Investigation of an inherited cerebellar malformation in the Eurasier breed dog

von Filipa de Faria Pedro Bernardino

Inaugural-Dissertation zur Erlangung der Doktorwürde
der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität
München

Investigation of an inherited cerebellar malformation in the Eurasier breed dog

von Filipa de Faria Pedro Bernardino

aus Guimarães, Portugal

München 2015

Aus dem Zentrum für Klinische Tiermedizin der Tierärztlichen Fakultät
der Ludwig-Maximilians-Universität München

Lehrstuhl: Innere Medizin der kleinen Haustiere und Heimtiere

Arbeit angefertigt unter der Leitung von: apl. Prof. Andrea Fischer

Gedruckt mit Genehmigung der Tierärztlichen Fakultät
der Ludwig-Maximilians-Universität München

Dekan: Univ.-Prof. Dr. Joachim Braun

Berichterstatter: Prof. Dr. Andrea Fischer

Korreferent: Univ.-Prof. Dr. Dr. Habil. Joris Peters

Tag der Promotion: 18. Juli 2015

For my beloved family.

"Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning."

Albert Einstein

TABLE OF CONTENTS

TABLE OF ABBREVIATIONS

AMA	Anterior membranous area
CAMRQ	Cerebellar ataxia, mental retardation and dysequilibrium syndrome
CA8	Carbonic anhydrase gene 8
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CVH	Cerebellar vermian hypoplasia
DES	Dysequilibrium syndrome
DNA	Deoxyribonucleic acid
DWLM	Dandy-Walker like malformation
DWM	Dandy-Walker malformation
DWV	Dandy-Walker variant
e.g.	Example given
FLAIR	Fluid attenuated inversion recovery
GL	Granular layer
GLC	Granular layer cells
MRI	Magnetic resonance imaging
PC	Purkinje cells
PCL	Purkinje cell layer
PMA	Posterior membranous area
VLDLR	Very low density lipoprotein receptor
VLDLR-CH	Very low density lipoprotein receptor associated cerebellar hypoplasia

WDR81	WD repeated domain gene 81

I. INTRODUCTION

Cerebellar malformations can be inherited or acquired from insults during cerebellar development. Viral infection of the developing cerebellum is the most frequently documented etiologic factor for cerebellar malformations in the veterinary literature, but is much more commonly reported in cats and only rarely in dogs (SCHATZBERG et al., 2003; RÉSIBOIS et al., 2007). There are only rare case reports in the veterinary literature which indicate that cerebellar hypoplasia may occur as an inherited disorder in dogs. These are mainly observations in three dog breeds: wire-haired Fox terriers, Irish setters and Chow-Chows (PALMER et al., 1973; KNECHT et al., 1979; DE LAHUNTA & GLASS, 2009). Anecdotal reports have described sporadic cases of vermis hypoplasia/agenesis with or without associated focal or generalized hypoplasia of the cerebellar hemispheres in dogs and the presence of a Dandy-Walker malformation was frequently discussed in these reports (KORNEGAY, 1986; DOW, 1940; SCHMID et al., 1992; NOUREDDINE et al., 2004; CHOI et al., 2007; LIM et al., 2008; SCHMIDT et al., 2008; DE LAHUNTA & GLASS, 2009; KOBATAKE et al., 2013).

In humans a classic DWM is defined as (1) complete or partial agenesis of the cerebellar vermis with an upward displacement and rotation of the remnants of the vermis, (2) cyst-like dilation of the fourth ventricle, and (3) enlargement of the posterior fossa, with upward displacement of the tentorium cerebelli osseum, transverse sinuses, and torcula. Hydrocephalus may occur in up to 80% (DANDY & BLACKFAN, 1914; TAGGART & WALKER, 1942; BENDA, 1954; SHEKDAR, 2011; SPENNATO et al., 2011). In many human cases, no substantial enlargement of the posterior fossa is observed, which has led to the introduction of the term “Dandy-Walker variant” (PATEL & BARKOVICH, 2002; SPENNATO et al., 2011); however no consensus on this terminology exists in the human literature, and the use of this term has been discouraged (NIESEN, 2002; PARISI & DOBYNS, 2003; BOLDUC & LIMPEROPOULOS, 2009; MALINGER et al., 2009; GAREL et al., 2011; SHEKDAR, 2011; SPENNATO et al., 2011). Recent evidence rather suggests that DWM and related malformations, such as inferior vermian hypoplasia and mega cisterna magna, may represent a continuum and a classification system based on embryonic development and

genotype has been proposed (BARKOVICH et al., 2009; BARKOVICH, 2012).

Therefore, the aim of the present investigation was to describe the clinical phenotype and imaging findings of a familial non-progressive ataxia which was reported in purebred Eurasier dogs, and to investigate the relationship between the dogs. An inferior cerebellar hypoplasia with some resemblance to a Dandy-Walker-like malformation (DWLM) was consistently identified in the affected dogs. We proposed autosomal recessive inheritance and a VLDLR gene mutation was recently identified based on the data provided in this investigation (GERBER et al., 2015).

II. REVIEW OF THE LITERATURE

1. The history of the Eurasier Breed

The Eurasier dog breed is quite a recent breed. It started with the crossbreeding of two ancient breeds: the Chow-Chow from Asia and the European Wolfsspitz. The idea came from Julius Wipfel, with the goal of creating "a polar dog with beautiful and attractive colours and a strong, but at the same time charming temperament". The first generation of the breed, at the time still called "Wolf-Chow", was born on the 22nd of June in 1960. The name of the breed "Eurasier" was introduced only in 1973. One of the most important litters, the "B-litter vom Jägerhof", was born on the 17th of December in 1962. Today we can state that there are no "true" Eurasiers that are not traced back to these two dogs. This litter and its relatives were continually crossed with a high level of inbreeding until the fifth generation. For instance, one stud "Droll vom Jägerhof" was used almost as the only stud dog in the breeding scheme from 1967 until 1971. The last generation from this stud dog had what was considered the desirable phenotype and temperament for the breed. With the inbreeding, it was assumed that more than 90% of all dogs available for breeding could be traced back to the first generation of Jägerhof-blood. Consequently, measures to increase the gene pool had to be established and outcrosses were introduced. First, a new breed, although without consensus between breeders, was introduced into the breeding-program in 1972: the Samoyed. Second, also in 1972, a new Chow-Chow female and a new Wolfsspitz male were crossed and also introduced into the breeding program. And third, a new Chow-Chow stud dog was introduced and crossed with the first generation after the introduction of the Samoyed breed. Later, between 1980 and 2003, several dogs from foundation breeds as well as some "Eurasiers" out of independent breeding programs were introduced. Also in the 80's, a completely new line of "Wolf-Chow" dogs was started in the "Unland"-kennel. The latest outcrossings to a Wolfsspitz and a Samoyed were done in 2012, so the total number of founders of the breed is up to roughly 27 dogs (FEDER, 2000).

