THESIS

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2024

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# BEAGLE DOG´S MODEL USED IN INVESTIGATION OF AUTISM

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Budapest, 2024

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## 1. Abbreviations

AAT: animal-assisted therapy

ABA: Applied Behavior Analysis

ADOS: Autism Diagnostic Observation Schedule

ADI-R: Autism Diagnostic Interview-Revised

ASD: Autism Spectrum Disorder

bShank3: Beagle Shank3

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9) gene

CSF: cerebrospinal fluid

DNA: Deoxyribonucleic Acid

DSM-5: Diagnostic and Statistical Manual of Mental Disorders

EIBI: Early Intensive Behavioral Intervention

FMRP: Fragile X Mental Retardation Protein

FXS: Fragile X syndrome

GABA: Gamma-Aminobutyric Acid

ICD-10 or ICD-11: International Classification of Diseases

M-CHAT: Modified Checklist for Autism in Toddlers

MMR: measles, mumps, and rubella vaccination

MSC: mesenchymal stem cell

PFC: prefrontal cortex

PMS: Phelan-McDermid syndrome

PSD: postsynaptic density

RNA: guide ribonucleic acid

SCQ: Social Communication Questionnaire

SHED: human exfoliated deciduous teeth

SSRIs: Selective Serotonin Reuptake Inhibitors

TEACCH: Treatment and Education of Autistic and Related Communication- Handicapped Children

VPA: Valproic Acid

## 2. Abstract

Autism spectrum disorder (ASD) is a neurological disease. People of all genders, races, ethnicities, and economic backgrounds can be diagnosed with ASD. Researchers don't know the primary causes of ASD. The development of animal models of autism is one approach researchers use to study potential causes of autism.

This thesis provides a comprehensive overview of ASD, including a detailed description of its various types, typical symptoms, and causes. Possible treatments for ASD are also discussed.

In addition, the thesis focuses on the crucial role of animal models in autism research. The second part provides a detailed analysis of various animal models used in ASD research and their importance.

The most significant part of the thesis is dedicated to the beagle as an animal model in autism research. The thesis specifically discusses the Shank three-gene model and the potential of stem cells from human exfoliated deciduous teeth.

## 3. Autism spectrum disorder (ASD)

### 3.1 Overview

Autism Spectrum Disorder (ASD) is a neurological condition that affects an individual's social interactions, communication, and behavior. It is referred to as a "spectrum" of disorders due to the wide range of symptoms and severity levels that it can manifest in. Each individual with ASD is unique, and their challenges and strengths may vary significantly. It can be diagnosed at any age, but it's usually considered a developmental disorder because symptoms typically appear in the first two years of life (1).

Individuals with this disorder often have difficulty communicating and interacting with others. They may also exhibit restricted interests and repetitive behaviors. Unusual responses to sensory stimuli, such as hypersensitivity or hyposensitivity to lights, sounds, textures, or smells, are typical for ASD (1).

Animal models are commonly used to investigate ASD and are of great importance. Various animal models exist, but the beagle dog model is highly valued because dogs adapt better to human behavior (1).

The three types of ASD differ in the following ways. Classical or childhood autism is mainly defined by a lack of communication skills and motor impairment, especially if the disability is more significant. Atypical autism is characterized by not all typical signs being present, and the first signs may be visible at three years of age instead of 12 or 24 months. Children with atypical autism tend to have more severe cognitive restrictions. Asperger Syndrome is different from classical and atypical autism due to a lack of delay in communication skills and cognitive development. Individuals with Asperger have a normal range of intelligence but may have motor impairments.

### 3.1.1 Kanner Syndrom

The Kanner Syndrom is also known as classical autism, infantile autism, or childhood autism, which was described by Leo Kanner in 1943.
Classical autism is characterized by characteristic abnormalities in social interaction, language, and communication as well as restricted, repetitive behavior patterns. It is often, but not always, accompanied by a mental disability (2). Children with the condition typically display symptoms from an early age, but some may not show signs until their second or third year of life. They may lose their language skills and begin to isolate themselves socially (2).

Children with classical autism display specific signs, such as a lack of their own language or delayed language development. Echolalia is a term used to describe the repetition of words and sentences by a conversation partner, as well as the constant repetition of similar noises or sounds. It is common for children to exhibit self-harming behaviors such as hitting their heads or biting their fingers, hands, or wrists. However, it is important to note that these actions should not be equated with suicidal tendencies. Additionally, some children may display aggressive behavior, but this does not necessarily indicate hostility. It could be the result of impulsive actions that lead to injuries or damages.

### 3.1.2 Asperger-Syndrome

The Asperger syndrome was named after the Austrian pediatrician Hans Asperger. In 1943, he described his findings as an autistic psychopathy. Characteristic signs are particularly evident in social interaction and repetitive behaviors, as seen in individuals with Kanner Syndrome. The language spoken by the children sounds pedantic and formal, and their movements appear to be unskillful (3).
Children with Asperger syndrome are often socially isolated, and they are getting bullied because of their unique behavior (3).
Repetitive behavior is a significant aspect of Asperger's syndrome as people often seek security in their daily routines. Any sudden changes to their routine can lead to excessive demands and nervous behavior (3).

Children with Asperger's syndrome often exhibit superior grammatical and stylistic language skills. Hans Asperger noted that these children often stand out because they use adult-like and pedantic expressions, along with unusual intonation or prosody. This is why Asperger's syndrome is also referred to as Little Professor Syndrome.
Individuals with Asperger's syndrome may exhibit peculiarities in their motor skills, such as awkward movements, clumsiness, and coordination difficulties, which are not typically present in other forms of autism. This may also include a stilted or hopping gait (3).

### 3.1.3 Atypical autism

Atypical autism is a type of autism that differs from the classical form. It is characterized by children displaying autistic traits, but not exhibiting all the typical signs. This variant is typically diagnosed in or after a child's third year of life. It is also known by the alternative name of "intelligence reductions with autistic traits".
Severe cognitive restrictions characterize atypical autism, so the common ASD signs are not always seen. Children with autism may have cognitive restrictions that make it difficult to speak.
The causes of atypical autism are mainly genetic in nature, resulting from brain damage, biochemical imbalances, and impaired cognitive processes (4).

