapitonov & Jurka, 2008). Structurally, these elements exhibit similarities to retroviruses and are directly associated with endogenous retroviruses (ERVs) that have become integrated into the germ line during evolutionary processes (Platt et al., 2018; Johnson, 2019). LTR retrotransposons can spread within the genome through copying and may play an important role in shaping genome organization (Bhat et al., 2022; Lawson et al., 2023). In addition, it has been reported that some LTR sequences can acquire functions as gene regulatory elements over time, thereby contributing to the regulation of gene expression (Platt et al., 2018; Li et al., 2022; Lawson et al., 2023).

Figure 2 "Copy-and-paste" retrotransposition mechanism of retrotransposons



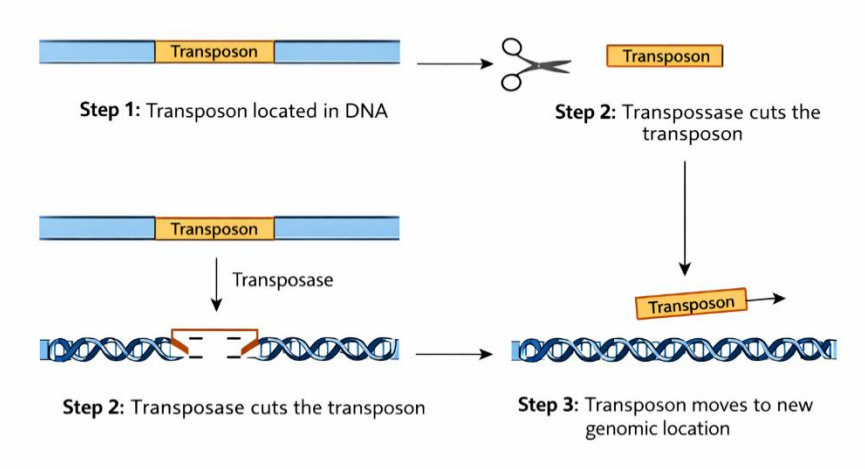
Reference: Kazazian, 2004

Class II – DNA Transposons

DNA transposons are mobile genetic elements that move within the genome through a mechanism that occurs directly at the DNA level (Feschotte & Pritham, 2007; Wicker et al., 2007). These elements are generally transferred from their original genomic location to another region through a "cut-and-paste" mechanism (Muñoz-López & García-Pérez, 2010; Bhat et al., 2022). This process is usually mediated by the transposase enzyme encoded by the transposon itself (Feschotte & Pritham, 2007; Wicker et al., 2007). The transposase enzyme recognizes the terminal inverted repeat (TIR) sequences located at both ends of the transposon and facilitates the excision of the transposon from the genome and its integration into a new target site (Muñoz-López & García-Pérez, 2010; Platt et al., 2018).

During their movement within the genome, DNA transposons can cause the formation of short repeat sequences at the target DNA site, and these structures are referred to as target site duplications (TSDs) (Wicker et al., 2007; Bhat et al., 2022). Although the active movement of DNA transposons is limited in many animal genomes today, these elements are thought to have played an important role in genome evolution in the past (Feschotte & Pritham, 2007). In addition, it has been shown that some DNA transposons have been "domesticated" by the host genome during evolutionary processes, thereby contributing to the emergence of new gene functions (Sinzelle et al., 2009).

Figure 3 "Cut-and-paste" transposition mechanism of DNA transposons



Reference: Kazazian, 2004

Structure of Transposons and Their Effects on Genome Functions

The diverse effects of transposons on genome organization, gene expression, and evolutionary processes are largely associated with their structural features and the transposition mechanisms they

employ (Feschotte & Pritham, 2007; Chénais et al., 2012). The structural organization of transposons not only determines their ability to move within the genome but also shapes the nature and extent of their interactions with the host genome (Wicker et al., 2007; Muñoz-López & García-Pérez, 2010).

Structural Features of Transposons

Although the structure of transposons varies according to their classes, many transposons share certain common structural components. These structural components play a critical role in the molecular mechanisms that enable transposons to move within the genome (Wicker et al., 2007).

Terminal Repeat Sequences

Many transposons contain terminal repeat sequences at their ends, and these sequences represent important components of the transposition mechanism (Muñoz-López & García-Pérez, 2010). Particularly, DNA transposons possess inverted repeat sequences known as terminal inverted repeats (TIRs), which are recognized by the transposase enzyme and enable the excision of the transposon from the genome and its transfer to a new genomic region (Su et al., 2019). In retrotransposons, LTR sequences are generally present, and these regions play important roles in the initiation of transcription and in integration processes within the genome (Wicker et al., 2007; Muñoz-López & García-Pérez, 2010).

Coding Genes

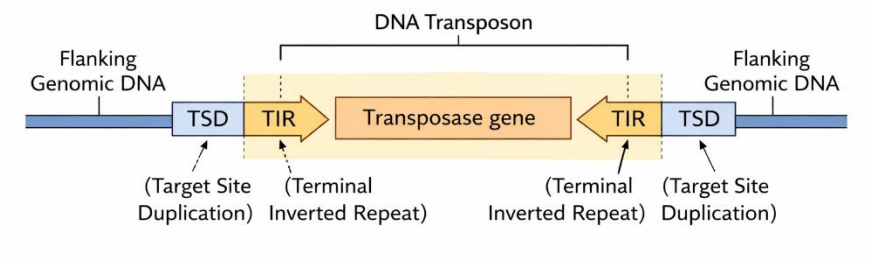
Some transposons possess gene regions that encode the enzymes required for their own movement. Among these genes are regions that encode enzymes such as transposase, reverse transcriptase, and endonuclease (EN) (Muñoz-López & García-Pérez, 2010). For example, LINE elements can carry out their own mobilization by encoding proteins with reverse transcriptase and EN

activities (Finnegan, 1997; Eickbush & Jamburuthugoda, 2008). In contrast, some transposons, particularly SINE elements, do not encode these proteins and instead rely on the enzymatic mechanisms provided by other mobile elements in order to move within the genome (Weiner, 2002).

Target Site Duplications

During the integration of transposons into the genome, short repeat sequences are observed to form at the target DNA site. These short repeat sequences are referred to as target site duplications (TSDs) and are considered one of the characteristic molecular signatures of the transposition event. The formation of TSDs occurs as a result of the cleavage and rejoining of DNA during the integration of the transposon into the target DNA region (Feschotte & Pritham, 2007; Muñoz-López & García-Pérez, 2010).

Figure 4 Structural organization of DNA transposons



Reference: Wicker et al., 2007

Functional Effects of Transposons in Animal Genomes

Genomic analyses conducted in different animal species have shown that transposons not only contribute to the determination of genome size but also play roles in many biological processes, including the evolution of gene regulatory networks, the generation of genetic diversity, and the emergence of phenotypic variations (Mita & Boeke, 2016; Bourque et al., 2018; Li et al., 2022). Although

the genomic distribution and functional effects of these elements may vary among species, it is known that retrotransposons constitute the dominant class of mobile elements in many animal genomes (Watkins, 2018; Platt et al., 2018).

Distribution of Transposons in Animal Genomes

The distribution of transposons in animal genomes shows significant variation among species. In mammalian genomes, LINE, SINE, and LTR retrotransposons are reported to be particularly widespread and constitute a substantial portion of the genome (Goodier & Kazazian, 2008; Watkins, 2018). For example, LINE-1 elements are present in numerous copies in mammals, and in some genomes they are still known to remain active (Richardson et al., 2015). These active elements can generate new insertions within the genome, thereby contributing to dynamic changes in genome structure.

Studies conducted on domestic animal genomes have also demonstrated that transposons have a broad genomic distribution. In the dog genome, sequences derived from transposons have been reported to constitute approximately 31% of the genome; among these elements, SINE elements known as SINEC_Cf are particularly widespread and have reached high copy numbers within the genome (Kirkness et al., 2003; Wang & Kirkness, 2005; Kim & Doo, 2019). Similarly, in the cat genome, LINE and SINE elements have been reported to constitute a substantial portion of the genome and to be concentrated in certain gene regions; repetitive elements have been estimated to account for approximately 55.7% of the entire cat genome (Pontius et al., 2007; Tamazian et al., 2014).

Genomic analyses conducted in livestock species have also shown that transposons may exert significant effects on the genome organization of these animals. In the cattle genome, interspersed repetitive elements have been reported to constitute approximately

46.54% of the genome, with LINE elements accounting for 23.29% and SINE elements for 17.66% of this content (Bovine Genome Sequencing and Analysis Consortium, 2009). It has also been reported that bovine-specific BovB LINE elements and ART2A SINE elements exhibit regional accumulation patterns, particularly near the major histocompatibility complex (MHC) regions containing immune system genes and near certain genes involved in metabolic processes (Adelson et al., 2009). In the sheep genome, repetitive elements have been reported to constitute approximately 47.74% of the genome, with LINE elements accounting for 28.3% and SINE elements for 11.3% (Qiao et al., 2022). These retrotransposon sequences have also been reported to accumulate around gene regions associated with growth, development, and adaptation, potentially contributing to the generation of genomic diversity (Jiang et al., 2014; Pan et al., 2019).

In avian species, the distribution of transposons is considerably more limited compared to mammals. While approximately 45% of the human genome, about 40% of the mouse genome, and roughly 31% of the dog genome consist of transposon-derived sequences (Kazazian, 2004; Kim & Doo, 2019), this proportion remains only about 9.4% in the chicken genome, with DNA transposons accounting for merely 0.8% of the genome (ICGSC, 2004). This situation is considered to be one of the main reasons why avian genomes exhibit a more compact structure compared with mammalian genomes (ICGSC, 2004). Nevertheless, retrotransposon and DNA transposon sequences present in the chicken genome are thought to exert certain effects on genome organization and gene regulation (Bourque et al., 2018; Chuong et al., 2017).