In purebred dogs, a desirable ancestor is usually identified and descendants are often repeatedly bred together. Thus this common ancestor appears several times on both maternal and paternal sides for several generations in the pedigree,

causing a small and decreasing gene pool within the several breeds (RUSBRIDGE et al., 2005). The Eurasier breed dog is not an exception, with a stud being used almost exclusively for several generations in the breeding program (FEDER, 2000). Once a breeding line is established, it continues generation after generation, with no or few occasional crosses with dogs without a common ancestor within several generations in the pedigree, these are called "outcrosses" (RUSBRIDGE et al., 2005).

This lack of outcrosses creates a strong familial relationship within purebred dog populations, leading to the accumulation of certain inherited diseases in nearly each breed and to the eradication of other diseases, as a result many inherited diseases occur in a breed-specific manner (WANG & ZOGHBI, 2001). Inherited diseases appear as a consequence of random mutations in one founder animal and they occur from generation to generation, whenever this founder animal is used in the breeding program (MELLERSCH, 2014). For instance, in recessive mutations, clinically affected animals only appear when inbreeding occurs and the affected dog inherits an identical copy of the mutation from both parents (MELLERSCH, 2014).

Recognition of a genetic background of a disease is therefore often possible in dogs but difficult in other species (WANG & ZOGHBI, 2001). Since the canine genoma was completely sequenced in 2004, the methods available to dissect the origin of the inherited traits in dogs have become both increasingly sophisticated and cost effective (MELLERSCH, 2014).

2. The caudal fossa: the cerebellum and the fourth ventricle

Understanding the embryologic development of the cerebellum and the fourth ventricle is essential to understanding caudal fossa malformations, including Dandy-Walker malformation (DE LAHUNTA & GLASS, 2009; SHEKDAR, 2011).

2.1. The development of the cerebellum

Cerebellar development occurs in many distinct stages, which are controlled by a multiple genetic cascade (BIRAN et al., 2012). The structures of the brain develop from the neural tube. Fusion and closure of the neural tube in the cranial region form the three primary brain vesicles: forebrain (prosencephalon), midbrain

(mesencephalon) and the hindbrain (rhombencephalon) (DE LAHUNTA & GLASS, 2009; SHEKDAR, 2011). The rhombencephalon divides posteriorly into two secondary vesicles through a marked constriction called "isthmus rhombencephali", the dorsal vesicle being the metencephalon and the ventral the myelencephalon (DE LAHUNTA & GLASS, 2009; SHEKDAR, 2011). The cerebellum develops from the alar plate region located in the dorsal portion of the metencephalon, and the pons develops from the floor and lateral walls of the metencephalon (SHEKDAR, 2011). Each alar plate extends dorsally and medially in the roof plate, fusing to each other (SHEKDAR, 2011). This primary growth of each alar plate is called the rhombic lip and it consists of proliferating cells from the germinal layer adjacent to the fourth ventricle (SHEKDAR, 2011). The rhombic lips enlarge and fuse in the median plane, covering the rostral half of the fourth ventricle and overlapping the pons and the medulla (SHEKDAR, 2011). In humans the cerebellum develops rostrally to posteriorly, with the more rostral parts remaining in the midline and forming the cerebellar vermis, while the more posterior parts move laterally and form the cerebellar hemispheres (PARISI & DOBYNS, 2003). In humans, the cerebellar vermis (paleocerebellum) develops and reaches complete foliation by the fourth month of gestation, while the large cerebellar hemispheres (neocerebellum) develop completely 30-60 days later than the vermis (PARISI & DOBYNS, 2003).

The completely formed cerebellum is composed by the medulla, which is located centrally, surrounded by the cerebellar cortex. In the median cross-section the cerebellum resembles tree branches, giving rise to the name *arbor vitae*, which results from the folding of the cerebellar cortex into the medulla during development, the white matter remaining in the centre and the grey matter in the periphery (DE LAHUNTA & GLASS, 2009). The cerebellar cortex is formed by three layers, named from external to internal: 1) the molecular layer (ML) composed of granule neuronal axons (Stellate cells and Basket cells) and the dendritic zones of the Purkinje cells (PC); 2) the Purkinje cell layer (PCL) composed, as the name indicates, of PC; and 3) the granular layer (GL) composed of granule layer cells (GLC) and Golgi cells (DE LAHUNTA & GLASS, 2009). The total population of PC is well established before the fetus is born, while the GLC continue to divide and migrate until late in the gestation or even after birth in some species (DE LAHUNTA & GLASS, 2009). For example in the horse and

farm animals the GLC complete the population of the GL before birth, while in dogs and cats this division and migration can occur up until the 10th week after birth (DE LAHUNTA & GLASS, 2009) and in humans up until the 20th month of life (PARISI & DOBYNS, 2003). This degree of cerebellar development is directly correlated with the amount of motor function and coordination in the newborn (DE LAHUNTA & GLASS, 2009). For this reason newborn animals, such as the foal and other farm animals, walk coordinately immediately after birth, while the kitten, puppy and human baby require a longer period to be able to walk coordinately (DE LAHUNTA & GLASS, 2009). This long duration of development and maturation makes the cerebellum vulnerable to a wide spectrum of developmental malformations (ADAMSBAUM et al., 2005; GAREL et al., 2011).

2.2. The development of the fourth ventricle

The development of the fourth ventricle has been well studied in the mouse model (SHEKDAR, 2011). In this specie, as well as in humans, the middle portion of the roof of the fourth ventricle, which contains the choroid plexus, is initially connected anteriorly with the cerebellar structures by the anterior membranous area (AMA), and posteriorly with the medulla by the posterior membranous area (PMA) (SHEKDAR, 2011). The AMA normally disappears, being completely incorporated into the developing choroid plexus in the early fetal development (SHEKDAR, 2011). The PMA expands, causing a caudally protrusion of the fourth ventricle called Blake's pouch, that initially does not communicate with the surrounding subarachnoid space (SHEKDAR, 2011). The area of Blake's pouch connecting with the cerebellar vermis becomes progressively thicker, whereas the other areas surrounding it become progressively thinner, causing the permeabilization of the Blake's pouch and giving rise to the foramen of Magendie (FRIEDE, 1975; SHEKDAR, 2011). Hence, the communication between the cerebrospinal fluid (CSF) from the fourth ventricle with the subarachnoid space is made through the foramen of Magendie, located in the middle of the membranous roof of the fourth ventricle, and the foramen of Luschka, located in the lateral recesses of the fourth ventricle (FRIEDE, 1975). A study comparing the patency of these foramen in 27 vertebrate species demonstrated that the foramen of Luschka was present in all species, but the foramen of Magendie was absent in some species, such as the dog, cat, goat, rat, rabbit and mouse (FRIEDE, 1975).