## 3.2 Signs and symptoms of ASD

Individuals with Autism Spectrum Disorder exhibit a range of symptoms in their social behavior and communication. First signs can occur in early childhood, typically in the first 12 to 24 months.
Autism can be accompanied by a complete lack of language skills or difficulties understanding language. Most individuals can acquire general language skills.
In terms of social communication, they may display limited or inconsistent eye contact and a lack of interest in sharing emotions, interests, or enjoyment of objects. Children with ASD often show atypical non-verbal communication, such as not pointing or bringing objects to express their interests, and tend to imitate others instead of initiating contact with their peers.

Miscommunication poses a significant challenge, especially when facial expressions, movements, and gestures are misinterpreted. People often talk about their favorite subject without noticing that others are not interested or giving others a chance to speak. In many cases, individuals with ASD may speak with a singsong or flat, robot-like tone of voice. Struggling to understand others' perspectives or predict their actions can be challenging.
Repetitive behavior is a significant sign of ASD. It is characterized by individuals repeating certain behaviors or having unusual behaviors, such as swinging their upper body or snapping their fingers. These actions are called stereotypes. It is also often seen that children line up toys or play with spinning coins. They may also develop an intense interest in specific topics like numbers, details, or facts that last for a long time. Minor changes in their routine can cause distress and make them struggle with transitions (5).

People with ASD often have difficulty processing sensory input, such as light and sound. It is also common for individuals to experience sleep problems and irritability.
However, it's important to note that they also possess unique strengths, such as the ability to learn things in detail and retain information for extended periods. So, while there may be challenges, it's essential to recognize and nurture these positive qualities as well (1, 5).

### 3.3 Causes and Related Factors

Various scientific fields are extensively researching autism to identify its possible causes or triggers.
However, studies suggest that a combination of genetic and environmental factors can affect a person's development, ultimately leading to ASD. Some factors that are associated with an increased likelihood of developing ASD include: Having a sibling with ASD, older parents, very low birth weight, or certain genetic conditions such as Down Syndrome or Fragile X Syndrome (7).
Scientists have discovered that submicroscopic changes in chromosomes play a significant role in genetic disorders. One such change is called copy number variation, a form of structural variation of the gene. Autism is often linked to such changes, where gene duplication or deletion occurs. These changes happen during the formation of egg or sperm cells (meiosis) and are usually new (de novo). However, if a child inherits such a deviation from a parent, they can pass it on to their own offspring with a probability of 50 percent. It

is possible for a deviation that contributes to autism to occur only once in a child and not be passed on, or it can affect several members of a family across different generations (15).

Neuropathological studies have found differences in cerebellar architecture and connectivity, limbic system abnormalities, and alterations in the cortical regions of the frontal and temporal lobes (9). Focal disruption of cortical laminar architecture was observed in the majority of subjects. This indicates problems with cortical layer formation and neuronal differentiation (9). ASD patients have a significantly smaller Gyrus parahippocampalis (Fig. 1., red area) related to empathy activity compared to people without ASD. The Parahippocampus is a part of the temporal lobe of the brain that includes the "Parahippocampal Place Area." This area is responsible for recognizing buildings and landscapes. If the Parahippocampus area is damaged, such as due to a stroke, a person may have difficulty recognizing buildings or people. Additionally, the Parahippocampus plays an important role in associative processing, which helps in recognizing social and communication connections (6, 7).

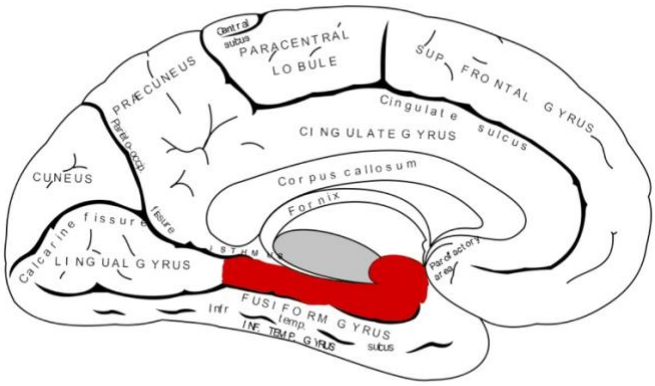


*Fig. 1. Medial surface of the left cerebral hemisphere. The author of this review redrew this figure. Original figure source: (Brodmann, 2016)*

A study of neocortical architecture in young children found disruption of cortical laminar architecture in most subjects, indicating issues with cortical layer formation and neuronal differentiation.

Brain overgrowth in terms of cortical size and in terms of increased extra-axial fluid have been described in children with ASD. Individuals who develop ASD have increased cerebrospinal fluid (CSF) in the subarachnoid space from 6 to 24 months of age (10). Studies

have shown that children with ASD tend to have larger brains. In fact, research has revealed that the brain volume of two-year-olds who later developed ASD was greater than that of their peers who did not. These findings suggest that increased brain volume may be an early indicator of ASD (11).

Familial recurrence, in terms of genetic and environmental factors related to the timing of birth, has a significant role in the development of ASD. The risk of ASD recurrence in siblings is 10.1% when compared to control groups. However, the risk of recurrence is higher in second-born children (11.5%) compared to later-born siblings (7.3%). Therefore, it can be concluded that there is a familial recurrence of ASD with factors related to the timing of birth (12).

There are certain risk factors associated with ASD. These include both shorter and longer inter-pregnancy intervals as well as premature birth. These factors have been found to increase the risk of ASD and other neurodevelopmental disorders. Healthcare professionals need to be aware of these risk factors so that appropriate measures can be taken to reduce the risk of ASD in at-risk individuals (14).

Research has indicated that there is a significant association between Autism and certain obstetric factors. Specifically, studies have found that preterm delivery, low birth weight, uterine bleeding, and cesarean delivery are among the vital obstetric factors that are associated with Autism. These findings have important implications for the management of pregnancy and delivery, as well as for the early identification and intervention of ASD. It is crucial for healthcare providers to be aware of these obstetric risk factors and to take appropriate measures to minimize them to reduce the risk of Autism (9).

Despite the hype surrounding the Lancet article that was published in 1998 and later retracted, there is no scientific evidence that vaccines, thimerosal, or mercury are linked to ASD. Research has shown that there is no increased risk of ASD after receiving the measles, mumps, and rubella (MMR) vaccination. Additionally, there is no significant difference in the frequency of autism between vaccinated and unvaccinated children (16, 17).