Table 1 Proportion of transposon-derived sequences and prominent elements in selected animal genomes

Species	Approx. TE proportion	Prominent elements
Human (*Homo sapiens*)	~45%	LINE-1, Alu
Mouse (*Mus musculus*)	~40%	LINE-1, B1/B2
Dog (*Canis lupus familiaris*)	~31%	SINEC_Cf
Cat (*Felis catus*)	~55.7%	LINE-1, various SINE elements
Cattle (*Bos taurus*)	~46.54% (LINE 23.29%, SINE 17.66%)	BovB LINE, ART2A SINE
Sheep (*Ovis aries)*	~47.74% (LINE 28.3%, SINE 11.3%)	Various retrotransposon families
Chicken (*Gallus gallus*)	~9.4%	Various LINE elements and low DNA transposon content

Reference: Kirkness et al., 2003; ICGSC, 2004; Kazazian, 2004; Wang & Kirkness, 2005; Pontius et al., 2007; Bovine Genome Sequencing and Analysis Consortium, 2009; Adelson et al., 2009; Jiang et al., 2014; Tamazian et al., 2014; Chuong et al., 2017; Bourque et al., 2018; Pan et al., 2019; Kim & Doo, 2019; Qiao et al., 2022

Transposon-Derived Mutations and Phenotypic Effects

The movement of transposons within the genome can lead to the formation of genetic mutations (Kazazian, 2004). The integration of transposons into gene regions or regulatory regions of genes may disrupt gene function or alter gene expression; such insertion events may result in gene inactivation, frameshift mutations, or abnormal gene expression (Kazazian, 2004; Feschotte & Pritham, 2007). Transposon-derived mutations have been reported to be associated with various diseases in both humans and animals (Kazazian, 2004;

Cordaux & Batzer, 2009). For example, several cases of hemophilia A, Duchenne muscular dystrophy, and β-thalassemia caused by LINE-1 and Alu element insertions have been reported in humans (Kazazian, 2004; Cordaux & Batzer, 2009).

Studies conducted in animal models have also shown that transposons may play an important role not only in genetic diseases but also in the emergence of phenotypic variations (Feschotte & Pritham, 2007; Feschotte, 2008). For example, in the mouse genome, retrotransposons have been reported to contribute to the formation of certain inherited mutations, and one of the best-known examples of this phenomenon is the agouti viable yellow (Avy) allele observed in mice. This phenotype is associated with the presence of an IAP retrotransposon inserted into the upstream regulatory region of the *agouti (ASIP)* gene (Kazazian, 2004; Feschotte & Pritham, 2007). This retrotransposon provides its own promoter sequence, causing the normally restricted expression of the *agouti* gene to occur constitutively in all tissues. As a result, mice may develop phenotypes characterized by yellow coat color, a predisposition to obesity, and various metabolic alterations.

Similarly, some retrotransposon insertions in dogs have been shown to be associated with morphological and phenotypic traits. For example, a SINE insertion occurring in a region near the *premelanosome protein (PMEL, also known as SILV)* gene has been demonstrated to be associated with the Merle coat color phenotype in dogs (Clark et al., 2006). This phenotype is commonly observed in certain dog breeds such as Australian Shepherd, Shetland Sheepdog, Border Collie, and Dachshund; while it is characterized by a mottled coat color in heterozygous individuals, homozygous individuals may develop hearing loss and visual impairments due to pigmentation defects (Clark et al., 2006). In addition, a retrotransposon-mediated gene duplication involving a retrogene of the *fibroblast growth factor 4 (FGF4)* gene has been shown to be

associated with the chondrodystrophy (short-legged) phenotype observed in some dog breeds (Parker et al., 2009). This trait is particularly evident in breeds such as Dachshund, Basset Hound, Pembroke Welsh Corgi, and Pekingese.

The effects of transposons on phenotypic traits are also clearly observed in livestock species. In the cattle genome, ruminant-specific retrotransposons have been shown not only to exhibit regional accumulation patterns but also to directly influence gene expression by affecting promoter and enhancer regions of genes at a functional level (Feschotte, 2008; Chuong et al., 2017). Indeed, CRISPR experiments have demonstrated that ruminant-specific retrotransposons regulate critical immune genes such as *interferon alpha and beta receptor subunit 2 (IFNAR2)* and *interleukin 2 receptor subunit beta (IL2RB)* through TE-derived enhancers (Kelly et al., 2022). In the sheep genome, the ruminant-specific BovB retrotransposon has been reported to affect the expression of the *bone morphogenetic protein 2 (BMP2)* gene, which is associated with fat-tail development; this finding indicates that transposons may play a direct role in the emergence of adaptive phenotypic traits (Pan et al., 2019).

On the other hand, the callipyge phenotype observed in sheep is an inherited trait characterized by pronounced muscle hypertrophy in the hind limbs and loin muscles, and it has been associated with regulatory alterations in the *delta like non-canonical notch ligand 1–iodothyronine deiodinase 3 (DLK1–DIO3)* genomic region located on chromosome 18 (Freking et al., 2002). These findings indicate that transposons are not merely elements that move within the genome but may also play important roles in both the development of diseases and the evolution of phenotypic diversity among species (Feschotte, 2008; Chuong et al., 2017).

Table 2 Transposon-Derived Mutations and Phenotypic Effects in Animals

Species	**Affected region**	**Transposon type**	**Phenotypic outcome**
Human (*Homo sapiens*)	Various gene regions	LINE-1, Alu	Genetic diseases such as hemophilia A, Duchenne muscular dystrophy, and β-thalassemia
Mouse (*Mus musculus*)	*ASIP* (Agouti) gene	IAP retrotransposon	Yellow coat color, predisposition to obesity, and metabolic alterations
Dog (*Canis lupus familiaris*)	*PMEL* (SILV) gene	SINE	Merle coat color; hearing and visual impairments in homozygous individuals
	FGF4 retrogene	Retrotransposon-mediated gene duplication	Chondrodystrophy (short-legged phenotype)
Sheep (*Ovis aries*)	Region surrounding the *BMP2* gene	BovB retrotransposon	Fat-tail development
	DLK1–DIO3 genomic region	Regulatory changes	Callipyge phenotype (hind limb muscle hypertrophy)
Cattle (*Bos taurus*)	IFNAR2, IL2RB gene regions	TE-derived enhancer	Regulation of immune gene expression

Reference: Freking et al., 2002; Kazazian, 2004; Feschotte & Pritham, 2007; Cordaux & Batzer, 2009; Clark et al., 2006; Parker et al., 2009; Chuong et al., 2017; Pan et al., 2019; Kelly et al., 2022

Role of Transposons in the Regulation of Gene Expression

Transposons can play an important role in the regulation of gene expression within the genome (Chuong et al., 2017). These elements can influence gene expression through various mechanisms; some transposon sequences may provide regulatory regions similar to promoters or enhancers, thereby altering the

transcription levels of nearby genes (Feschotte, 2008; Chuong et al., 2017). In addition, certain transposon sequences can provide transcription factor binding sites and thus contribute to the evolution of gene regulatory networks (Chuong et al., 2017).

The effects of transposons on gene expression may also occur through epigenetic mechanisms. It has been shown that transposon sequences can induce changes in DNA methylation and chromatin structure, and the host genome generally establishes epigenetic modifications such as DNA methylation and histone modifications in these regions in order to suppress transposon activity (Bourque et al., 2018). However, these epigenetic changes may sometimes also influence the expression of genes located near transposons and can alter the expression patterns of certain genes in different tissues or at different developmental stages (Feschotte, 2008; Bourque et al., 2018).

In addition, some retrotransposon-derived sequences have been shown to play roles in the regulation of genes that are active during placental development in mammals. In particular, endogenous retrovirus (ERV) sequences have been reported to occur in the promoter or enhancer regions of certain genes expressed in trophoblast cells and to functionally bind key transcription factors involved in placental development (Chuong et al., 2013; Chuong et al., 2017). Furthermore, it has been demonstrated that many microRNA (miRNA) and long non-coding RNA (lncRNA) sequences originate from transposon-derived sequences (Piriyapongsa et al., 2007). These RNA molecules may participate in the post-transcriptional regulation of gene expression and in epigenetic control mechanisms (Piriyapongsa et al., 2007; Feschotte, 2008).

Role of Transposons in the Evolution of Animal Genomes

Transposons are considered genetic elements that play an important role in genome evolution (Feschotte, 2008). These elements proliferate within the genome, contributing to increases in genome size and potentially playing a role in the reshaping of genome architecture (Bourque et al., 2018). Transposons may also contribute to the emergence of new genes through mechanisms such as gene duplication, exon shuffling, and the formation of novel regulatory sequences (Feschotte, 2008; Chuong et al., 2017). These processes may contribute to the development of new gene functions in the genome and enhance the ability of organisms to adapt to environmental conditions (Feschotte, 2008; Chuong et al., 2017).

The formation of retrogenes mediated by retrotransposons can also contribute to the emergence of new gene copies. For example, the formation of an *FGF4* retrogene observed in dogs is considered an example of transposon-mediated gene duplication (Parker et al., 2009). The movement of transposons can also contribute to exon shuffling processes; in this process, LINE-1 elements can carry their 3′ flanking sequences to new genomic locations, allowing exons from different genes to be rearranged into new combinations and thereby contributing to the emergence of novel protein structures (Moran et al., 1999).

In addition, some transposon sequences may be reutilized by the host genome during evolutionary processes, thereby contributing to the emergence of new gene functions. This process is referred to as transposon domestication or exaptation (Modzelewski et al., 2022). For example, it has been demonstrated that syncytin proteins, which play a role in placental development in mammals, have independently arisen in different mammalian lineages through the co-option of endogenous retrovirus (ERV) envelope proteins (Garcia-Perez et al., 2016). Therefore, transposons are currently

regarded not only as elements that move within the genome but also as important evolutionary factors that contribute to the shaping of genome architecture and to the emergence of new biological functions (Feschotte, 2008; Chuong et al., 2017; Bourque et al., 2018).

Conclusion

Transposons constitute a fundamental component of animal genomes and play important roles in shaping genome structure, gene regulation, and evolutionary processes. Their structure, distribution, and activity vary considerably among species, and these differences contribute significantly to the diversity observed in genome size, chromosomal organization, and regulatory capacity. In particular, the predominance of retrotransposons in mammalian genomes has been associated with genome expansion and the diversification of regulatory sequences, whereas the relatively low transposon content in avian genomes reflects a more compact genomic architecture.