2.3. The anatomy of the cerebellum

The cerebellum is divided into two areas, the vermis located centrally in the median region, and the hemispheres located laterally on each side of the vermis (DE LAHUNTA & GLASS, 2009). The cerebellum can be further divided into two parts: 1) the cerebellar body composed by the vermis and hemispheres, and 2) the small flocculonodular lobe located ventrally and near the center of the cerebellum, separated from the rest of the cerebellum by the uvulonodular fissure (DE LAHUNTA & GLASS, 2009). The cerebellar body is further divided into cranial and caudal lobes by the primary fissure. The cranial vermis is composed by the lingula, lobules centralis and culmen, and the caudal vermis is composed by the declive, folium, tuber, pyramis, nodulus and uvula, separated by the uvulonodular fissure. The fourth ventricle is located directly adjacent to the nodulus (DE LAHUNTA & GLASS, 2009). The left and right hemispheres are composed caudally to cranially by the ansiform lobule, dorsal paraflocculus, ventral paraflocculus and flocculus, and in the median caudal plane by the paramedian lobule. The flocculus connects on each side laterally by the peduncle flocculonodular with the nodulus (DE LAHUNTA & GLASS, 2009). Three cerebellar peduncles connect the cerebellum to the brainstem: the rostral peduncle, with mainly efferent processes to the mesencephalon, the middle peduncle, with afferent processes from the transverse fibers of the pons, and the caudal peduncle, with afferent processes from the spinal cord and medulla (DE LAHUNTA & GLASS, 2009). The cerebellar nuclei are located in the cerebellar medulla and are named from lateral to medial, lateral, interposital and fastigial cerebellar nuclei (DE LAHUNTA & GLASS, 2009).

These afferent pathways are mainly constituted by the climbing and mossy fibers, while the PC are the sole output (efferent) pathway from the cerebellar cortex (DE LAHUNTA & GLASS, 2009; BIRAN et al., 2012). The climbing fibers originate mainly from the inferior olivary nuclei and synapses directly with the dendrites of the PC (DE LAHUNTA & GLASS, 2009; BIRAN et al., 2012). Aspartate is the neurotransmitter released in these synapses (DE LAHUNTA & GLASS, 2009). The mossy fibers originate from various spinal cord, brain stem and deep cerebellar nuclei, and synapses indirectly the PC, through the relay cells, the GLC and parallel fibers (DE LAHUNTA & GLASS, 2009; BIRAN et al., 2012). Acetylcholine is the neurotransmitter released in these synapses (DE LAHUNTA

& GLASS, 2009). The afferent and efferent pathways of the cerebellum are resumed in Tables 1 and 2, respectively.

Table 1. The cerebellar afferent pathways (DE LAHUNTA & GLASS, 2009)

Cerebellar afferents	**General proprioception**	**Special proprioception**	**Special somatic (visual and auditory)**	**Upper motor neuron**
Origin	1. Spino-cerebellar tracts 2. Cuneo-cerebellar tracts	Vestibulo-cerebellar axons (vestibulo-cochlear nerve or vestibular nuclei)	1. Tectocerebellar axons 2. Visual and auditory areas of cerebral cortex	Brainstem nuclei: 1. Red, 2. Olivary, 3. Pontine; 4.Reticular formation
Cerebellar peduncle	1. Caudal and rostral (small number) 2. Caudal	Caudal	1. Rostral and 2. Middle contralateral	1. Rostral, 2. contra-lateral caudal, 3. middle contra-lateral, 4. caudal

Table 2. The cerebellar efferent pathways (DE LAHUNTA & GLASS, 2009)

Cerebellar efferents	**Purkinje cells**	**Neurons of the cerebellar nuclei**		
Origin	Flocculonodular lobe	Fastigial nucleus	Interposital nucleus	Lateral nucleus
Synapse	Vestibular nuclei	Vestibular nuclei, reticular formation	Red nucleus, reticular formation	Red nucleus, reticular formation, pallidum ventral nucleus of the thalamus
Cerebellar peduncle	Caudal	Rostral	rostral	Rostral

2.4. The cerebellar function

The cerebellum regulates motor function, the correct position of the head, the neck and the body, in rest and in motion, the muscular tonus and maintains the equilibrium (DE LAHUNTA & GLASS, 2009). During routine neurological examination, the motor function is the main cerebellar function evaluated (DE LAHUNTA & GLASS, 2009). However it is well known in humans the involvement of the cerebellum in sensory systems, learning and higher cognitive functions, and emotions (SCHMAHMANN & SHERMAN, 1998; PATEL & BARKOVICH, 2002; ADAMSBAUM et al., 2005; SCHMAHMANN & CAPLAN, 2006; DE LAHUNTA & GLASS, 2009; ECONOMOU & KATSETOS, 2012; DE SMET et al., 2013, CAN et al., 2014). The term "cerebellar cognitive affective syndrome" has been used to describe the non-motor functional deficits in human patients (SCHMAHMANN & SHERMAN, 1998). For this reason the cerebellum can be divided into three different functional areas according to their embryological development (DE LAHUNTA & GLASS, 2009; SHEKDAR, 2011) (Table 3). Functional areas are also distinguished according to the longitudinal areas of the cerebellar cortex and related nuclei (DE LAHUNTA & GLASS, 2009) (Table 4). Since primates have very skilled limb and hand movements, their lateral zone is extremely developed. This is also observed in a few species of exotic animals (DE LAHUNTA & GLASS, 2009).