### 3.4 Diagnosing ASD

The diagnosis of ASD involves a comprehensive evaluation conducted by a qualified healthcare professional, typically a developmental pediatrician, child psychiatrist, pediatric neurologist, or clinical psychologist. The diagnostic process typically involves the following components:

Developmental History: The healthcare professional will collect information from caregivers or parents regarding the individual's developmental milestones, behaviors, and symptoms. The questions may cover early social interactions, communication skills, play behaviors, and concerns about atypical development.

It is essential to observe and assess an individual's social communication skills, repetitive behaviors, and sensory sensitivities in different environments such as clinical settings, home, or school. Professionals trained to evaluate these areas may use observational tools like the Autism Diagnostic Observation Schedule (ADOS) systematically to conduct assessments.

Parent or Caregiver Interview: Structured interviews, such as the Autism Diagnostic Interview-Revised (ADI-R), are frequently employed to collect comprehensive information from parents or caregivers about the individual's early development, current symptoms, and family history. These interviews offer valuable insights into the presence and intensity of symptoms related to ASD (11).

Tools like the Modified Checklist for Autism in Toddlers (M-CHAT) or the Social Communication Questionnaire (SCQ) can help identify individuals who may need further evaluation for ASD. These tools can help detect warning signs or areas of concern that require a more thorough assessment (11).

It is crucial to undergo a medical evaluation to eliminate the possibility of any underlying medical conditions or genetic syndromes that might be linked with ASD or have similar symptoms. This evaluation may comprise physical examination, genetic testing, neurological assessment, and screening for other developmental or psychiatric disorders.

The diagnosis of ASD is determined by following standardized diagnostic criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) or the International Classification of Diseases (ICD-10 or ICD-11). These criteria include deficits in social communication and interaction and the presence of restricted, repetitive patterns of behavior, interests, or activities. The diagnosis considers the severity of symptoms and level of impairment (18).

Multidisciplinary Approach: When assessing for ASD, a multidisciplinary team, which includes professionals from various disciplines, such as psychology, speech-language pathology, occupational therapy, and education, is often involved. Collaboration among team members helps ensure a comprehensive evaluation and appropriate intervention planning (11).

It is crucial to understand that ASD is a complex condition that presents a wide range of symptoms and varying levels of functionality. Therefore, conducting a comprehensive and personalized assessment is essential to ensure an accurate diagnosis and develop personalized interventions and support strategies for each individual. Early diagnosis and intervention can significantly improve outcomes for individuals with ASD (9, 18).

### 3.5 Treatment and Therapies

The treatment of ASD involves a mix of interventions that are customized to address the needs and challenges of everyone. There is no single cure for ASD, but different therapeutic approaches and strategies can help people with ASD improve their social communication skills, manage their behaviors, and enhance their overall quality of life (19).
Behavioral interventions refer to a range of techniques used to modify behavior patterns. One such technique is Applied Behavior Analysis (ABA). ABA is an evidence-based therapy that focuses on teaching and reinforcing positive behaviors while reducing problem behaviors. This structured approach involves breaking down complex skills into smaller, more manageable steps, providing repeated practice, and employing reinforcement techniques to facilitate learning (19).

Treatment and Education of Autistic and Related Communication- Handicapped Children (TEACCH) is an educational support concept that is designed for both children and adults with autism. Its purpose is to enhance the quality of life for individuals with autism and to help them cope with daily life. The concept assumes that people with autistic traits can learn through structure and visualization (19).

Early Intensive Behavioral Intervention (EIBI) involves intensive, one-on-one therapy delivered early in life, typically during preschool years, to promote skill acquisition and reduce the severity of ASD symptoms (20).

Training parents is an essential aspect of dealing with ASD. Parent training programs aim to educate, guide, and support parents and caregivers in understanding ASD, implementing effective strategies at home, and advocating for their child's needs. In addition, parent support groups can provide emotional support and practical advice from other families who have faced similar challenges (19).

Managing medication is an important aspect of treating ASD. Depending on the symptoms, a healthcare professional may prescribe medications such as selective serotonin reuptake inhibitors (SSRIs), antipsychotics, stimulants, and mood stabilizers to alleviate hyperactivity, aggression, anxiety, or depression. However, it's important to note that medication should be used cautiously and only under the supervision of a healthcare professional (19).

Therapy dogs facilitate social engagement, communication, emotional connection, and everyday participation of children with autism (19).

The use of dogs in therapy for individuals with ASD is known as animal-assisted therapy (AAT) or specifically, canine-assisted therapy. While AAT encompasses a variety of animals, dogs are among the most used animals due to their social nature, trainability, and ability to form bonds with humans. Here are some ways in which dogs can be involved in the treatment of individuals with ASD (25).

Dogs can provide emotional support and companionship to individuals with ASD. Their presence can help reduce stress and anxiety, provide comfort during challenging situations, and offer unconditional love and acceptance.

Therapy dogs can help improve social interaction and communication skills in individuals with ASD. For instance, interaction with a therapy dog could encourage verbal communication, eye contact, and social engagement that might be difficult for the individual otherwise (25, 26, 27).

Interacting with a dog can help individuals with ASD regulate sensory input and improve sensory processing skills through activities such as petting, grooming, or playing with the dog.
Activities such as walking, playing fetch, or doing agility training with a dog can help individuals with ASD improve their motor skills, coordination, and physical fitness.
Individuals with ASD can benefit from forming a bond with a therapy dog, promoting trust, empathy, and social connection (25).

It's important to note that while therapy dogs can provide valuable benefits, they are not a substitute for evidence-based interventions such as behavioral therapy, speech therapy, and occupational therapy. Therapy involving dogs should be conducted under the guidance of trained professionals who are knowledgeable about both ASD and animal-assisted interventions.

## 4. Animal models of autism

### 4.1 Overview of Animal Models

Animal models are frequently employed in scientific research to study diverse aspects of ASD. Although no single animal model can completely replicate all aspects of ASD, these models enable researchers to explore specific genetic, neurobiological, and behavioral traits linked to the disorder (31).
Researchers use genetic manipulations in animal models to investigate the genetic basis of ASD and its effects on brain development and behavior. They use techniques such as gene knockout, gene overexpression, or CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9) gene editing to introduce or disrupt specific genes linked with ASD or similar neurodevelopmental conditions.
Animal models of ASD are utilized to test potential treatments and interventions. Researchers can develop and test novel therapies by targeting specific genes or pathways implicated in ASD to ameliorate ASD-like symptoms and improve outcomes.