Accumulating evidence indicates that transposons influence genome function through multiple mechanisms, including the generation of mutations, genomic rearrangements, and modifications in gene expression. These effects can lead to phenotypic variation, contribute to adaptive traits, and in some cases play a role in the emergence of genetic diseases. In addition, transposon-derived sequences may be co-opted by the host genome to serve regulatory or functional roles, further highlighting their importance in genome evolution.

Rather than being merely mobile DNA elements, transposons are now understood as dynamic genomic components that contribute to regulatory evolution and biological diversity. Continued advances in genomic technologies, particularly long-read sequencing and single-cell approaches, are expected to provide deeper insights into the tissue-specific and developmental dynamics of transposon

activity. Such knowledge will further improve our understanding of genome function and may also support future applications in areas such as animal breeding, disease resistance, and reproductive biology.

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FELINE IMMUN GENE EXPRESSION AND TRANSCRIPTOMICS

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Introduction

Immune gene expression profiling (GEP) is an important molecular approach that provides dynamic, real-time insights into the functional state of immune cells, extending beyond the static genetic information of an organism. Gene expression analyses reveal the transcriptomic responses of cells at a given time point under physiological or pathological conditions such as infection, cancer, or inflammation. In this way, it becomes possible to characterize immune responses at the molecular level in situations where conventional diagnostic methods may be insufficient. Particularly in precision medicine and personalized immunotherapy, the analysis of gene expression signatures has become a valuable tool for evaluating the status of immune cells within the tumor microenvironment and for predicting therapeutic responses. For example, the assessment of

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interferon-gamma–related gene signatures can be used to predict clinical responses to certain immunotherapies (Ravi et al., 2025; Sun et al., 2024; Jamieson & Maker, 2017).

Molecular immunology studies in veterinary medicine have advanced considerably over the past four decades. Early investigations were largely based on serological tests and a limited number of immune parameters; however, the development of high-throughput transcriptomic and multi-omics approaches has made it possible to investigate the immune system at a much more detailed molecular level. In this process, the sequencing of domestic animal genomes and the establishment of comprehensive omics databases for different species have played a key role. In particular, the characterization of feline and canine genomes has enabled system-level analyses of genes associated with immune responses and has facilitated a better understanding of the molecular mechanisms underlying disease pathogenesis (Fu et al., 2023; Van de Walle & Harman, 2025). Such studies also contribute to the comparative investigation of animal and human diseases within the framework of the One Health concept, thereby supporting the development of novel therapeutic strategies (Van de Walle & Harman, 2025).

The importance of immune gene expression studies in cats becomes particularly evident in understanding the pathogenesis of infectious diseases. Feline infectious peritonitis (FIP) is a fatal disease associated with the replication of feline coronavirus (FCoV) within macrophages and is characterized by excessive activation of the host immune response (Zwicklbauer et al., 2025; Moyadee et al., 2025). In cats that develop FIP, a marked increase in the expression of genes encoding pro-inflammatory cytokines has been reported, with dramatic upregulation of cytokines such as interleukin-1beta (IL-1β), interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α). In contrast, decreased expression of genes associated with T lymphocytes (T cells) - mediated immunity and peripheral

lymphopenia are frequently observed (Malbon et al., 2010). Similarly, during feline immunodeficiency virus (FIV) infection, antiviral defense genes are initially activated; however, as the infection progresses, gene expression changes associated with immune suppression and impaired T-cell function emerge. In this context, activation of antiviral genes such as MX dynamin-like GTPase 1 (MX1) and radical S-adenosyl methionine domain containing 2 (RSAD2, Viperin), together with increased programmed death-ligand 1 (PD-L1) expression, has been suggested to contribute to T-cell exhaustion (Ertl & Klein, 2014; Mehrbod et al., 2015).

The investigation of the immune system at the genetic level in cats represents an important area of research not only in veterinary medicine but also in human medicine. In particular, FIV infection shares many pathogenetic and immunological similarities with human immunodeficiency virus (HIV) infection, making cats a valuable biological model for studying this disease (Lin, 1992; Smithberg et al., 2008). In addition, the evaluation of immune gene expression using peripheral blood or tissue samples provides important insights that may contribute to the development of next-generation biotherapeutic approaches, effective vaccines, and cancer therapies specifically designed for cats. Understanding feline immune responses at the molecular level also enables comparative investigations of disease mechanisms across species and provides an important scientific framework that supports both animal health and translational medical research.

Mechanisms of the Immune System in Cats

The immune system of cats provides a multilayered and coordinated defense mechanism against invading pathogens such as viruses, bacteria, fungi, and parasites. This defense system is generally composed of two main components: innate immunity and

adaptive immunity. These two systems do not function independently; rather, they operate in continuous interaction, enabling the recognition and elimination of pathogens as well as the development of immunological memory (Tizard, 2024; Lin, 1992).

The innate immune system constitutes the first line of defense that is activated upon encountering pathogens. This system includes various cell types such as macrophages, neutrophils, dendritic cells, and natural killer (NK) cells. Innate immune cells recognize microorganisms through receptors belonging to the pattern recognition receptors (PRRs) family, which detect pathogen-associated molecular patterns (PAMPs). Among these receptors, Toll-like receptors (TLRs), RIG-I–like receptors (RLRs), and NOD-like receptors play important roles. Activation of these receptors initiates processes such as phagocytosis, the production of inflammatory cytokines, and antiviral defense mechanisms. In addition, the innate immune system limits pathogen replication and supports the development of adaptive immune responses through various molecular effectors, including interferons, chemokines, and antimicrobial peptides (Tizard, 2024; Akira et al., 2006).

The adaptive immune system represents a more specialized defense mechanism characterized by high antigen specificity and the ability to generate immunological memory. The principal cells of this system are lymphocytes, which are mainly divided into two major groups: T cells and B cells. After recognizing antigens, B cells differentiate into plasma cells that produce antibodies known as immunoglobulins. In cats, four major immunoglobulin (Ig) classes have been identified: IgG, IgM, IgA, and IgE. These antibodies contribute to the immune response through mechanisms such as pathogen neutralization, opsonization, and activation of the complement system (Tizard, 1998; Lin, 1992).

T cells play a central role particularly in immune responses directed against intracellular pathogens. Cytotoxic T cells ($CD8^+$ T cells) are capable of directly eliminating virus-infected cells or cells harboring intracellular pathogens, whereas helper T cells ($CD4^+$ T cells) play an essential role in regulating immune responses. Through the production of cytokines, helper T cells activate macrophages, B cells, and other immune cells, thereby determining the direction and magnitude of the immune response (Tizard, 2024).

Communication among the different components of the immune system is largely mediated by cytokines and chemokines. Cytokines are small proteins that facilitate signal transmission between immune cells and play a crucial role in regulating inflammatory responses. For example, pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) initiate inflammatory responses during infection, whereas certain cytokines such as interleukin-10 (IL-10) contribute to the regulation and resolution of inflammation. The balanced regulation of this cytokine network is critical for the effective and controlled functioning of the immune system (Tizard, 1998).

In conclusion, the immune system in cats provides effective protection against pathogens through the coordinated action of innate and adaptive immune mechanisms. The regulation of this system at the molecular level occurs through the expression of immune-related genes and the activation of cellular signaling pathways. Therefore, the analysis of gene expression profiles is essential for understanding the functioning of the feline immune system, as it provides important insights into the regulation of host immune responses and the mechanisms underlying disease pathogenesis.

Differences Between the Feline Immune System and That of Other Species

Although the immune system of cats is largely similar to that of other mammals in terms of its fundamental organization, it exhibits certain species-specific structural and functional characteristics. These differences may influence the immune responses of cats to particular infections and their susceptibility to various diseases.

One of these differences is the presence of pulmonary intravascular macrophages (PIMs), which play a role in the clearance of circulating foreign particles. In many mammalian species, Kupffer cells in the liver are primarily responsible for removing microorganisms and particulate matter from the bloodstream. In cats, however, pulmonary intravascular macrophages located in the lungs have been reported to play a more prominent role in this process. Some researchers have suggested that this characteristic may contribute to a greater susceptibility of cats to systemic inflammatory conditions such as endotoxemia and septic shock compared with other species (Tizard, 1998; Lin, 1992).

Another characteristic feature of the feline immune system is the relatively limited diversity of the antibody repertoire. Molecular analyses have shown that the immunoglobulin heavy-chain variable region in cats is largely based on the immunoglobulin heavy chain variable region family 3 (VH3). In addition, it has been reported that the majority of immunoglobulin light chains in cats are of the lambda (λ) type, accounting for approximately 80–90% of the total. In contrast, in humans and mice the distribution between kappa (κ) and λ light chains is more balanced. This observation suggests that antibody diversity in cats may arise from a different genetic organization compared with that of other species (Tizard, 1998; Steiniger et al., 2017).

Cats may also exhibit certain differences from other species with respect to innate immune responses. For example, lipopolysaccharide (LPS), an important component of the bacterial cell wall, is known as a potent immune stimulant that induces lymphocyte proliferation in many mammalian species. However, feline lymphocytes have been reported to show a very limited proliferative response to LPS, or in some cases no response at all. This characteristic suggests that innate immune receptors or cellular signaling pathways in cats may be regulated differently compared with those in other mammals (Lin, 1992).

One of the key structures that determine the genetic basis of the immune system in cats is the major histocompatibility complex (MHC). In cats, this genomic region is referred to as the feline leukocyte antigen (FLA). It has been suggested that the FLA region exhibits more limited polymorphism compared with that observed in some other mammalian species. This characteristic has been proposed to contribute to a relatively delayed immune response in allogeneic tissue transplantation in cats compared with other species (Tizard, 1998; Lin, 1992).

Species-specific structural differences have also been identified in the CD4 molecule in cats. The feline CD4 molecule has been reported to contain an additional disulfide bond and distinct glycosylation sites compared with the human CD4 molecule. These structural features are thought to influence T-cell receptor signaling and viral entry mechanisms (Tizard, 2024).

When these features are considered together, it becomes evident that although the feline immune system shares the fundamental principles of immune organization with other mammals, it also exhibits certain molecular and cellular differences. Understanding these differences provides important insights into the

pathogenesis of infectious diseases in cats and contributes to the identification of species-specific immunological mechanisms.