Table 3. Functional areas of the cerebellum based on embryological development (DE LAHUNTA & GLASS, 2009; SHEKDAR, 2011)

Classification	**Embryological development**		
Functional area	Archicerebellum	Paleocerebellum	Neocerebellum
Anatomic structures	Flocculonodular lobe	Rostral cerebellar lobe and adjacent vermis	Caudal cerebellar lobe and adjacent vermis
Function	Vestibular functions	Spinal cord functions and postural tonus	Regulation of skilled motions

Table 4. Functional areas of the cerebellum according to the longitudinal areas of the cortex and related nuclei (DE LAHUNTA & GLASS, 2009)

Classification	**Longitudinal areas of the cortex and related nuclei**		
Functional area	Medial zone	Intermediate zone	Lateral zone
Anatomic structures	Vermis, fastigial nuclei	Paravermal cortex, interposital nuclei	Lateral portions of the hemispheres, lateral nuclei
Function	Postural tone and equilibrium of the entire body	Muscle and postural tone to regulate skilled movements	Regulation of skilled movements of the limbs

2.5. Clinical signs of cerebellar disease

Animals with cerebellar disease present with cerebellar ataxia, characterized by a hypermetric gait. In severe cases it can be associated with falling and rolling. In the resting posture a broad-base stance on the thoracic limbs, truncal ataxia, and tremors of the head and neck, as well as intention tremors during eating or drinking can be noticed (DE LAHUNTA & GLASS, 2009). Paradoxical vestibular disease may occur with involvement of the flocculus, the nodulus or the caudal cerebellar peduncle. This results in abnormal positional nystagmus, with inconsistency in the direction, which may also change with different positioning of the head, and head tilt (DE LAHUNTA & GLASS, 2009). In addition, deficits in the cranial nerves during neurological examination including an absence of the menace reaction may be noticed (DE LAHUNTA & GLASS, 2009). The menace reaction is a learned reaction in puppies and kittens, usually appearing after the 10^{th} to 12^{th} week after birth. It requires the peripheral and the central pathways of the visual system, which end up at the facial nuclei in the medulla, to be intact. Diffuse cerebellar disorders, especially those involving the interposital and lateral cerebellar nuclei, have been often related to cause absence of the menace reaction (DE LAHUNTA & GLASS, 2009).

In human patients as previously mentioned cerebellar disease is associated not only with motor functional deficits but also with cognitive, social and/or behavioral dysfunctions (SCHMAHMANN & CAPLAN, 2006; BIRAN et al., 2012; CAN et al., 2014). It may actually contribute to the long term cognitive,

language and behavioral dysfunctions seen in 25 to 50% of preterm infants (SCHMAHMANN & CAPLAN, 2006; BIRAN et al., 2012). These dysfunctions may also occur in the absence of typical motor functional deficits (SCHMAHMANN & CAPLAN, 2006).

2.6. Causes of congenital cerebellar malformation

The most frequently documented non-inherited etiology of congenital cerebellar malformation in the veterinary literature is viral infection of the developing cerebellum (DE LAHUNTA & GLASS, 2009). Intrauterine parvovirus infection affecting the cerebellar germinal cell layer is an established cause of cerebellar hypoplasia in cats (SCHATZBERG et al., 2003). In dogs parvovirus has only been amplified from the cerebellum in some rare cases of cerebellar hypoplasia (SCHATZBERG et al., 2003) or demyelination of the white matter of the cerebellum (SCHAUDIEN et al., 2010), but it was never amplified from the cerebellum in cases with midline malformations and vermian defects resembling DWLM, cerebellar abiotrophy or hydranencephaly (SCHATZBERG et al., 2003). Herpesvirus was reported in a Malamute puppy with cerebellar ataxia and blindness that survived infection in the first weeks of life (PERCY et al., 1971). Post-mortem examination of this puppy revealed cerebellar and retina dysplasia, with poor differentiation and relative acellularity of the inner GL and ML, and also a reduced number of PC, with the ventral folia of the cerebellar vermis and paramedian lobules being most affected (PERCY et al., 1971). Evidence for inherited cerebellar hypoplasia in dog breeds is rare in the veterinary literature and limited to a few individuals in wire-haired Fox terriers, Irish setters and Chow-Chows (PALMER et al., 1973; KNECHT et al., 1979; DE LAHUNTA & GLASS, 2009). In cattle, congenital cerebellar hypoplasia and atrophy is commonly seen after an in utero infection of the bovine fetus with the bovine diarrhea virus (DE LAHUNTA & GLASS, 2009). In pigs cerebellar hypoplasia is one of the malformations seen after an in utero infection with the hog cholera virus (DE LAHUNTA & GLASS, 2009). Schmallenberg virus causes cerebellar hypoplasia in lambs, goats and calves, as well as other malformations of the brain and spinal cord (CONRATHS et al., 2013). Experimental studies in rats injected with Escherichia coli at gestational day 17, demonstrated a decrease of the PC density and volume of the cerebellum cortex (BIRAN et al., 2012). Exposure to bacterial endotoxin (lipopolysaccharide) caused diffuse cerebellar white matter damage in a

fetal preterm sheep (BIRAN et al., 2012). In humans, cytomegalovirus and rubella infection during CNS development is a well-known cause of cerebellar damage in the newborn (WAKELING et al., 2002; PARISI & DOBYNS, 2003; NIESEN, 2002; SPENNATO et al., 2011; BIRAN et al., 2012). Maternal exposure to nicotine, cocaine and ethanol (alcohol) during pregnancy causes cerebellar damages, which are thought to be responsible for the neurobehavioral deficits observed in the offspring (BIRAN et al., 2012). The maternal exposure to nicotine via infusion during the gestational period caused a significant decrease in the PC of the cerebellar cortex in animal models (BIRAN et al., 2012). A study with nonhuman primate specie showed that exposure to alcohol during the gestational period had a permanent dose-related deficit in the number of PC, although its mechanism still remains unclear (BIRAN et al., 2012). An important area of study is the effect of exposure to glucocorticoid during the development of the cerebellum, because the cerebellum has the highest levels of glucocorticoid receptors in the brain, which are localized in the EGL (BIRAN et al., 2012). A decreased volume in cerebral and cerebellar tissue was observed in preterm newborns after postnatal therapy with dexamethasone (BIRAN et al., 2012). The same was observed with clinically routine doses of hydrocortisone, but not with pretenatal use of betamethasone (BIRAN et al., 2012). Animal models showed reduced cerebellar growth, characterized by neuronal apoptosis and inhibition of the proliferation of immature and precursors of the GLC, after exposure to all glucocorticoids (hydrocortisone, dexamethasone, and corticosterone) (BIRAN et al., 2012). Many experimental data revealed that the cerebellum during its phase of rapid growth is especially vulnerable to undernutrition (BIRAN et al., 2012). There are studies in fetal sheep and guinea pig demonstrating that placental insufficiency can cause decreased cerebellar growth and differentiation (BIRAN et al., 2012). With advanced neonatal brain imaging it has become increasingly recognizable that an injury and/or an impaired development of the cerebellum, involving both white and grey matter, can be a consequence of a premature birth (BIRAN et al., 2012).