Animal models with genetic manipulations help to understand the neurobiological mechanisms of ASD, providing insights into the brain development, synaptic function, neural circuitry, and neurotransmitter systems affected in ASD (24).

While animal models are valuable tools for research, ethical considerations are paramount. Researchers must adhere to strict ethical guidelines and animal welfare standards when conducting genetic manipulations and experiments involving animals.

Overall, genetic manipulations in animal models play a crucial role in advancing our understanding of the genetic basis of ASD and in the development of potential treatments and interventions. However, it's important to recognize the limitations of animal models and the need for translational research to validate findings in humans and ultimately improve outcomes for individuals with ASD (24).

### 4.2 Rodent model

The use of rodent models is crucial in the progress of comprehending ASD. These models serve as indispensable instruments in autism research, furnishing invaluable insights into the fundamental neurobiology, genetics, and behavioral characteristics linked with ASD. By studying rodent behavior and brain function, experts can better understand the biological mechanisms that contribute to ASD. This knowledge can pave the way for the development of novel treatments and interventions that can enhance the quality of life for individuals with ASD (29, 30).

The Fmr1 knockout mouse is a standard rodent model used in scientific research. This model involves genetic deletion or mutation of the Fmr1 gene, which is linked to Fragile X syndrome (FXS). FXS is a genetic disorder that is a primary cause of inherited intellectual disability and ASD. The expansion of trinucleotide repeats (CGG) in the Fmr1 gene causes FXS, which results in the loss of function of Fragile X Mental Retardation Protein (FMRP), a vital protein for typical brain development and synaptic function.

Research has shown that mice with a deficiency in the Fmr1 gene, which is linked to Fragile X Syndrome, exhibit a variety of behavioral, cognitive, and neurobiological abnormalities that are remarkably like those seen in people with FXS and Autism Spectrum Disorder. These abnormalities include difficulties with social interaction, repetitive behaviors, hyperactivity, learning and memory impairments, and changes in anxiety-related behavior.

The Fmr1-deficient mice have been utilized as a model for investigating the mechanisms that underlie these disorders and for testing potential therapeutic interventions. The behavioral traits observed in these mice have offered valuable insights into the pathophysiology of FXS and ASD and have played a significant role in developing targeted treatments for these conditions (29, 30).

From a neurobiological perspective, mice with a knocked-out Fmr1 gene frequently display changes in synaptic plasticity, dendritic spine morphology, neurotransmitter systems (specifically glutamatergic signaling), and brain development, particularly in regions associated with social behavior and cognition. Additionally, these mice exhibit atypical sensory processing, including altered reactions to auditory and tactile stimuli.

The Fmr1 knockout mouse model has proven invaluable in advancing our understanding of the underlying pathophysiological mechanisms involved in both FXS and ASD. Extensive research has uncovered potential targets for therapeutic intervention in FXS and related neurodevelopmental disorders. Furthermore, Fmr1 knockout mice are utilized to assess the efficacy of treatments and therapies for FXS and ASD. Using these mice, we have gained valuable insights into synaptic dysfunction, molecular signaling pathways, and gene-environment interactions associated with ASD.

The Fmr1 knockout mouse model has been a breakthrough in advancing our knowledge of the molecular, cellular, and behavioral aspects of both Fragile X syndrome and autism spectrum disorder. It has proven to be a precious tool for preclinical research and therapeutic development and has significantly contributed to our understanding of these conditions. (29, 30).

The model has been used to test pharmacological interventions and behavioral therapies aimed at ameliorating FXS and ASD-like symptoms.

The Fmr1 knockout mouse model has proven invaluable in advancing our understanding of the underlying pathophysiological mechanisms involved in both FXS and ASD. Extensive research has uncovered potential targets for therapeutic intervention in FXS and related neurodevelopmental disorders. Furthermore, Fmr1 knockout mice are utilized to assess the efficacy of treatments and therapies for FXS and ASD. Using these mice, we have gained

valuable insights into synaptic dysfunction, molecular signaling pathways, and gene-environment interactions associated with ASD (29, 30).

The Shank3 knockout mouse model plays an integral role in scientific research focused on ASD and similar neurodevelopmental conditions. This model is developed by genetically removing or altering the Shank3 gene in mice, leading to the absence or loss of function of the SHANK3 protein. The Shank3 gene encodes the SHANK3 protein, a crucial component of the postsynaptic density (PSD) in excitatory synapses. Deletions or mutations in the Shank3 gene have been correlated with Phelan-McDermid syndrome (PMS), a rare genetic disorder that is linked to intellectual disability, developmental delay, and ASD (32, 33, 34). The Shank3 knockout mouse model is precisely engineered to replicate the genetic mutations seen in those diagnosed with PMS. It is a valuable tool for researchers to explore the impact of Shank3 deficiency on synaptic physiology, brain function, and behavior. This model is instrumental in the study of the pathophysiological mechanisms linked to ASD and other related neurodevelopmental disorders. (32, 33, 34).

The Shank3 knockout mouse model is designed to mimic the genetic alterations observed in individuals with PMS, which enables researchers to investigate the effects of Shank3 deficiency on brain function, behavior, and synaptic physiology. This model is beneficial in studying the pathophysiological mechanisms underlying ASD and related neurodevelopmental disorders (32).

It is often observed that Shank3 knockout mice exhibit behavioral, cognitive, and neurobiological abnormalities akin to neurodevelopmental disorders, including ASD. These mice often show deficits in social interaction, communication, and repetitive behaviors, as well as impairments in learning and memory. On the neurobiological front, alterations in synaptic function, dendritic spine morphology, neurotransmitter systems (especially glutamatergic signaling), and brain development in regions involved in social behavior and cognition are commonly observed in Shank3 knockout mice.
The utilization of the Shank3 knockout mouse model in research studies has played a crucial role in identifying promising therapeutic targets for PMS and other related neurodevelopmental disorders. This model has been utilized to evaluate various pharmacological interventions, gene therapy approaches, and behavioral therapies aimed at reducing Shank3 deficiency and mitigating ASD-like symptoms. Additionally, research

conducted with Shank3 knockout mice has yielded valuable information on synaptic dysfunction, molecular signaling pathways, and gene-environment interactions contributing to ASD (32, 33, 34).

In conclusion, the Shank3 knockout mouse model is valuable in comprehending the biological and behavioral traits of both Phelan-McDermid syndrome and ASD. This understanding can lead to the development of innovative therapeutic approaches for these conditions, ultimately improving the quality of life for those affected by neurodevelopmental disorders. The model holds the potential to advance our knowledge in this area significantly (32).