Feline Immune Genetics: Structural Architecture and Evolutionary Differences

The immune system of cats is largely similar to that of other placental mammals in terms of its fundamental organization. However, during the course of evolution, pathogen-driven selective pressures and species-specific adaptations have led to significant structural differences in certain immune gene families. These differences are particularly evident in antigen presentation pathways, innate immune receptors, and antiviral defense mechanisms (Yuhki et al., 2003; Yuhki et al., 2007; Yuhki et al., 2008). In cats, immune-related genes are generally studied within functional groups such as the MHC, innate immune receptors, cytokines, and antimicrobial peptides.

MHC (FLA) Genes

In cats, MHC refers to a genomic region known as the feline leukocyte antigen (FLA), which contains genes that play a central role in both innate and adaptive immune responses. In the domestic cat, the MHC region spans approximately 3.3 Mb and is located on chromosome B2 (Morris, 2009). Based on their functional characteristics, MHC genes are classified into three main groups: Class I, Class II, and Class III.

Class I genes encode molecules that are expressed on most nucleated cells and present intracellularly derived peptides to $CD8^+$ cytotoxic T cells. This mechanism plays a critical role in the recognition and elimination of virus-infected cells. In cats, the Class I region is approximately 1.8 Mb in length and contains a total of 72 genes, of which about 19 are functional Class I genes (Yuhki et al., 2007).

Class II genes are primarily expressed in antigen-presenting cells such as macrophages, dendritic cells, and B cells, and they present extracellularly derived antigens to $CD4^+$ helper T cells. This process is critical for the initiation of adaptive immune responses and for the activation of B cells leading to antibody production. In cats, the Class II region spans approximately 700 kb and contains 44 genes, of which about 31 have been reported to be actively expressed (Yuhki et al., 2003).

The Class III region, in contrast, does not directly participate in antigen presentation but contains numerous genes involved in regulating immune responses, including those encoding complement proteins, cytokines, and tumor necrosis factors (Morris, 2009).

Although the overall organization of the feline MHC is similar to that of the human MHC, several important differences have been identified. In humans, the Class II region contains the DR, DQ, and DP gene families (Beck et al., 1999), whereas in cats the DQ gene family has been completely lost and the DP genes have been reported to be nonfunctional (Beck et al., 2001). In contrast, gene duplication events have occurred within the DR gene family in cats, resulting in the presence of multiple DRB genes (Yuhki et al., 2008). These structural differences may have certain implications for immune responses. The absence of the DQ gene family has been suggested as a potential factor that may limit antigen presentation diversity in cats. However, the expansion of the DR gene family and the enlargement of the Class I gene region may contribute to the recognition of a broader range of antigens, thereby partially compensating for this limitation. Consequently, the organization of the feline MHC is considered an important genetic feature that has evolved as part of species-specific adaptations shaping immune responses.

Comparative genomic studies conducted in species belonging to the order Carnivora have also revealed the presence of certain shared evolutionary features within the MHC region. For example, the presence of a similar chromosomal breakpoint between the tripartite motif containing (TRIM) TRIM39 and TRIM26 genes in the MHC region of both cats and dogs suggests that this structural arrangement may have emerged during the early evolutionary history of the Carnivora lineage (Yuhki et al., 2007; Murphy et al., 2001). However, the gene content of the MHC region varies considerably among species. In dogs, the DQ gene family in the Class II region has been retained, with functional genes such as DQA1 and DQB1 present (Debenham et al., 2005). In contrast, the loss of the DQ gene family in cats represents a notable difference. On the other hand, the expansion of the DR gene family in cats may result in antigen presentation being concentrated through specific MHC molecules.

Studies conducted among felid species have also demonstrated that MHC diversity can vary considerably between species. For example, cheetahs have been reported to exhibit very low MHC genetic diversity, which allows allograft tissues to be more readily tolerated between individuals (O'Brien et al., 1985; Drake et al., 2004). In domestic cats, however, a higher degree of MHC polymorphism has been observed compared with cheetahs, which may contribute to the development of immune responses against a broader range of pathogens.

Studies investigating the relationship between MHC genes and disease susceptibility in cats remain limited. In a study examining FIP, no significant association was found between DRB alleles and disease development (Addie et al., 2004). Nevertheless, investigating the potential roles of MHC genes in susceptibility to diseases such as diabetes, FIV, feline leukemia virus (FeLV), and other infectious diseases is considered an important area of research

for improving feline health and advancing comparative immunology (Morris, 2009).

Innate Immune Receptors (TLRs and NKRs)

The innate immune system operates through various receptor families that enable the early recognition of pathogens. These receptors detect PAMPs, thereby initiating inflammatory and antiviral immune responses. Among the innate immune receptors in cats, TLRs and NK cell receptors play particularly important roles.

Toll-Like Receptors (TLRs)

TLRs represent one of the most important families of PRRs involved in pathogen recognition. In cats, genes encoding TLR1 through TLR9 have been identified. Some of these receptors are located on the cell surface, whereas others are situated within intracellular endosomal compartments, allowing the recognition of different microbial structures (Ignacio et al., 2005).

The TLR1, TLR2, TLR4, and TLR6 genes are expressed on the cell surface and are primarily involved in the recognition of bacterial cell wall components. In contrast, TLR3, TLR7, TLR8, and TLR9 are located in endosomal compartments and detect viral or bacterial nucleic acids (Ignacio et al., 2005; Khair et al., 2022). Activation of these receptors leads to the production of interferons and various pro-inflammatory cytokines, thereby initiating innate immune responses.

The feline TLR4 gene has been reported to be highly expressed particularly in the lungs, bladder, and peripheral blood lymphocytes (Asahina et al., 2003). This observation suggests that TLR4 may play an important role in the immune response against bacterial infections in cats.

Molecular comparisons have revealed that feline TLR genes share approximately 81–89% sequence similarity with human TLR

genes (Ignacio et al., 2005). However, the molecular regulation of antiviral immunity in cats has not yet been fully elucidated, and research in this area is still ongoing (Robert-Tissot et al., 2011).

It has also been demonstrated that certain viral infections can alter TLR expression in cats. For example, during FIV infection, TLR expression in lymphocytes has been reported to vary depending on the cell type. During the active phase of infection, increased messenger RNA (mRNA) levels of TLR3, TLR7, and TLR8 have been observed (Robert-Tissot et al., 2011). Similarly, infection with feline infectious peritonitis virus (FIPV) has been reported to increase the expression of TLR9, TNF-α, and interferon beta (IFN-β) (Khair et al., 2022). These findings suggest that TLR-mediated signaling pathways may play an important role in the pathogenesis of viral infections in cats.

Natural Killer (NK) Cell Receptors (NKRs)

NK cells are important innate immune cells capable of recognizing and eliminating infected or transformed cells. The activity of NK cells are regulated by various activating and inhibitory receptors located on the cell surface. The genes encoding these receptors are primarily clustered in two regions of the mammalian genome: the Natural Killer Complex (NKC) and the Leukocyte Receptor Complex (LRC).

Genes located within the NKC region mainly encode members of the killer cell lectin-like receptor (KLR) family, which contain C-type lectin-like domains (Jelinek et al., 2023). In contrast, genes within the LRC region encode receptors that contain extracellular domains belonging to the immunoglobulin superfamily. This group includes the killer cell immunoglobulin-like receptors (KIR) and the leukocyte immunoglobulin-like receptors (LILR) families (Trowsdale et al., 2001).

Members of these receptor families typically interact with MHC class I molecules, thereby contributing to the regulation of NK cell activity. The balance between activating and inhibitory signals determines the ability of NKRs to recognize and eliminate infected or abnormal cells (Lanier, 1998; Carrillo-Bustamante et al., 2016; Guethlein et al., 2015).

The KIR gene family has expanded in many mammalian species and exhibits a high degree of polymorphism. This gene family is particularly diverse in primates and cattle (Guethlein et al., 2015). In contrast, a different pattern is observed in species belonging to the order Carnivora. In the genomes of domestic dogs and domestic cats, the KIR gene family has not expanded, and in some species it may even be absent. For example, KIR genes have been reported to be absent in the dog genome, whereas in cats only a single KIR gene or pseudogene has been identified (Hammond et al., 2009; Jelinek et al., 2023).

Another receptor family located within the LRC region is the leukocyte LILR gene family. LILR receptors are expressed in many immune cell types, including monocytes, macrophages, B and T lymphocytes, granulocytes, NK cells, mast cells, and dendritic cells (Storm et al., 2021). The best-characterized ligands for these receptors are MHC class I molecules, although they have also been shown to interact with certain pathogen-derived proteins and host-derived immunomodulatory molecules (Jones et al., 2011; Burshtyn & Morcos, 2016). However, the functions of the LILR gene family have not yet been fully characterized, particularly in species other than humans. Evidence from studies in humans and mice suggests that these receptors may play roles in various pathological processes, including autoimmune diseases, viral infections, neurodegenerative disorders, and cancer (Storm et al., 2021; Al-Moussawy et al., 2022). Therefore, LILR receptors are considered potential biomarkers and

immunotherapeutic targets (Gao et al., 2018; Takeda & Nakamura, 2017).

The organization of innate immune receptors in felid genomes has not yet been fully characterized. In particular, detailed investigation of genes located within the LRC region may contribute to a better understanding of innate immune mechanisms in cats.

Cytokines and Antimicrobial Peptides

Interferons (IFNs)

IFNs represent one of the most important components of innate immunity against viral infections in mammals. PAMPs, such as viral nucleic acids, are detected by receptors belonging to the PRRs family within host cells. Among these receptors, TLRs, RLRs, and cytoplasmic DNA sensors play key roles (Akira et al., 2006; Yoneyama & Fujita, 2010). Activation of these receptors leads to the activation of transcription factors including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein-1 (AP-1), and interferon regulatory factors (IRF3 and IRF7). These transcription factors subsequently stimulate the expression of type I interferons and various pro-inflammatory cytokines (Schindler et al., 2007).