2.7. Classification of cerebellar diseases

In domestic animals congenital cerebellar diseases are usually classified according to the underlying pathobiology, either as congenital malformation, which are characterized by a non-progressive course and usually observed when the cat or

puppy tries to stand and walk, between the 6th and 8th week of age (DE LAHUNTA & GLASS, 2009); or as cerebellar cortical abiotrophy, characterized by different ages of onset and progressive ataxia (DE LAHUNTA & GLASS, 2009; URKASEMSIN & OLBY, 2014). The later, also known as cerebellar cortical degeneration, cerebellar ataxias, cerebellar degeneration, and cerebellar cortex degeneration, are characterized by postnatal neurodegeneration that occurs mainly in the cerebellar cortex, being further divided into diseases that primarily affect the PC or the GLC (URKASEMSIN & OLBY, 2014). Most of these disorders are inherited in an autosomal recessive pattern including the Old English Sheepdog (STEINBERG et al., 2000; AGLER et al., 2014), Gordon Setter (DE LAHUNTA & GLASS, 2009; AGLER et al., 2014), Scottish Terrier (URKASEMSIN et al., 2010), Finnish Hound (KYÖSTILÄ et al., 2012), Rhodesian Ridgeback (CHIETTO et al., 1994), Beagle (FORMAN, et al., 2012), and Australian Kelpie (SHEARMAN et al., 2011). An X-linked inheritance has been described in the English Pointer (O'BRIEN, 1993). There are also numerous sporadic single reports in several breeds, which may also have a hereditary origin (URKASEMSIN & OLBY, 2014).

Several classifications in human medicine have been proposed to describe cerebellar malformations, which usually are a part of a more complex malformation involving the midbrain and hindbrain; however, none has been satisfactory enough to be universally accepted (PARISI &DOBYNS, 2003; BARKOVICH et al., 2009; SHEKDAR, 2011).

One was proposed by PATEL AND BARKOVICH (2002) based on imaging findings. The cerebellar malformations were classified into hypoplasia and dysplasia. Hypoplasia was defined as a small or incompletely formed but structurally normal cerebellum, whereas dysplasia was defined as a disorganized cerebellum. Furthermore, these malformations were divided into generalized, involving both cerebellar hemispheres and the vermis, and focal, restricted to either a single hemisphere or vermis (PATEL & BARKOVICH, 2002).

Another one was proposed by Tortori-Donati in 2005 and was based on morphological features of the posterior fossa (SHEKDAR, 2011). Here the cerebellar malformations were divided into cystic and non-cystic malformations (SHEKDAR, 2011). These malformations were then further classified into focal (involving only the vermis or only the cerebellar hemispheres), diffuse (involving

both cerebellar hemispheres and the vermis), and combined (involving the cerebellum and brainstem) (SHEKDAR, 2011).

Cerebellar malformations in humans are however in a much more complex scheme of classification of midbrain-hindbrain malformations included (PARISI & DOBYNS, 2003; BARKOVICH et al., 2009). PARISI AND DOBYNS (2003), described a scheme based as much as possible on the embryological derivation of midbrain and hindbrain. According to this classification, DWM and CVH were within the same group of malformations. BARKOVICH AND COLLEAGUES (2009) proposed a new classification scheme based whenever possible upon embryology and genetics. Dandy-Walker malformation and CVH are classified within the same group of mesenchymal-neuroepithelial signaling defects (BARKOVICH et al., 2009).

3. Inferior vermian hypoplasia

In humans, inferior vermian hypoplasia (IVH), cerebellar vermian hypoplasia or isolated cerebellar hypoplasia are used often in the literature to describe the same pathology (PATEL & BARKOVICH, 2002, ADAMSBAUM et al., 2005: LIMPEROPOULOS et al., 2006, 2008). There are, however, doubts as to whether they represent the same or a different pathology (MALINGER et al., 2009). There is also a confusing overlap between the classification of DWV and IVH, with these two apparently distinct entities being used as synonyms (NIESEN, 2002; PARISI & DOBYNS, 2003; ADAMSBAUM et al., 2005; MALINGER et al., 2009; MACKILLOP, 2011; SPENNATO et al., 2011). This confusion between these different entities raise several questions in regard to prognosis and genetic counseling of the parents during pregnancy (ADAMSBAUM et al., 2005, MALINGER et al., 2009). Recent genetic data demonstrated that deletions or duplications of specific genes may result in a wide spectrum of cerebellar and posterior fossa malformations, suggesting that these pathologies share the same pathogenesis (MILLEN & GLEESON, 2008; ALDINGER et al., 2009).

3.1. Imaging diagnosis

LIMPEROPOULOS AND COLLEAGUES (2008) considered IVH when caudal growth of the inferior vermis over the fourth ventricle remained incomplete after the 18th to 20th week of gestation. However, MALINGER AND COLLEAGUES

(2009) suggested that MRI used to diagnose the different forms of vermian hypoplasia should not be performed before the 24th week of gestation. Magnetic resonance imaging is evaluated in the midsagittal plane. A small but anatomically normal vermis, a normal-sized or hypoplastic cerebellar hemispheres, a normal-sized posterior fossa, and fourth ventricle with a normal triangular shape are identified with IVH (ADAMSBAUM et al., 2005; LIMPEROPOULOS et al., 2008; MALINGER et al., 2009). Supratentorial anomalies such as ventriculomegaly, agenesis of the corpus callosum and simplified cortical gyration, can be observed together with IVH (ADAMSBAUM et al., 2005; LIMPEROPOULOS et al., 2008).