The VPA (valproic acid) - induced rodent model is a well-established method in autism research. It entails administering VPA, a medication typically used to manage epilepsy and bipolar disorder, to pregnant rodents at a critical point in their embryonic development. Researchers then study the effects of VPA on the offspring, offering valuable insights into the condition of autism. (35).
It has been observed that VPA can interfere with natural neurodevelopmental processes if consumed during crucial stages of embryonic development. Studies on rodents have indicated that exposure to VPA during gestation can result in behavioral, neuroanatomical, and neurochemical abnormalities in their offspring, which bear similarities to specific characteristics of ASD in humans (35, 36).

Children who are exposed to VPA before birth frequently display behavioral traits that mirror the primary symptoms of ASD. These traits may include difficulties with social interaction, communication, repetitive behavior, and cognitive challenges. Additionally, offspring exposed to VPA in utero may exhibit neuroanatomical abnormalities, such as changes in brain volume, abnormal cell proliferation, and disruptions in synaptic connectivity. Furthermore, VPA-induced rodent models of autism suggest that there may be neurochemical imbalances, including disruptions in neurotransmitter systems like glutamate and Gamma-Aminobutyric Acid (GABA) (35, 36, 37).

The VPA-induced rodent model of autism has gained widespread use in the study of the complex mechanisms behind ASD. By examining potential genetic, neurobiological, and environmental factors that may contribute to the disorder, researchers can gain valuable

insights into the underlying causes of ASD. This model is beneficial in exploring the effects of prenatal VPA exposure on brain development, behavior, and gene expression patterns in offspring. Furthermore, it serves as a valuable tool in testing potential therapeutic interventions for ASD, including pharmacological agents, behavioral therapies, and environmental interventions designed to alleviate ASD-like symptoms (36, 37).
Although the VPA-induced rodent model of autism has yielded insightful information regarding the origins and mechanisms of ASD, it is crucial to recognize its limitations. The model fails to entirely replicate the intricate and diverse nature of ASD observed in humans, and it is imperative to exercise caution when applying the findings to clinical situations. Despite this, the model continues to be an advantageous resource for examining aspects of ASD and evaluating potential treatments (38).

### 4.3 Songbird model

Songbirds, focusing on zebra finches, are a valuable research tool for studying social behavior, communication, and vocal learning in ASD research. Although songbirds do not exhibit ASD-like symptoms in their natural state, their capacity to learn and produce intricate vocalizations resembles the development of human speech and language. Below is an introduction to the songbird model regarding autism research (38,39,40).

Zebra finches can learn intricate vocalizations by imitating sounds, like how humans acquire language. The neural circuits and genetic mechanisms involved in vocal learning of songbirds share resemblances with those responsible for human speech development (38).

Songbirds are known for their diverse social behaviors, including courtship displays, mate selection, and parental care. Additionally, they rely heavily on vocalizations for communication and social interactions within their social groups. These behaviors highlight the complex social dynamics within avian communities and the importance of vocal communication in facilitating these interactions. Understanding these social behaviors and the role of vocalizations in social communication can provide valuable insights into the evolutionary and ecological processes that have shaped the behavior of these fascinating birds (38, 39, 40).

Researchers can now use genetic tools to modify specific genes in songbirds, enabling them to study the effects of genetic changes on vocal learning, social behavior, and brain development (39).

The neural circuitry involved in vocal learning among songbirds is well-characterized, featuring specialized brain regions like the song control nuclei. By delving into the structure and function of these areas in songbirds, researchers can glean valuable insights into the neurobiology of vocal communication and its implications for ASD (39).

Although songbirds do not typically display behaviors like those seen in individuals with ASD, scientists have employed various experimental techniques, including early life encounters, medication interventions, and genetic alterations, to induce ASD-like traits in these birds. The intention behind these investigations is to replicate specific characteristics of ASD, such as social impairments, repetitive actions, and abnormalities in sensory processing (38, 39, 40).

Utilizing the songbird model holds promise in evaluating potential therapeutic interventions for ASD. This may include pharmacological agents, behavioral therapies, and neural stimulation techniques. By examining the effects of these interventions on vocal learning, social behavior, and brain function in songbirds, researchers can gain valuable insight into their efficacy and safety (38,39,40).

Although the songbird model has limitations in autism research, such as variations in brain structure and complexity compared to humans, it provides distinct benefits for studying vocal learning, social behavior, and communication. Through its integration with other animal models and human studies, the songbird model enriches our comprehension of the biological and behavioral mechanisms that underlie ASD and holds promise for creating innovative interventions and treatments (38).

### 4.4 Dog model

The domestic dog, scientifically known as Canis familiaris, has become a popular subject for comparative research in various fields such as genetics, molecular biology, social

cognition, and psychology. This is mainly because of the dog's unique evolutionary history and adaptation to a similar social environment, resulting in significant socio-cognitive changes. Moreover, dogs are now considered suitable models for age-related cognitive impairments, neurological and non-neurological diseases, and obesity. They share causative genes with humans and respond similarly to treatments for human diseases. Dogs are ideal models for comparative and translational neuroscience. They surpass commonly used laboratory animals like rats and mice based on their adaptation to human-made environments, providing higher face and construct validity.

Various dog breeds, including beagles and retrievers, have been examined as possible animal models for ASD due to their social behavior, communication abilities, and genetic similarities to humans. Canine models provide researchers with a more ecologically relevant framework to investigate the genetic and neural mechanisms underlying ASD-like behaviors (43, 44).

Therapy dogs mainly, have been utilized in interventions for individuals with ASD to supply emotional assistance, encourage social interaction, and mitigate anxiety and stress. Interacting with dogs may help individuals with ASD enhance their social skills, communication, and emotional regulation (44).

Animal-assisted therapy programs involving dogs have been tailored to meet the specific needs and goals of people with ASD. Trained therapy dogs can work alongside therapists to create structured interventions tailored to the individual's unique strengths and challenges (27).

While research on the effectiveness of animal-assisted interventions for individuals with ASD is ongoing, early studies have reported positive outcomes in areas such as social interaction, emotional regulation, and stress reduction. More research is needed to understand the mechanisms behind these benefits better (27).