The type I interferon family primarily consists of IFN-α, IFN-β, and IFN-ω proteins (Bekisz et al., 2004). The IFN-α gene family is encoded by multiple genes, whereas IFN-β and IFN-ω are generally encoded by a smaller number of genes. These cytokines activate antiviral signaling pathways through a receptor complex composed of IFNAR1 and IFNAR2 (Uze et al., 2007). As a result of interferon signaling, the production of antiviral effector molecules such as Mx proteins, PKR, OAS, and ISG15 increases, and these proteins inhibit different stages of viral replication (Sadler & Williams, 2008). In addition, interferons regulate the functions of

dendritic cells, NK cells, B cells, and T lymphocytes, thereby establishing an important link between innate and adaptive immune systems (Colonna et al., 2004).

Studies on the interferon system in cats have primarily focused on the characterization of type I interferon genes. The first feline interferon cDNA was cloned by Nakamura and colleagues in 1992, and this protein was identified as IFN-ω (Nakamura et al., 1992). Due to its strong antiviral activity, recombinant feline IFN-ω became one of the first biotechnological antiviral agents used in the treatment of viral diseases in cats.

Genomic analyses have identified 13 IFN-α and 13 IFN-ω gene subtypes in cats. Cell culture studies have demonstrated that these proteins exhibit antiviral activity. Structural analyses have shown that feline IFN-α and IFN-ω proteins display a high degree of sequence similarity, and that feline IFN-ω is more closely related to feline IFN-α than to IFN-ω proteins of other species. This finding suggests that the interferon gene family in felid species may have undergone a distinct evolutionary organization. In addition, feline IFN-ω has been shown to increase OAS activity, induce Mx protein expression, and stimulate ISG15 gene expression (Bracklein et al., 2006; Tanabe et al., 2008).

The interferon response in cats plays an important role in the control of viral infections. For example, during FCoV infection, viral RNA can be recognized by endosomal receptors such as TLR7, which stimulates the production of type I interferons and activates antiviral defense mechanisms (Robert-Tissot et al., 2011). However, some viruses are able to evade the immune system by suppressing the interferon response. Such mechanisms are thought to play a role in the pathogenesis of severe viral diseases such as FIP.

Antimicrobial Peptides (AMPs)

AMPs are small, cationic molecules synthesized by epithelial cells and leukocytes throughout the animal kingdom and represent important effector molecules of innate immunity (Zasloff, 2002). While these peptides are constitutively expressed in certain tissues, their expression levels can increase in response to inflammatory stimuli or pathogen exposure. The primary function of AMPs is to exert direct antimicrobial activity against bacteria, viruses, fungi, and parasites (Zasloff, 2002; Lehrer, 2004). However, these molecules are not limited to direct microbicidal effects; they also participate in various immunological functions such as chemotaxis, promotion of wound healing, regulation of apoptosis, and modulation of Toll-like receptor signaling pathways (Zasloff, 2002; Lehrer, 2004).

In mammals, two major AMP families are recognized: defensins and cathelicidins. Cathelicidins are characterized by the presence of a conserved cathelin/propeptide domain, whereas the C-terminal mature peptide region can vary considerably among species (Zanetti et al., 1995; Zaiou et al., 2003; Verbanac et al., 1993). Consequently, the structure and biological properties of cathelicidins may differ between species.

In cats, only a single gene belonging to the cathelicidin family has been identified, and this gene is referred to as feCath. Interestingly, humans, mice, rats, and dogs also possess only a single cathelicidin gene (Zanetti, 2005). In contrast, multiple cathelicidin genes have been identified in certain species such as cattle, sheep, and pigs, and this diversity has arisen through gene duplication events (Zanetti, 2005). The sequence and structural characteristics of the feline feCath peptide show high similarity to cathelicidins found in dogs, mice, and humans. This peptide has been reported to belong to the group of linear α-helical cathelicidins. In contrast, some

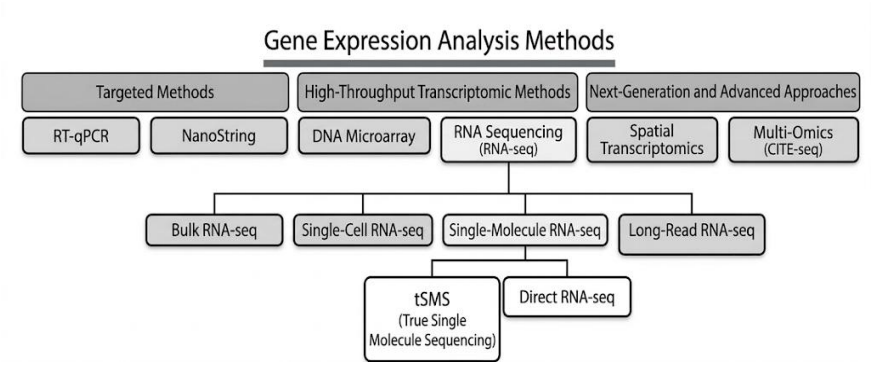
species such as cattle and pigs possess different cathelicidin subtypes, including proline-rich peptides, tryptophan-rich peptides, or peptides containing disulfide bonds. Therefore, although the cathelicidin system in cats exhibits a more limited repertoire compared with species that possess multiple cathelicidin genes, its organization is more similar to that observed in humans and dogs (Leonard et al., 2011; Kościuczuk et al., 2012).

Gene Expression Methods

The investigation of immune gene expression levels represents an important research approach for understanding the physiological state of cells, immune responses to pathogens, and inflammatory processes at the molecular level. Today, a variety of techniques are used in gene expression analysis, ranging from targeted methods to large-scale transcriptomic approaches. Each of these methods differs in terms of sensitivity, scope, and data generation capacity.

The study of immune gene expression in cats has become an important area of research, particularly for understanding the pathogenesis of viral infections, chronic inflammatory diseases, and immunodeficiency syndromes. In diseases such as FIP, FIV infection, and FeLV infection, examining host immune responses at the transcriptional level contributes to the elucidation of disease mechanisms. For this purpose, the expression levels of cytokines, interferon-stimulated genes, and chemokines involved in immune responses are analyzed using various molecular methods. Currently, techniques such as reverse transcription-quantitative real-time PCR (RT-qPCR), microarray analysis, and RNA sequencing (RNA-seq) are widely used in studies investigating the molecular regulation of immune responses in cats.

Figure 1. Gene expression analysis methods



References: Stanton, 2001; Gleeson et al., 2020; Amarasinghe et al., 2020; Richter, 2021; Thind et al., 2021; Cesano, 2015; Lang, 2024

Reverse Transcription-Quantitative PCR (RT-qPCR)

RT-qPCR is one of the most widely used targeted methods for RNA expression analysis. In this method, RNA isolated from cells is first converted into complementary DNA (cDNA) through reverse transcription, after which PCR amplification is performed using gene-specific primers. During the reaction, the fluorescence signal generated is measured in real time, allowing the quantitative determination of the expression level of the target gene (Stanton, 2001; Jozefczuk & Adjaye, 2011).

The RT-qPCR method has a broad dynamic measurement range and provides high sensitivity and accuracy. In addition, the short analysis time and the lack of need for additional post-PCR processing are among its important advantages (Bustin, 2000; Klein et al., 2001). For this reason, RT-qPCR is frequently preferred in studies comparing the expression levels of specific immune-related genes. However, the method also has certain limitations. The reliability of RT-qPCR results may be affected by RNA quality, reverse transcription efficiency, primer design, amplification efficiency, reference gene selection, and data normalization. In

addition, measurements obtained at very high cycle thresholds may be less reliable. Furthermore, overlap in the emission spectra of fluorescent dyes can limit multiplex analyses (Klein, 2002).

The RT-qPCR method is widely used in many studies investigating immune responses in cats to determine the expression levels of cytokines, interferon-stimulated genes, and antiviral defense genes. It is particularly frequently applied in studies aiming to characterize immune responses during FIP and FIV infections.

NanoString nCounter Technology

The NanoString nCounter system is a digital gene expression analysis technology based on the direct counting of mRNA molecules without the need for amplification. In this system, gene-specific hybridization probes and fluorescent barcodes are used to identify and directly count target RNA molecules (Geiss et al., 2008; Cesano, 2015).

One of the major advantages of this method is the ability to analyze hundreds of genes simultaneously in a single experiment. In addition, the ability to work with small amounts of RNA and the absence of amplification steps help reduce analytical errors. However, NanoString technology is limited to predefined gene panels and is therefore not suitable for large-scale exploratory transcriptome analyses (Cesano, 2015).

DNA Microarray Technology

DNA microarray technology is a high-throughput transcriptomic method that enables the simultaneous analysis of the expression levels of thousands of genes. In this approach, numerous DNA probes are immobilized on a chip surface, and fluorescently labeled cDNA molecules hybridize to these probes. In this way, the expression levels of a large number of mRNA transcripts present in cells can be analyzed simultaneously (Stanton, 2001).

This method has become an important tool for determining large-scale gene expression profiles, investigating disease mechanisms, and performing biological pathway analyses (Xue et al., 2015; Donlin et al., 2019). However, the requirement for prior knowledge of target gene sequences and technical limitations such as cross-hybridization may reduce the sensitivity of microarray analyses in certain situations (Fahmideh et al., 2023).

RNA Sequencing (RNA-seq)

RNA-seq is a powerful transcriptomic analysis method that enables the sequencing of RNA molecules present in cells using next-generation sequencing technologies. Through RNA-seq, gene expression levels, alternative splicing events, and novel transcripts can be identified with high accuracy (Richter, 2021).

RNA-seq–based transcriptomic analyses allow a comprehensive investigation of immune responses in cats. Using these approaches, interferon signaling pathways activated during viral infections, chemokine expression, and gene networks associated with inflammatory responses can be characterized in greater detail.

Bulk RNA Sequencing (Bulk RNA-seq)

Bulk RNA-seq is based on the sequencing of RNA molecules obtained collectively from all cells within a tissue or cell population. This method determines the average level of gene expression and allows differential gene expression analyses to be performed between different biological conditions (Thind et al., 2021; Kalantari-Dehaghi et al., 2025).

Although bulk RNA-seq provides comprehensive datasets, it cannot distinguish cellular heterogeneity in tissues composed of multiple cell types. As a result, expression changes occurring in rare cell populations may sometimes remain undetected.