3.2. Progressive and non-progressive hereditary ataxias

In humans, inferior cerebellar vermian hypoplasia is one of the many features characterizing some progressive hereditary cerebellar ataxias (PALAU & ESPINÓS, 2005; MILLEN & GLEESON, 2008; MANCUSO et al., 2014). Hereditary cerebellar ataxias in humans are a heterogeneous group of neurological disorders. There have been identified at least 36 types of autosomal dominant cerebellar ataxia, 20 autosomal recessive ataxia, 2 X-linked ataxias, and several associated with mitochondrial impairment (MANCUSO et al., 2014). Their course is frequently chronic and progressive. However, although very rarely, genetic ataxias can present as episodic ataxias (MANCUSO et al., 2014). Clinically, human patients may present symptoms exclusively related to the cerebellar malformations, e.g. ataxia, abnormal eye movements, various type of nystagmus, dysarthria and dysmetria, or present neurological changes related to pyramidal, extrapyramidal, sensory and cognitive dysfunctions and also symptoms related to some other systemic malformations (PALAU & ESPINÓS, 2005; MANCUSO et al., 2014). Some examples of hereditary cerebellar ataxias, characterized by the presence of inferior cerebellar vermian hypoplasia, which may or may not be associated with other malformations (e. g. brainstem atrophy) are ataxia with oculomotor apraxia, autosomal recessive ataxia type I, and spinocerebellar ataxia (types 1-3, 6, 7, 17) (PALAU & ESPINÓS, 2005; MANCUSO et al., 2014).

Dysequilibrium syndrome (DES) is a genetically and phenotypically heterogeneous group of diseases, characterized by a non-progressive, autosomal recessive cerebellar ataxia, and mental retardation, with or without quadrupedal

gait (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; TÜRKMEN et al., 2008; TÜRKMEN et al., 2009; BOYCOTT et al., 2009; KOLB et al., 2010; GULSUNER et al., 2011; ALI et al., 2012). Inferior vermian hypoplasia is one of the main neuroimaging features of DES, as well as hypoplasia of the cerebellar hemispheres, simplified cortical gyration and decreased size of the brainstem, mainly in the pons (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; KOLB et al., 2010; ALI et al., 2012). The term CAMRQ is also used to describe this condition consisting of cerebellar ataxia, mental retardation and dysequilibrium syndrome. Three types of CAMRQ have been described: CAMRQ1 associated with mutations of the very-low density lipoprotein receptor (VLDLR) gene (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; KOLB et al., 2010; ALI et al., 2012); CAMRQ2 associated with mutations of the carbonic anhydrase gene 8 (CA8) (TÜRKMEN et al., 2009); and CAMRQ3 associated with mutations of the WD repeat domain gene 81 (WDR81) (GULSUNER et al., 2011).

3.3. Prognosis and Outcome

The outcome in human patients with IVH appears to be associated with the presence of supratentorial anomalies (LIMPEROPOULOS et al., 2006). LIMPEROPOULOS AND COLLEAGUES (2006) demonstrated that in isolated IVH, the development outcomes of children are associated with an overall good outcome, with only mild developmental delays in a subset of infants. Approximately one quarter of the children in this study showed impairment in gross and fine motor skills, deficits in expressive language, behaviour and social skills, as well as in cognition (LIMPEROPOULOS et al., 2006).

4. Dandy-Walker malformation

The inicial descriptions of Dandy-Walker malformation (DWM) were done by Virchow in 1863, Sutton in 1887 and Tusari in 1891 (DOW, 1940). However, its recognition and acceptance was the work of Dandy in 1914 (DANDY & BLACKFAN, 1914). This malformation was subsequently studied in more detail by Taggart in 1942 (RAYBAUD, 1982). The terminology “Dandy-Walker malformation” was suggested by Benda in 1954 to describe this as a clearly

defined entity (BENDA, 1954).

DWM is a relatively common malformation in humans, occurring in at least 1 out of 5000 live born infants (PARISI & DOBYNS, 2003). Despite experience over a century with DWM, the knowledge about its etiology, pathogenesis, classification, outcomes and related malformations remains very limited (STOLL et al., 1990; NIESEN, 2002; PATEL & BARKOVICH, 2002; WAKELING et al., 2002; PARISI & DOBYNS, 2003; WEIMER et al., 2006; IMATAKA et al., 2007; SPENNATO et al., 2011).

In dogs there were few reports describing the presence of cerebellar vermis hypoplasia/aplasia with or without involvement of the cerebellar hemispheres and a DWLM was frequently discussed. The breeds reported included miniature Schnauzer (CHOI et al., 2007; DE LAHUNTA & GLASS, 2009), Golden Retriever (SCHMIDT et al., 2008), Boston Terrier (DOW, 1940; NOUREDDINE et al., 2004), Cocker Spaniel (LIM et al., 2008), Labrador Retriever, Bull Terrier, Weimaraner, Dachshund, Mixed breed (KORNEGAY, 1986), Beagle, Silky Terrier (PASS et al., 1981), and Wire-Haired Miniature Daschund (KOBATAKE et al., 2013). Hydrocephalus has also been recognized in some of these dogs ((DOW, 1940; KORNEGAY, 1986; NOUREDDINE et al., 2004; CHOI et al., 2007; LIM et al., 2008).

4.1. Pathogenesis

The pathogenesis of DWM is a matter of discussion and has not been defined up to now. Initially, it was proposed that congenital obstruction of the foramina of Luschka and Magendie was responsible for the cystic dilatation of the fourth ventricle, which per se was responsible for the enlargement of the posterior fossa (SASAKI-ADAMS et al., 2008). In later studies, two other theories were proposed. Firstly, it was suggested that DWM may result from an abnormal development of the rhombencephalon, which leads to an incomplete formation of the vermis. Secondly, it was suggested that DWM may be the result of a defect within the tela choroidea, which leads to the cystic dilation of the fourth ventricle (SASAKI-ADAMS et al., 2008). Hence, the most convincing explanation is that DWM is the result of a developmental arrest in the hindbrain with persistence of the AMA (FRIEDE, 1975; SASAKI-ADAMS et al., 2008; SHEKDAR, 2011). The AMA persists, not fusing with the choroid plexus, and takes place between the caudal edge of the developing vermis and the cranial edge of the developing

choroid plexus (FRIEDE, 1975; SHEKDAR, 2011). The AMA expands due to CSF pulsations, rather than the nonopening of the cerebellar foramina, as suggested earlier (FRIEDE, 1975; SHEKDAR, 2011). The AMA distends progressively forming a cyst that displaces and rotates the hypoplastic vermis superiorly and in counterclockwise manner (FRIEDE, 1975; SHEKDAR, 2011). The enlargement of the posterior fossa and the consequent elevation of the tentorium cerebelli osseum and torcula are the end result of the non-migration of the straight sinus from the vertex to the lambda (FRIEDE, 1975; SHEKDAR, 2011). This may result from an error occurring during development, or from the mechanical interference of the cystic enlargement of the fourth ventricle (FRIEDE, 1975; SHEKDAR, 2011). Recent genetic studies have suggested that the surrounding mesenchyme may also play a significant role in the cerebellar development during embryogenesis. Thus mutations involving the cerebellum and the surrounding mesenchyme seem to be necessary for the development of a DWM complex, and the extent of the mutation seems to be directly correlated with the severity of the malformation (MCCORMACK et al., 2002; GRINBERG et al., 2004; JALALI et al., 2008; ALDINGER et al., 2009; BARKOVICH, 2012). Based on this information DWM should be included in the group of mesenchymal-neuroepithelial signaling defects (BARKOVICH, 2012). From this embryogenetic point of view retrocerebellar subarachnoid cysts, enlargement of the cisterna magna (mega cisterna magna) and persistent Blakes pouch cyst should be considered a part of the DWM. The molecular and biological pathways involved in these disorders have not, however, been yet identified (BARKOVICH, 2012).