## 5. Beagle dog´s model of autism

### 5.1 Beagle dog as animal model in autism research

Choosing suitable animal models to study a specific human condition is crucial yet challenging. The challenge lies in identifying animal behaviors that closely resemble and reflect the intricate human symptoms and have similar biological and etiological mechanisms. This is commonly referred to as construct or translational validity. Moreover, these animal models must demonstrate treatment responses comparable to those seen in humans, known as predictive validity.

Over the past few years, there has been an increasing fascination with utilizing domestic dogs (Canis familiaris) as a comparative and translational neuroscience model, specifically emphasizing autism research. The canine model is an appealing alternative because of its complex and compelling dog-human social interactions.

This is mainly because dogs possess numerous genetic, behavioral, and brain structure similarities to humans, making them exceptional for scrutinizing intricate neurobiological mechanisms contributing to various human behavior and cognition facets.

The utilization of canine models in scientific research is a precious approach due to their exceptional social cognitive abilities that display a broad spectrum of variability among individuals. In comparison to rodents, dogs exhibit more notable phenotypic similarities, rendering them functionally analogous to the human condition and more likely to share a similar etiology (44).

Dogs are being increasingly recognized as a valuable model for studying age-related cognitive impairments, various neurological disorders such as Alzheimer's disease, and non-neurological diseases like diabetes and obesity. Moreover, canine epilepsy has been proposed as a promising comparative model for human epilepsy. Furthermore, canine and human diseases often share phenotypic manifestations and causative genes and respond to treatments similarly.

Furthermore, dogs possess comparable drug metabolism to humans and a high reproduction rate, making them an ideal model for studying various human health conditions and developing novel treatments. As such, the use of dogs in scientific research has become increasingly popular in academia and industry, providing researchers with a valuable tool

for investigating the biological mechanisms of disease and developing new therapies (27, 44).

Traditional rodent models have long been a valuable tool in experimental research, particularly in the study of ASD. These models have generated a wealth of data, providing researchers with valuable insights into the molecular genetics of ASD. By simulating core features of human autism in mice, these models have allowed researchers to understand the disorder's underlying mechanisms better (31).

The study of genetic mutant mice has significantly contributed to our understanding of the molecular and neural circuit mechanisms that underlie ASD. By studying the genetic mutations in mice that are like those found in humans with ASD, researchers have identified specific genes and neural circuits that may contribute to the development of the disorder. However, while these mouse models have helped advance our knowledge of ASD, they have limitations in preclinical studies (31).

The notable contrast between human and rodent social behavior and structure is a significant issue. Rats and mice cannot mimic the intricacy of human social behavior, which increases the likelihood of oversimplified comparisons between human and rodent conduct.

This is because rodents and humans have observable differences in brain and behavior, making it challenging to accurately translate findings from mouse models to humans.

Therefore, the applicability of ASD mouse models in preclinical studies is restricted to specific aspects of the disease, and researchers must use caution when interpreting their results.

The study of several characteristics of ASD that require a higher level of social-cognitive functioning is challenging or infeasible in rodents. In addition, inducing a broad range of ASD symptoms that occur spontaneously in humans is a prerequisite when studying these animals. Although rodent models of ASD can drive our comprehension of fundamental behavioral regulatory mechanisms, the contrasting nature of human and rodent social cognition (i.e., the set of mental operations and social-communicative signals used in social interactions with others) substantially limits the impact of rodent models on elucidating the intricate social cognitive aspects of human ASD (41, 44).

Recent research indicates that despite the evolutionary differences between dogs and humans, domestic dogs use eye contact and gaze similarly to humans, particularly infants, in interspecies situations. Additionally, dogs can be studied in their natural environment and tested in less restrictive conditions than nonhuman primates, mainly in captivity or semi-

natural conditions. For instance, eye-tracking experiments with head-unrestrained dogs have been conducted. Furthermore, the experimental paradigms used to investigate dogs' social skills are well-suited and even identical to those used to evaluate children's social skills. This unique positioning of the dog model is significant in bridging the gap between nonhuman primate models and human ASD populations.

When considering the use of a dog model to study human ASD symptoms across the developmental spectrum, it is essential to consider certain factors. Retaining juvenile and puppy-like behavioral traits into adulthood resulting from dog domestication suggests that dogs' cognitive developmental level is more like that of children than adults. Hence, the dog may be a viable model for children with ASD. However, it must be kept in mind that human ASD is a lifelong condition, and diagnosis tends to remain stable over time, although there may be improvement in symptoms with age, according to longitudinal studies. Therefore, while the dog may be suitable for studying ASD symptoms primarily in children, it may not be a comprehensive model for all ages (41, 44).

### 5.2 Shank3 gene models

The SHANK3 protein is vital in building and maintaining the connections between nerve cells, also known as synapses, in the brain. It is primarily located in the postsynaptic density of excitatory synapses and is critical in their formation, maturation, and stability. However, if the SHANK3 gene malfunctions, it can result in Phelan-McDermid syndrome or 22q13.3 deletion syndrome, which can lead to severe developmental delays, autistic behavior, expressive language, speech delay, and hypotonia (43).

Recent research has identified several SHANK3 mutations in patients with autism spectrum disorder, suggesting that SHANK3 plays a significant role in the development of ASD. The SHANK3 gene comprises five unique CpG-islands, and its tissue-specific expression is controlled by deoxyribonucleic acid (DNA) methylation in an epigenetic manner. Rodent studies have found various SHANK3 variants, and their expression is regulated by DNA methylation of intragenic promoters. Based on cumulative evidence, it appears that SHANK3 variants and their epigenetic regulation play a critical role in the pathogenesis and neuropathology of ASD (43).

The canine species are excellent subjects studying social cognition as they strongly bond with humans and display comparable social behaviors. Utilizing the CRISPR/Cas9 editing process, a dog model has been developed successfully with Shank3 mutations, which showcases a spectrum of autism-like tendencies such as social limitations and heightened anxiety.

CRISPR/Cas9 is a new technology that allows scientists to change DNA in living things. It has two parts: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), part of bacterial DNA, and Cas9, an enzyme that can cut DNA. Scientists use a small ribonucleic acid (RNA) molecule called a guide RNA to help Cas9 cut the DNA in the right place. This technology can make specific changes to DNA, like adding new DNA or changing how particular genes work.
CRISPR/Cas9 is very useful for studying how genes work and finding new ways to treat diseases caused by genetic mistakes. It is easy to use and very precise.