Single-molecule RNA Sequencing (scRNA-seq)

scRNA-seq is an approach based on the sequencing of individual RNA or cDNA molecules without PCR amplification. By avoiding amplification steps, this method can reduce amplification-related biases and enable more accurate transcript quantification. Single-molecule RNA-seq approaches can be broadly divided into two main categories:

- cDNA-based single-molecule sequencing (True Single Molecule Sequencing – tSMS)
- Direct RNA Sequencing (Direct RNA-seq)

tSMS: In this approach, RNA is first converted into cDNA, which is then analyzed using single-molecule sequencing technologies. Platforms such as Helicos tSMS are examples of this category. This method has been used particularly for transcriptome profiling and differential gene expression analyses (Garalde et al., 2018).

Direct RNA-seq: In this method, RNA molecules are sequenced directly without cDNA synthesis. The direct RNA sequencing platform developed by Oxford Nanopore Technologies (ONT) is one of the best-known examples of this approach. This method offers important advantages for the detection of RNA modifications and the analysis of full-length transcripts (Liu & Graber, 2006; Gleeson et al., 2020).

Long-read RNA Sequencing

Long-read sequencing technologies (such as PacBio SMRT and Oxford Nanopore) enable the sequencing of RNA transcripts in their full length. Through this approach, alternative splicing events, isoform diversity, and complex transcript structures can be analyzed more accurately (Amarasinghe et al., 2020).

Spatial Transcriptomics

Spatial transcriptomics is a novel transcriptomic approach that enables the analysis of gene expression while preserving not only which genes are expressed but also their spatial locations within tissues. Through this method, gene expression profiles can be studied together with the spatial organization of cells within a tissue (Lang, 2024).

Spatial transcriptomics has become an important research tool particularly in cancer biology, developmental biology, and studies investigating the organization of the immune system within tissues. However, high costs and complex data analysis remain among the factors limiting the widespread application of this technology (Lang, 2024).

This approach is especially valuable for examining the distribution of immune cells within tissues and has the potential to contribute significantly in the future to the understanding of inflammatory diseases and the tumor microenvironment in cats.

Multi-omic Approaches and CITE-seq

Multi-omic approaches are methods that enable the simultaneous analysis of multiple types of molecular data from the same biological sample. Among these approaches, CITE-seq technology has gained particular attention because it allows the simultaneous measurement of both gene expression and protein abundance at the single-cell level (Stoeckius et al., 2017).

The CITE-seq method utilizes oligonucleotide-barcoded antibodies to analyze protein expression together with RNA data. By integrating these datasets, this approach enables a more accurate characterization of cellular phenotypes and provides important contributions to single-cell transcriptomic studies (Nicolet & Wolkers, 2022; Song et al., 2025).

General Evaluation of Immune Gene Expression Studies in Cats

When studies investigating immune gene expression in cats are considered collectively, it becomes evident that host immune responses exhibit significant transcriptional changes during infection, inflammation, and metabolic diseases. A substantial proportion of these studies has focused particularly on viral infections, with FCoV and FIP representing some of the most commonly used disease models for investigating immune responses at the molecular level in cats (Gelain et al., 2006; Safi et al., 2017; Malbon et al., 2019; Malbon et al., 2020; Lee et al., 2025). Overall, these studies indicate that interferon-mediated antiviral responses constitute one of the fundamental components of immune defense in cats. Many studies have reported a marked increase in the expression of interferon-related genes (MX1, viperin, IFIT family genes, STAT1, and STAT2) and chemokines (CXCL10 and CCL8) during infection (Robert-Tissot et al., 2011; Safi et al., 2017; Malbon et al., 2019; Malbon et al., 2020). In addition, increased expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α has been shown to play a significant role particularly in the pathogenesis of FIP (Foley et al., 2003; Kipar et al., 2006; Safi et al., 2017).

Studies investigating the pathogenesis of FIP indicate that the balance of cytokines plays a critical role in disease development. For example, some studies have reported that high TNF-α and low IFN-γ expression are associated with disease progression, whereas elevated IFN-γ levels may be associated with a protective immune response against infection (Kiss et al., 2004; Gelain et al., 2006).In addition, gene expression analyses performed in lymphoid tissues such as the mesenteric lymph nodes, spleen, and bone marrow have revealed that inflammatory cytokines and Toll-like receptor–mediated signaling pathways are particularly activated in cats that develop FIP (Kipar et al., 2006; Malbon et al., 2019). RNA-seq–based transcriptomic analyses have further demonstrated that during

FIP, immune processes such as chemokine-mediated cell migration, interferon signaling, and neutrophil activation are strongly activated, whereas T cell–mediated immune responses may be suppressed (Malbon et al., 2020; Lee et al., 2025).

In addition to viral infections, parasitic diseases also represent important models in studies investigating immune gene expression in cats. RNA-seq analyses conducted in Toxoplasma gondii infection have demonstrated widespread transcriptional changes across different tissues and have shown that signaling pathways such as JAK–STAT, NF-κB, MAPK, and NOD-like receptor pathways are activated (Cong et al., 2018). Similarly, in Cytauxzoon felis infection, genes associated with the interferon response (IFIT3, ISG15, STAT1), chemokines (CXCL9, CXCL10), and cytotoxic immune responses, including granzyme genes, have been reported to be significantly upregulated during the acute phase of infection (Scimeca & Reichard, 2023). These findings indicate that immune responses against parasitic infections in cats are also largely regulated through interferon- and cytokine-mediated signaling pathways.

Immune gene expression analyses are not limited to infectious diseases but also provide important insights into chronic inflammatory and metabolic disorders. For example, RNA-seq analyses conducted in feline chronic gingivostomatitis (FCGS) have shown a marked increase in the expression of genes associated with T-cell signaling, leukocyte migration, and cytokine–receptor interactions in the oral mucosa (Vapniarsky et al., 2020). Similarly, in inflammatory bowel disease (IBD), increased expression of cytokines such as IL-6, IL-10, IL-12p40, TNF-α, and TGF-β has been reported in the intestinal mucosa (Van et al., 2006).

In the context of metabolic diseases, one study investigating type 2 diabetes in cats found that peripheral blood monocytes

exhibited differential expression of genes associated with inflammation, oxidative phosphorylation, and cellular stress responses (O'Leary et al., 2017). Together, these studies demonstrate that immune gene expression analyses contribute not only to understanding the pathogenesis of infectious diseases but also to elucidating the molecular mechanisms underlying chronic inflammatory and metabolic disorders.

Studies investigating immune gene expression in cats also show considerable diversity in terms of the biological samples used. In many studies, peripheral blood and peripheral blood mononuclear cells (PBMCs) have been used to evaluate systemic immune responses (Gelain et al., 2006; Robert-Tissot et al., 2011; Lee et al., 2025). In addition, studies using bronchoalveolar lavage cells, alveolar macrophages, peritoneal effusion cells, and various tissue samples have revealed tissue-specific characteristics of immune responses (Ritchey et al., 2001; Foley et al., 2003; Kipar et al., 2006; Safi et al., 2017). These findings indicate that immune responses may exhibit different transcriptional profiles not only at the systemic level but also depending on the local tissue microenvironment.

In recent years, the use of high-throughput transcriptomic technologies has enabled a more detailed understanding of the feline immune system at the molecular level. In particular, RNA sequencing and scRNA-seq approaches have revealed the heterogeneity of immune cell populations and gene expression profiles specific to individual cell subtypes. scRNA-seq analyses performed on the peripheral blood of healthy cats have demonstrated that distinct immune cell populations—including T cells, neutrophils, monocytes, B cells, plasmacytoid dendritic cells, and mast cells—can be transcriptionally identified (Ramarapu et al., 2024). These findings indicate that the molecular characteristics of circulating immune cells in cats are largely similar to those observed in other mammals.

Overall, studies investigating immune gene expression in cats indicate that the immune response is highly dynamic and exhibits disease-specific transcriptional regulation. However, a substantial proportion of existing studies have been conducted with limited sample sizes or have focused on targeted analyses of specific gene groups. In the future, the application of single-cell transcriptomics, spatial transcriptomics, and multi-omics approaches will enable a more detailed investigation of different immune cell subpopulations and tissue microenvironments. Such advanced molecular approaches may contribute to a more comprehensive understanding of the regulation of the feline immune system and may facilitate the development of novel diagnostic biomarkers and targeted therapeutic strategies.

Conclusion

The immune system in cats provides effective protection against pathogens through the coordinated action of innate and adaptive immune mechanisms. In recent years, advances in genomic and transcriptomic technologies have enabled a more detailed investigation of these immune responses at the molecular level. In particular, analyses of immune gene expression have provided important insights into how host responses are regulated during infectious diseases, chronic inflammatory processes, and metabolic disorders. These studies indicate that immune responses in cats are largely regulated through interferon-mediated antiviral mechanisms, cytokine and chemokine signaling pathways, and cellular immune processes.

A review of the current literature shows that most studies on immune gene expression in cats have primarily focused on viral infections. Diseases such as FCoV and FIP represent some of the most frequently investigated models in this field, and studies have demonstrated that interferon-related genes, pro-inflammatory

cytokines, and chemokines change significantly during infection. However, studies conducted in different pathological conditions such as parasitic infections, chronic inflammatory diseases, and metabolic disorders have also revealed that immune responses exhibit disease-specific transcriptional regulation. These findings indicate that gene expression analyses are not only valuable for understanding the pathogenesis of infectious diseases, but also represent an important tool for elucidating the molecular mechanisms underlying inflammatory and immune-mediated disorders.

Studies investigating immune gene expression in cats also demonstrate considerable methodological diversity. In earlier studies, RT-PCR and qPCR-based targeted gene analyses were predominantly used, whereas in recent years high-throughput transcriptomic methods, such as RNA-seq and scRNA-seq, have become increasingly common. These new technologies allow the gene expression profiles of immune cell subpopulations to be characterized in greater detail and help reveal the cellular heterogeneity of the immune system. In particular, analyses performed at the single-cell level have shown that the molecular characteristics of circulating immune cells in cats are largely similar to those observed in other mammals, providing important insights for comparative immunology. However, a significant proportion of the existing studies have been conducted with limited sample sizes, and many investigations have remained restricted to targeted analyses focusing on specific cytokines or antiviral genes. In addition, studies examining how immune responses are regulated within different tissue microenvironments are still relatively limited. In the future, the use of single-cell transcriptomic approaches, spatial transcriptomics, and multi-omics technologies will enable a more detailed characterization of immune cell subpopulations and tissue-specific immune responses in cats. Such advanced molecular

approaches will not only improve our understanding of the fundamental biology of the immune system, but will also contribute significantly to the identification of novel diagnostic biomarkers and the development of targeted immunotherapeutic strategies.