4.2. Etiology

A review of the human literature identified environmental factors, including prenatal exposure to rubella (NIESEN, 2002; PARISI & DOBYNS, 2003; SPENNATO et al., 2011), toxoplasmosis, cytomegalovirus (NIESEN, 2002; WAKELING et al., 2002; SPENNATO et al., 2011), alcohol (PARISI & DOBYNS, 2003; SPENNATO et al., 2011), isotretinoin, coumadin, and maternal diabetes, to be associated with DWM (STOLL et al., 1990; SPENNATO et al., 2011). Dandy-Walker malformation can also be found as part of a Mendelian disorder or be associated with a chromosomal abnormality (STOLL et al., 1990; NIESEN, 2002; WAKELING et al., 2002; PARISI & DOBYNS, 2003; WEIMER

et al., 2006; IMATAKA et al., 2007; SPENNATO et al., 2011). Although familial cases of DWM are rare and the inheritance mode is not well understood, a number of theories, ranging from sporadic genetic aberrations to more classical Mendelian inheritance, have been proposed to explain it (BRAGG et al., 2006). Few reports of siblings with DWM highly suggest an autosomal recessive pattern of inheritance (STOLL et al., 1990; ABDEL-SALAM et al., 2006). However an X-linked recessive or a multifactorial inheritance is also possible (WAKELING et al., 2002). An autosomal dominant inheritance of DWM associated with occipital encephalocele was described in two families (JALALI et al., 2008). The fact that DWM can appear associated with other clinically recognizable genetic syndromes, such as Meckel-Gruber, Walker-Walburg, PHACE syndrome (PARISI & DOBYNS, 2003; IMATAKA et al., 2007; SPENNATO et al., 2011), or Down syndrome (NIGRI et al., 2014), is highly suggestive of an inherited condition.

4.2.1. Genetic and chromosomal abnormalities

In six human patients with DWM the first critical region in the chromosome of 3q2 was identified. This region is composed of two adjacent zinc fingers in the cerebellum genes, ZIC1 and ZIC4. Mice with a heterozygous deletion of these two linked genes have a phenotype that is similar to DWM, providing a mouse model for this malformation (GRINBERG et al., 2004). Another case report in a human patient with an interstitial deletion of 3q22.3-q25.2, also suggests that the contiguous deletion of ZIC1 and ZIC4 may be responsible for the DWM phenotype (LIM et al., 2011). The effects of these interstitial deletions in the phenotype of DWM can be extremely variable ranging from mild cerebellar vermian hypoplasia (CVH) to classic DWM (GRINBERG et al., 2004; MILLEN & GLEESON, 2008). In a recent study, however, deletions of both ZIC1 and ZIC4 genes were identified in 4 patients, who did not display any features of DWM, and in another 11 patients without deletions of these genes, a diagnosis of isolated or syndromic DWM was confirmed (FERRARIS et al., 2013). These findings indicate that deletions in the chromosome 3q2, including ZIC1 and ZIC4 genes, can be or not associated with DWM and that penetrance of these genes may be incomplete (FERRARIS et al., 2013). A second locus in chromosome 6p25.3 associated with DWM was identified. Deletions or duplications encompassing the gene FOXC1 were also associated with cerebellar and posterior

fossa malformations (ALDINGER et al., 2009; HALDIPUR et al., 2014). Again the phenotype was extremely variable ranging from mild CVH or mega cisterna magna (MCM) to classic DWM. A mouse model was also provided (ALDINGER et al., 2009). These data demonstrate that loss or duplication of FOXC1 (ALDINGER et al., 2009) and the deletion of both ZIC1 and ZIC4 genes (MILLEN & GLEESON, 2008) may result in cerebellar and/or posterior fossa malformations, with a wide spectrum of phenotypes. This fact suggests that isolated CHV, MCM and DWM may share the same pathogenesis in at least some affected individuals (MILLEN & GLEESON, 2008; ALDINGER et al., 2009). A *de novo* 2.3-Mb deletion of the chromosome 8p21.2-p21.3 associated with a down regulation of the gene FGF17, known to be involved in the cerebellar development, was also identified in a patient with severe growth mentation, seizures and classic DWM (ZANNI et al., 2011). Several studies have identified deletions in the long arm of chromosome 13 in patients with DWM, suggesting that the gene (s) associated with this malformation may also be located on this chromosome (MCCORMACK et al., 2002; MADEMONT-SOLER et al., 2010). Recently a small deletion on this chromosome associated to DWM was reported, reducing the critical region to 13q32.2-32.3 on this chromosome (MADEMONT-SOLER et al., 2010). Among the few genes of this deleted region, ZIC2 and ZIC5 seemed the most plausible candidates (MADEMONT-SOLER et al., 2010). A linkage to chromosome 2q36.1 was identified in families with autosomal dominant inheritance of classic DWM which was associated with an occipital encephalocele (JALALI et al., 2008). Families with autosomal dominant inheritance of DWM without an occipital encephalocele have not been reported to date (JALALI et al., 2008).

Abnormal karyotypes are found in up to 55% of fetuses with an antenatal diagnosis of DWM (IMATAKA et al., 2007). Some of the chromosomal anomalies identified in Dandy-Walker include trisomy 18 (in 20 of 78 cases of DWM), 9 (at least 20 case reports of DWM) and 13 (in 4 of 50 cases of DWM) (PARISI & DOBYNS, 2003; IMATAKA et al., 2007; SASAKI-ADAMS et al., 2008); triploid (PARISI & DOBYNS, 2003; IMATAKA et al., 2007); tetraploid (IMATAKA et al., 2007); partial trisomy 3q, 3 p, 7q, 8q, 11q (WEIMER et al., 2006; IMATAKA et al., 2007); partial duplication of 5q, 8p, 8q, and 11q (NIESEN, 2002; PARISI & DOBYNS, 2003; IMATAKA et al., 2007); and

deletion of 2q, 3q, 6p (PARISI & DOBYNS, 2003; WEIMER et al., 2006; IMATAKA et al., 2007), 13q (4 case reports of DWM) and 5p (IMATAKA et al., 2007). However the most common chromosomal defects observed are trisomy 18, triploidy and trisomy 13 (IMATAKA et al., 2007).