Researchers have been exploring the potential of creating a canine model for studying ASD by manipulating the Shank3 gene, which is one of the most found genetic defects in ASD patients. Using the CRISPR/Cas9 gene editing technique, scientists have been able to produce multiple lines of Beagle Shank3 (bShank3) mutants, which have been propagated for a few generations. A battery of behavioral assays was developed and used in controlled experimental settings for the mutant dogs to validate this approach. The study results indicate that bShank3 mutants exhibit apparent social behavior deficits, including a tendency to withdraw from social interactions and a reduced level of interaction with humans. They also show heightened anxiety in different experimental settings. The study demonstrates the feasibility of producing many mutant animals in a reasonable time frame, and the unique behavioral findings support the validity and value of using a canine model to investigate the pathophysiology of ASD and potentially other psychiatric disorders (44 - 50).

A research study utilized Shank3 mutant dog models to explore potential connections between Shank3 mutations and neuronal dysfunction. Using acute brain slices, scientists investigated the electrophysiological properties and synaptic transmission of pyramidal neurons in the prefrontal cortex (PFC). Biocytin and Golgi staining were utilized to examine dendrite elaboration and dendritic spine morphology in the PFC. Additionally, electron microscopy was used to investigate postsynaptic density.

The results established a protocol for electrophysiological recording of canine brain slices. They showed that excitatory synaptic transmission onto PFC layer 2/3 pyramidal neurons in Shank3 heterozygote dogs was impaired, along with reduced dendrite complexity and spine density compared to wild-type dogs. Postsynaptic density structures were also damaged in Shank3 mutants, but pyramidal neurons demonstrated hyperexcitability.
The study's limitations include the unclear causal links between impaired PFC pyramidal neuron function and behavioral alterations. Further experiments, such as manipulating PFC neuronal activity or restoring synaptic transmission in Shank3 mutant dogs, are necessary to evaluate PFC roles in altered social behaviors.
In conclusion, this study demonstrated the feasibility of utilizing canine brain slices as a model for studying neuronal circuitry and disease. Shank3 haploinsufficiency causes morphological and functional abnormalities in PFC pyramidal neurons, supporting the notion that Shank3 mutant dogs are new and valid animal models for autism research (47).

Autism spectrum disorder patients have been found to have an increased ability to distinguish low-level features of auditory stimuli and mutations in the SHANK3 gene, which encodes a synaptic scaffolding protein. However, it is currently unknown whether these SHANK3 mutations result in heightened neural processing of low-level auditory features. To investigate this possibility, a study was conducted on dogs with Shank3 mutations, examining the effects of these mutations on the early neural processing of pitch. Electroencephalograms were recorded while the dogs were exposed to deviant tones of varying frequencies and probabilities and other tones in a repetitive stream. The results showed that Shank3 mutant dogs exhibited larger amplitudes of early neural responses to deviant tones and a greater sensitivity to variations of anomalous frequencies within 100ms of tone onsets, independent of the probability of unnatural tones. These findings demonstrate the critical role of Shank3 in modulating early neural detection of novel sounds and provide new insights into the genetic basis of atypical auditory information processing in autism patients (48).

The pupillary response is a crucial process that plays a significant role in visual perception, social interaction, and emotional cognition. It has been extensively studied to better understand the neural mechanisms of neuropsychiatric disorders. However, there has been a significant lack of research on pupil response to social and non-social stimuli in animal

models of neurodevelopmental disorders like autism spectrum disorder and attention deficit hyperactivity disorder.

To solve this problem, a team of researchers has developed a pupilometer that utilizes a robust eye feature-detection algorithm to facilitate real-time pupillometry in dogs. A pilot study discovered that a brief light flash induced a less-pronounced and slower pupil dilation response in gene-edited dogs carrying mutations in Shank3, which is the ortholog of the human gene repeatedly identified in ASD patients.

Further studies revealed that obnoxious, loud firecracker sounds of up to 120 dB induced a more vital and extended pupil dilation response in Shank3 mutant dogs. In contrast, high-reward food caused a weaker pupillary response in Shank3 mutants than in wild-type control dogs. Additionally, it was found that Shank3 mutants showed compromised pupillary synchrony during dog-human interaction.

These findings of altered pupil response in Shank3 mutant dogs provide a basis for further understanding the underlying neural mechanisms of ASD and other psychiatric disorders. They also demonstrate the validity and value of the pupilometer for dogs, providing a practical paradigm for studying these conditions in animal models (49).

### 5.3 Stem cells from human exfoliated deciduous teeth

Stem cells from human exfoliated deciduous teeth (SHED) are a type of mesenchymal stem cell (MSC) that can be isolated from the dental pulp of deciduous teeth that fall out naturally. SHED shares many characteristics with other types of MSCs, including the ability to self-renew and differentiate into multiple cell lineages. These stem cells have shown great promise in various regenerative medicine applications, especially in treating neurological disorders and injuries (51, 52).

Stem cells from human exfoliated deciduous teeth have been used to treat a range of neuroinflammation-related conditions, including spinal cord injury, hypoxic-ischemic brain injury, and experimental autoimmune encephalomyelitis through their neuro-regenerative, anti-inflammatory, and immunomodulatory activities. These remarkable cells can restore damaged tissues and regenerate lost cells, significantly improving the patient's condition (51, 52).

Research into the clinical applications of SHED is ongoing, and continued research may lead to new and more effective treatments for a range of medical conditions. One such condition is ASD, a group of developmental disabilities characterized by impairments in social interaction and communication. Mesenchymal stem cell-based therapy has emerged as a promising approach to treating individuals with ASD. Stem cells from human exfoliated deciduous teeth have immune-modulatory and anti-inflammatory properties and have been found to hold earlier-developing characteristics, making them an ideal candidate for treating ASD.

A study was conducted on SHANK3 mutant beagle dogs to explore the potential of SHED transplantation as a therapy for ASD. The dogs were evaluated based on social interaction tests and randomly assigned to two groups. Six mutant dogs received six intravenous infusions of SHED and were monitored for three months to test social interaction and inflammatory cytokine levels (51, 52).

The study found that SHED infusion significantly improved the impaired social novel preference of SHANK3 mutant beagle dogs at 1- and 3-month follow-ups. The infusion also partially enhanced social interaction between the mutant dogs and human experimenters, including following, sniffing, and licking. Additionally, the study showed that stressed tail posture, which indicates social stress, was significantly alleviated (51, 52).