In conclusion, the investigation of immune gene expression in cats is of great importance for both veterinary medicine and comparative biomedical research. Cats are considered valuable model organisms, particularly for understanding the molecular mechanisms of viral infections and immune-mediated diseases. In the future, studies incorporating larger sample sizes, advanced transcriptomic technologies, and integrated multi-omics approaches will significantly expand our knowledge of the regulation of the feline immune system. Such advances will also facilitate the translation of this knowledge into novel applications benefiting both animal health and human medicine.

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MICROSATELLITES: APPLICATIONS IN VETERINARY MEDICINE

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Introduction

In recent years, the analysis of genetic material in veterinary medicine has evolved from being merely an academic research field into a central component of clinical diagnostics, animal breeding, and forensic applications. Microsatellite sequences, also known as simple sequence repeats (SSR), are located within the non-coding regions of the genome and exhibit a high degree of polymorphism, making them one of the most strategic tools driving this transformation. Particularly in livestock species, improvements in production traits and the accurate verification of pedigree records in companion animals can be achieved with high reliability due to the strong discriminatory power provided by microsatellite markers. In modern veterinary practice, microsatellites are not only tools for individual identification but also dynamic genetic elements that

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provide critical information for population genetics and the biodiversity management of endangered species. The role of microsatellite instability (MSI) in tumors of domestic animals contributes to the development of targeted treatment protocols in oncological cases. At the same time, the analysis of these sequences in pathogen genetics enables the identification of the sources and transmission dynamics of infectious disease outbreaks, forming the basis of molecular epidemiology. This chapter aims to examine the structural characteristics of microsatellites within the framework of veterinary medicine and to provide a comprehensive overview of their applications, including cross-species genetic transferability and the current implications of next-generation sequencing technologies in clinical diagnostics.

Microsatellites, as one of the most dynamic tools of modern molecular genetics and veterinary medicine, function as polymorphic beacons that illuminate the “dark regions” of the genome. Also known as Simple Sequence Repeats (SSR) or Short Tandem Repeats (STR), these genetic elements consist of nucleotide motifs of 1 to 6 base pairs that are repeated consecutively within the DNA sequence (Alves et al., 2024; Singh et al., 2023). First introduced into the scientific literature approximately 35 years ago by Litt and Luty, the concept of microsatellites has since driven revolutionary advances in fields such as animal breeding, population genetics, forensic science, and microbiological surveillance (Alves et al., 2024; Saeed et al., 2016). Within the discipline of veterinary medicine, microsatellites are not merely genetic markers; they also represent powerful biological datasets that play a key role in understanding disease resistance, production traits, and evolutionary adaptation processes in animal populations.

Molecular Basis and Genomic Organization of Microsatellites

The biological significance of microsatellites arises from their unique organization within the genome and their inherent mutational instability. Although these sequences are distributed throughout eukaryotic genomes, they are particularly enriched in

euchromatic regions, within both coding and non-coding DNA segments, as well as in organelle genomes (Hosseinzadeh-Colagar et al., 2016; Vieira et al., 2016). The genomic distribution of microsatellites is not random. Comparative studies conducted on model organisms such as Arabidopsis thaliana and rice have demonstrated patterned distributions of these sequences across the genome. These findings have strengthened the hypothesis that microsatellites may exert regulatory influences on gene expression and genome stability (Vieira et al., 2016).

Structural Classification and Motif Complexity

The characterization of a microsatellite locus is primarily based on the length of the repeated motif and the integrity of the repeat sequence. This classification ranges from mononucleotide repeats to hexanucleotide repeats, and the length and structure of the motif directly influence the polymorphism potential of the marker (Alves et al., 2024). In veterinary genetics, particularly in forensic investigations and parentage analyses, tetranucleotide repeats are frequently preferred. Their use increases data reliability because they produce fewer "stutter" artifacts during PCR amplification compared with shorter repeat motifs (Jan & Fumagalli, 2016; Vieira et al., 2016). The degree of structural perfection of microsatellites is another critical parameter affecting mutational stability. Perfect microsatellites consist of uninterrupted repeat units, whereas imperfect or interrupted microsatellites contain base substitutions or insertions within the repeat array. These interruptions generally lead to a reduction in mutation rates (Alves et al., 2024; Vieira et al., 2016). Such structural differences play an important role in the calibration of molecular evolutionary clock models used to reconstruct the genetic history of populations.

Mutational Mechanisms

The primary driving force behind allelic diversity in microsatellites is the "slipped-strand mispairing" (slippage)

mechanism that occurs during DNA replication. When DNA polymerase copies a repetitive sequence, a temporary dissociation may occur between the template strand and the newly synthesized strand, followed by reannealing at an incorrect position. If a loop forms on the newly synthesized strand, the number of repeat units increases, resulting in repeat expansion. Conversely, if the loop forms on the template strand, the number of repeats decreases, leading to repeat contraction (Hosseinzadeh-Colagar et al., 2016; Vieira et al., 2016). This polymerase error mechanism gives microsatellites a mutation rate typically ranging from approximately 10^{-3} to 10^{-4} per locus per generation, which is several orders of magnitude higher than the mutation rate observed in single nucleotide substitutions (Hennequin et al., 2001). In addition, recombination events such as unequal crossing-over and gene conversion also contribute to microsatellite length polymorphism (Vieira et al., 2016). This elevated mutation rate is a key property that enables the detection of subtle genetic differences among closely related individuals or subpopulations, making microsatellites highly informative markers in population genetics and veterinary genomic studies.

Marker-Assisted Selection and QTL Mapping in Animal Breeding

One of the most common applications of microsatellites in veterinary medicine and animal breeding is the identification of genetic regions that control economically important production traits, known as Quantitative Trait Loci (QTLs). Traits such as milk yield, meat quality, growth rate, and disease resistance are typically influenced by multiple genes and are therefore considered polygenic traits. High-density genetic maps based on microsatellite markers provide the fundamental framework for identifying the genomic coordinates of these complex traits. By enabling the localization of QTLs across the genome, microsatellite-based mapping approaches

have become essential tools for understanding the genetic architecture of economically important characteristics in livestock species (Ihara et al., 2004; Mavi & Cheema, 2018).

QTL Analysis and Applications in Breeding Programs

The placement of more than 3,800 microsatellite markers in cattle genetic maps at an average interval of 1.4 cM has greatly accelerated the assignment of hereditary phenotypes to specific chromosomal regions (Ihara et al., 2004). The primary objective of QTL studies is to enable the early identification of individuals with superior phenotypes through Marker-Assisted Selection (MAS) by evaluating their genetic profiles at the beginning of life (Mavi & Cheema, 2018). In particular, genome scans conducted in dairy cattle breeding programs using Granddaughter and Daughter designs have facilitated the discovery of major genes influencing milk production traits and milk composition (Mavi & Cheema, 2018). In these experimental designs, microsatellites function as co-dominant markers that allow the tracking of genomic segments transmitted from ancestral individuals to their offspring. For example, the BM1500 microsatellite locus has been reported to show a significant correlation with marbling and rib fat thickness in cattle, highlighting its potential utility as a genetic marker associated with carcass quality traits (Cunming et al., 2025).

Myostatin Mutation and the Double-Muscling Phenotype

The genetic basis of the double-muscling phenotype, characterized by muscle hypertrophy in cattle, represents one of the most striking examples of microsatellite-based linkage mapping in veterinary genetics. In the mid-1990s, studies conducted on breeds such as Belgian Blue and Piedmontese demonstrated that this trait is controlled by a single autosomal recessive locus (mh), which was mapped to bovine chromosome 2 (BTA2) using microsatellite markers (Charlier et al., 1995; Cunming et al., 2025). These mapping

studies ultimately led to the identification of the bovine homolog of the myostatin (MSTN) gene, previously characterized in humans. Subsequent research revealed an 11-base pair deletion (nt821) in Belgian Blue cattle that completely disrupts the function of the myostatin protein (McPherron & Lee, 1997). Today, this mutation is routinely monitored by breeders through microsatellite markers or direct mutation testing, allowing the optimization of meat production while maintaining genetic control of the trait.

Diagnosis of Hereditary Diseases in Veterinary Clinical Genetics

The widespread distribution of microsatellites throughout the genome plays a critical role in the diagnosis of both monogenic and polygenic hereditary diseases. This is particularly important in purebred dog and cat populations, where intensive inbreeding practices often increase the prevalence of genetic defects. The mapping of these inherited disorders and the identification of carrier individuals constitute a fundamental component of preventive veterinary medicine, enabling breeders and veterinarians to reduce the transmission of deleterious alleles within breeding populations (Charlier et al., 2008; Donner et al., 2018; Grobet et al., 1997).

Common Hereditary Diseases and Genetic Markers

Many hereditary diseases are associated with variations occurring within a specific gene or within a microsatellite sequence located in close proximity to that gene. This relationship allows indirect genetic testing to be performed even when the exact causal mutation has not yet been identified. In dogs, commercial genetic testing panels developed to monitor conditions such as Progressive Retinal Atrophy (PRA) and Exercise-Induced Collapse (EIC) combine microsatellite and single nucleotide polymorphism (SNP) data. These panels provide valuable guidance to breeders in selecting genetically healthy breeding animals (Donner et al., 2018). Similarly, in cattle breeding programs, monitoring lethal recessive

mutations that may lead to embryonic or fetal loss—such as Complex Vertebral Malformation (CVM) and Brachyspina syndrome—is of critical importance for maintaining herd health and protecting the economic sustainability of livestock production (Charlier et al., 2008).

Forensic Veterinary Medicine and Individual Identification

One of the most prominent applications of microsatellites in veterinary medicine is forensic genetics. Biological traces left by animals at crime scenes—such as hair, blood, feces, or saliva—can serve as "silent witnesses" that help establish the presence of suspects or victims at a particular location (Dawnay et al., 2008; Menotti-Raymond et al., 1999; Rogalska-Niżnik, 2025). Through the analysis of microsatellite markers, these biological samples can be used for individual identification, allowing investigators to link a specific animal to a crime scene or to reconstruct events related to criminal investigations involving animals.

Criminal Cases and DNA Fingerprinting Applications

The success of microsatellites in forensic investigations stems from their high discriminatory power in individual identification. The genotype of each individual is unique in terms of the combination of alleles present at microsatellite loci, which enables precise differentiation between individuals. This high level of genetic variability makes microsatellite-based DNA fingerprinting a powerful tool for linking biological evidence collected from crime scenes to a specific animal or individual.

- **Feline DNA and a Murder Investigation:** In the Shirley Duguay murder case in Canada, white cat hairs found on a jacket near the victim's body were analyzed using microsatellite markers and matched to the suspect's cat, "Snowball." This case represents the first internationally

recognized example in which animal DNA served as a key piece of evidence in securing a murder conviction (Menotti-Raymond et al., 1997).

- **Dog Blood Evidence in Gang-Related Attacks:** In a homicide case in Washington State, where a couple was murdered, blood stains from a dog found on the attackers' clothing were analyzed and showed a complete match at 10 STR loci with the DNA profile of the victims' dog, "Chief." This genetic evidence played a decisive role in the conviction of the perpetrators (Menotti-Raymond et al., 1997).
- **Livestock Theft (Rustling):** In the United Kingdom, the Animal and Plant Health Agency (APHA) uses genetic panels consisting of 14 microsatellite loci to determine the true parentage or origin of sheep and cattle in livestock theft cases, even when ear tags have been altered or replaced. These analyses have provided irrefutable forensic evidence, often leading to confessions by the offenders (Dawnay et al., 2008).

Parentage Verification and Probability of Exclusion

In purebred animal breeding, parentage verification (paternity testing) is a legal requirement to ensure the accuracy of pedigree records. The effectiveness of microsatellite-based panels is evaluated using the Cumulative Probability of Exclusion (CPE). For example, a canine panel containing 12 microsatellite loci (SSR markers) can exclude an incorrect parent with a probability of 99.999995% (Singh et al., 2023). In horses, STR panels recommended by the International Society for Animal Genetics (ISAG) provide an exclusion power exceeding 0.999, even in cases where one or both parents are unknown. These systems play a crucial role in maintaining the integrity and reliability of equine racing registries and breeding records (Kim et al., 2025).

Veterinary Microbiology, Parasitology, and Epidemiological Surveillance

The use of microsatellites is not limited to vertebrate genomes; they have also revolutionized the strain-level typing and epidemiological monitoring of bacterial and fungal pathogens. Understanding the genetic variation of pathogens is essential for identifying sources of infection and tracking the spread of antibiotic resistance within populations (Bart-Delabesse et al., 1998; Hennequin et al., 2001; Saeed et al., 2016). Microsatellite-based analyses provide powerful tools for distinguishing closely related strains and for investigating transmission pathways during infectious disease outbreaks, thereby supporting molecular epidemiological studies in veterinary medicine.

The Role of Microsatellites in Pathogen Typing

In bacterial pathogens, microsatellites are often involved in processes known as phase variation, which enable bacteria to evade host immune responses through alterations in surface protein expression. In species such as Haemophilus influenzae and Neisseria, tetranucleotide repeats located within virulence genes play a regulatory role in controlling this process (Saeed et al., 2016). In parasitic diseases that cause significant economic losses—such as coccidiosis—microsatellite typing of Eimeria strains is used to monitor the spread of drug-resistant variants and to support the development of effective vaccination strategies (Dewi et al., 2025; Shirley, 2000). Similarly, in Cryptosporidium infections, specific microsatellite markers, such as the ML1 242 allele, can help determine whether an infection originated from livestock operations or from wildlife reservoirs, thereby clarifying the zoonotic transmission pathways of the disease (Hunter et al., 2008).

Population Genetics, Wildlife Conservation, and Evolutionary Analyses

In wildlife biology, microsatellites serve as primary tools for preserving the genetic diversity of endangered species and for assessing the impacts of habitat fragmentation. In small populations, genetic variation often declines due to genetic drift and inbreeding, which can significantly limit a species' capacity to adapt to environmental changes (Moss et al., 2003; Presti & Wasko, 2014; Putman & Carbone, 2014). Microsatellite markers enable researchers to evaluate population structure, gene flow, and levels of genetic diversity within and among wildlife populations, providing essential data for the development of effective conservation and management strategies.

Examples of Wildlife Conservation and Management

- **African Wild Dog (*Lycaon pictus*):** In this critically endangered species, microsatellite studies have demonstrated that many populations remain genetically isolated and exhibit reduced genetic diversity. These findings have served as a scientific basis for translocation strategies and genetic rescue programs, where individuals are moved between populations to restore genetic variability (Putman & Carbone, 2014).
- **Gray Wolves (*Canis lupus*):** The genetic structure of wolf populations in North America has been extensively analyzed using microsatellite markers. These studies have mapped how anthropogenic barriers, such as roads and human settlements, disrupt gene flow between populations (Putman & Carbone, 2014).
- **Pacific Salmon:** Microsatellite markers have been used to trace the migration routes and detect genetic bottlenecks in salmon populations. These insights have contributed to optimizing both commercial fisheries management and the

designation of conservation zones (Putman & Carbone, 2014).

- **Illegal Parrot Trade:** A panel of 106 tetranucleotide SSR loci developed for seven endangered parrot species can determine whether confiscated birds originate from wild populations or licensed breeding facilities with an accuracy of 99.9%, providing strong genetic evidence against wildlife trafficking (Jan & Fumagalli, 2016).

Microsatellites are also highly effective in the identification of cryptic species—species that are morphologically similar but genetically distinct—as well as in the detection of hybrid individuals (Dawson et al., 2013). Such analyses are indispensable for the accurate inventory of biodiversity and for establishing appropriate conservation priorities.

The Transformation of Marker Technologies: Microsatellites vs. SNPs

Over the past decade, single nucleotide polymorphisms (SNPs) have gained increasing popularity, particularly due to the declining costs of high-throughput sequencing technologies and SNP genotyping arrays. Nevertheless, microsatellites continue to maintain a strong position in veterinary genetics for both technical and economic reasons (Guichoux et al., 2011; Laoun et al., 2020; Pérez-González et al., 2023). SNP markers clearly dominate applications such as genomic estimated breeding value (GEBV) prediction and large-scale commercial breeding programs (Ihara et al., 2004; McClure et al., 2013). However, microsatellites remain the gold standard in several contexts, particularly in the genetic characterization of local breeds in developing countries, forensic investigations, and wildlife conservation projects with limited financial resources. Studies have demonstrated that the population structure inferred from a panel of 21–30 microsatellite loci shows a 77–95% concordance (Pearson correlation) with results obtained

from datasets containing tens of thousands of SNP markers (Laoun et al., 2020).

Transitional Approaches: Microsatellite Allele Imputation

To facilitate the transition from microsatellite-based records to SNP-based genotyping systems in cattle breeding, techniques known as microsatellite (MS) allele imputation have been developed. Using this approach, the historical microsatellite alleles of an animal can be predicted by analyzing its SNP haplotype data. This method enables continuity between older microsatellite datasets and modern SNP-based genomic databases, thereby preserving valuable historical genetic information (McClure et al., 2013).

Technical Implementation: Genotyping Protocol and Data Analysis

For veterinarians and geneticists, the microsatellite analysis workflow follows a rigorous protocol, beginning with sample collection and continuing through allele sizing and data interpretation. Any error occurring at any stage of this process may lead to incorrect parentage assignments or may compromise the reliability of forensic evidence in legal investigations (Butler, 2005).

- **Sample Collection and DNA Isolation:** DNA is extracted from hair follicles, blood, tissue, or fecal samples using either commercial extraction kits or conventional laboratory methods (Grobet et al., 1997).
- **PCR Amplification:** Primers are designed to target the flanking regions surrounding the microsatellite locus. Through multiplex PCR, it is possible to simultaneously amplify 12–15 loci in a single reaction, significantly increasing analytical efficiency (Rogalska-Niżnik, 2025; Singh et al., 2023).
- **Capillary Electrophoresis:** Fluorescently labeled PCR products are separated using genetic analyzers, which

provide high-resolution fragment separation with precise sizing accuracy.

- **Allele Sizing:** The lengths of microsatellite alleles are determined in base pairs using an internal size standard. During this stage, accurate interpretation of artifacts—such as stutter bands (shadow bands appearing before or after the main peak) and large allele dropout (preferential amplification of smaller alleles leading to weak detection of larger alleles)—requires careful evaluation by experienced personnel to ensure reliable genotyping results (Hosseinzadeh-Colagar et al., 2016).

Future Perspectives

Over the past three decades, microsatellites have served as a cornerstone of genetic diagnosis, animal breeding, and forensic investigation in veterinary medicine. Although single nucleotide polymorphism (SNP) technologies and whole-genome sequencing (WGS) approaches are rapidly expanding, the distinctive characteristics of microsatellites—such as their high locus-specific polymorphism and their ability to be analyzed even from low-quality or degraded DNA samples—continue to make them indispensable genetic markers. In the future, the wider adoption of next-generation sequencing (NGS)-based microsatellite analyses, particularly SSR-Seq, will enable researchers to detect not only length polymorphisms but also single nucleotide variations within repeat motifs, thereby substantially enhancing the informational power of these markers (Alves et al., 2024). The application of microsatellites in veterinary medicine serves multiple objectives, including sustainable livestock production, conservation of endangered species, eradication of hereditary diseases, and the advancement of forensic justice. For scientists and veterinarians alike, these genetic markers will continue to function as reliable tools guiding efforts to preserve biodiversity and improve animal health at the genetic level. Moreover, the rapid development of emerging biotechnological tools capable of

processing microsatellite data more quickly and cost-effectively will further democratize their use in field conditions, expanding their accessibility and practical impact across veterinary and ecological research.

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