The infusion of SHED was able to rescue altered interferon-γ and interleukin-10 levels, indicating its potential as a safe and effective therapy for ASD. No allergic immune responses or other serious adverse events were observed during the period of SHED transplantation and follow-up visits (51, 52).

Stem cells from human exfoliated deciduous teeth offer attractive advantages over other sources of postnatal stem cells as they can be easily harvested through non-invasive surgical procedures. In SHED-treated mutant beagle dogs, some indicators changed, such as a significant increase in γ-glutamyl transpeptidase levels and lower phosphorus, calcium, and alkaline phosphatase levels compared to pre-treatment. However, there were no deviations outside of reference ranges in the complete blood count/biochemical examination before and after treatment. No serious adverse events were observed after the infusion of SHED (51, 52).

Recent research has shown that the transplantation of Stem Cells from Human Exfoliated Deciduous Teeth could hold great promise as a therapy for ASD. In an experimental study,

SHED transplantation was found to be effective in improving social behavior and reducing stress in dogs that had a genetic mutation in the SHANK3 gene, which resulted in behaviors similar to the core features of ASD observed in humans. The study also showed that the levels of serum interferon-γ and interleukin-10 in the dogs could be used to predict the severity of autism and the response to the therapy. These findings provide a valuable foundation for further research and development of treatment options for individuals with ASD and offer a glimmer of hope for those affected by this complex neurodevelopmental disorder (51, 52).

## 6. Conclusion

ASD is a complex and heterogeneous neurological condition that affects an individual's ability to communicate, socialize, and behave appropriately. As the name suggests, the symptoms and severity of ASD vary widely across individuals and are often referred to as a spectrum disorder. The condition can be diagnosed at any age, but it typically manifests in early childhood before age three. The symptoms of ASD can range from mild to severe and may include difficulties in verbal and non-verbal communication, repetitive behaviors, and difficulty with social interactions.

There are different variations of ASD. Kanner Syndrome, also known as classical autism, is distinguished by irregularities in social interaction and motor function. Atypical autism, on the other hand, is characterized by the fact that not all classic symptoms are present, and the initial signs appear later in life, typically after 12 to 24 months of age. A typical range of intellect characterizes Asperger Syndrome but may include motor impairments.

Researchers have taken various approaches to identify the causes of ASD. Genetic and environmental factors have been identified as key contributors to this condition. One approach that holds particular significance is the study of submicroscopic changes in chromosomes that are linked to ASD.

There are many effective methods to improve the quality of life for individuals with ASD. One such approach is canine therapy, which has shown promising results. It is essential to

acknowledge that ASD is a multifaceted condition and can only be treated by qualified professionals after careful consideration.

Animal models have proven to be very valuable in autism research. Scientists are manipulating genes to better understand the pathological background of autism and to explore possible treatments. Of course, ethical guidelines and animal welfare regulations must be strictly observed during experiments with animals. The use of rodent models is well-established, as is the use of songbirds. However, dogs are an increasingly popular choice due to their ability to adapt to human behavior and their evolutionary history. Dogs share causative genes with humans and respond similarly to treatments for human diseases. Therapy dogs have been used to provide social assistance, encourage interaction, and reduce anxiety and stress for individuals with ASD. Interacting with dogs may help people with ASD improve their social skills, communication, and emotional regulation. The use of beagle dog models has grown in importance in recent years. Canine models offer a unique opportunity to study complex dog-human social interactions. Dogs possess numerous genetic, behavioral, and brain structure similarities to humans and are, therefore, especially useful for studying the intricate neurobiological mechanisms contributing to various human behaviors and cognitive facets. Unlike rodents, dogs exhibit more notable phenotypic similarities, making them functionally analogous to the human condition and more likely to share a similar etiology.

Rodent models have long been valuable for experimental research, especially in autism research. Studies of genetic mutant mice have dramatically improved our understanding of the molecular and neural mechanisms underlying ASD.
However, a significant challenge arises from the striking differences between human and rodent social behavior and structure. Rats and mice cannot replicate the complexity of human social behavior, which raises the risk of oversimplifying comparisons between human and rodent behavior.

The usage of the shank3 gene dog model is a promising path in autism research. Stem cells extracted from human exfoliated deciduous teeth have demonstrated neuro-regenerative, anti-inflammatory, and immunomodulatory properties, making them a potential treatment for neuroinflammation-related conditions. Recent studies have shown that transplanting these stem cells may hold great promise for ASD therapy. Dogs that received this treatment

showed better behavior. In addition, serum levels of interferon-γ and interleukin-10 in dogs can be used to assess the severity of autism and predict response to therapy. These exciting findings offer a valuable foundation for further research and the development of effective treatment options for individuals with ASD.

In conclusion, the author of the thesis claims that the information presented has been thoroughly investigated. However, it is imperative that more veterinarians and human physicians are motivated to conduct further studies in this field to promote the use of canine models in autism research.

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## 8. Figure Bibliography

Figure 1: M. Brodmann (2016). Gyrus cinguli. https:// Gray727_cingulate_gyrus.png

## 9. Acknowledgments

I would like to thank my supervisor, Dr. Andrea Heinzlmann, for her support and advice.
I am grateful to Prof. Dr. Péter Sótonyi, the current head of the department, for allowing me to write my thesis at the department.
I am grateful to all the employees of the Department of Anatomy and Histology for the help I received from them.
Finally, I would like to thank my loved ones for their support and help in putting the pieces together, especially Jennifer Servos, who corrected this thesis grammatically.

## 10. Thesis Topic Declaration Form

**Appendix 1.** Thesis topic declaration form

University of Veterinary Medicine Student name: Sebastian Paus

THESIS TOPIC DECLARATION FORM

I hereby request approval from the Head of Department of the Department of/and.....
Anatomy and Histology
.....

to prepare a thesis based on a topic announced and supervised by said Department as follows.

Date: Budapest, 13.09.2022 S. Paus
Student signature

Thesis topic:

Autism spectrum disorder (ASD) is a developmental disability caused by differences in the brain. Canine model is an appealing alternative because of its complex and effective dog-human social interactions. On the base of the literature I would like to introduce the beagle dogs as an animal model to investigate the pathophysiology and develop treatments for ASD.

Title of Thesis (English title as well):

Beagle dog's model used in investigation of autism

.....

.....

Supervisor signature:

Andrea Gerencsér

Approved by:

Head of Department signature

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# 11. Copyright Declaration

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