Regarding sex chromosome abnormalities, there are reports of DWM associated with X-monosomy/Turner syndrome, X-pentasomy, autosomal X-linked recessive (WAKELING et al., 2002; BRAGG et al., 2006) and fragile X-syndrome (IMATAKA et al., 2007). An X chromosome locus has also been discussed to be responsible for DWM (WAKELING et al., 2002; BRAGG et al., 2006).

4.3. Controversy on the classification of DWM in humans

Classic DWM is defined as a complete or partial agenesis of the cerebellar vermis, resulting in an upward displacement and rotation of the remnants of the vermis, a cyst-like dilation of the fourth ventricle, and an enlargement of the posterior fossa causing an upward displacement of the transverse sinuses, tentorium cerebelli osseum and torcula (PATEL & BARKOVICH, 2002; SHEKDAR, 2011). Hydrocephalus is not a part of the essential criteria (SHEKDAR, 2011; SPENNATO et al., 2011). In some cases, however, a substantial enlargement of the posterior fossa with elevation of the tentorium cerebelli osseum, transverse sinuses and torcula was not observed, which led to the introduction of the term Dandy-Walker variant (DWV) (PATEL & BARKOVICH, 2002; SHEKDAR, 2011). This terminology is not without controversy (NIESEN, 2002; PARISI & DOBYNS, 2003; MALINGER et al., 2009; MACKILLOP, 2011; SPENNATO et al., 2011), and there are actually no specific criteria to classify/quantify the size of posterior fossa and vermian hypoplasia. Thus, the diagnosis of classic DWM and DWV is often established based on the experience of the neuroradiologists (SASAKI-ADAMS et al., 2008). Furthermore, it was often observed, particularly in prenatal diagnosis, that the term DWV was used synonymously for other pathologies, such as isolated vermian hypoplasia, inferior vermian hypoplasia, and vermian dysgenesis/agenesis as part of the molar tooth syndrome (ADAMSBAUM et al., 2005; MALINGER et al., 2009). For this reason, some authors have recommended abandoning the term “Dandy-Walker variant” (NIESEN, 2002; PARISI & DOBYNS, 2003; BOLDUC & LIMPEROPOULOS, 2009; MALINGER et al., 2009; GAREL et al., 2011; SPENNATO et al., 2011).

However and as mentioned, some genetic studies suggested that CVH, DWV, classic DWM and mega cisterna magna may share a common pathogenesis, because all these different phenotypes were observed with a specific loss of a gene (MILLEN & GLEESON, 2008) or the interstitial deletion of a chromosome (ALDINGER et al., 2009). This may explain the difficulty in distinguishing clinically between these malformations (MILLEN & GLEESON, 2008; ALDINGER et al., 2009). Moreover, there is evidence of a shared pathogenetic origin, based on the observations in monozygotic twins (SPENNATO et al., 2011).

4.4. Associated malformations

Dandy-Walker malformation can appear isolated or associated with other intra or extra-cranial abnormalities. Intra-cranial abnormalities include agenesis or dysgenesis of the corpus callosum (PATEL & BARKOVICH, 2002; KLEIN et al., 2003; ALEXIOU et al., 2010; SPENNATO et al., 2011), inter-hemispheric cysts, malformations of the cerebral gyration (polymicrogyria), encephaloceles, gray matter heterotopias, malformations of the dentate nucleus and of the brainstem, hamartomas (KLEIN et al., 2003; ALEXIOU et al., 2010; SPENNATO et al., 2011) and lissencephaly (NOTARIDIS et al., 2006). These intra-cranial abnormalities are present in 29% to 48% or 13% to 67% of the patients with DWM (BOLDUC & LIMPEROPOULOS, 2009). However, the most common anomaly is ventriculomegaly/hydrocephalus, observed in 36% to 76% of children with DWM (NIESEN, 2002; BOLDUC & LIMPEROPOULOS, 2009). A significant proportion (10-17% or 5-50%) of children with DWM have agenesis or dysgenesis of the corpus callosum (PARISI & DOBYNS, 2003; BOLDUC & LIMPEROPOULOS, 2009). In the veterinary literature concurrent hydrocephalus, agenesis or dysgenesis of the corpus callosum and polymicrogyria have also been described in patients with cerebellar malformations (MACKILLOP, 2011). The corpus callosum allows motor, sensorial and cognitive information to pass between the hemispheres, causing inhibition of concurrent activity in the other hemisphere (VASUDEVAN et al., 2012). Extra-cranial or systemic abnormalities are reported less common, affecting 9% to 40% of the children with DWM (BOLDUC & LIMPEROPOULOS, 2009). Various anomalies have been described, namely, cardiac, urogenital, intestinal, facial, and limb anomalies

(PASCUAL- CASTROVIEJO et al., 1991; KLEIN et al., 2003; ALEXIOU et al., 2010; SPENNATO et al., 2011). Syringomyelia has also been observed with DWM (SPENNATO et al., 2011). Dandy-Walker malformation can also be associated with other syndromes such as Meckel-Gruber, Walker-Warburg, PHACE syndrome (PARISI & DOBYNS, 2003; IMATAKA ET AL., 2007; SPENNATO et al., 2011), and Down syndrome (NIGRI et al., 2014).

4.5. Diagnosis of DWM

Imaging diagnosis including computed tomography, magnetic resonance imaging and ultrasound, have been used to identify DWM in human and veterinary medicine. However histopathology also plays a major role in the diagnosis and prognostication of DWM (KLEIN et al., 2003). Genetic testing is yet not available due to undefined etiology.

4.5.1. Imaging Diagnosis

Any cystic lesion in the posterior fossa, which causes a large degree of vermian rotation and compression may suggest the presence of vermian hypoplasia or aplasia, despite the presence of a completely normal cerebellar vermis, on the dorsal view of computed tomography (CT) or even MRI (KLEIN et al., 2003). Thus sagittal views of the cerebellar vermis are essential for the accurate analysis of the content of the posterior fossa (KLEIN et al., 2003). Even CT scans with sagittal reconstruction are inadequate because of their insufficient definition (KLEIN et al., 2003). For the moment, the same can be said about ultrasonography (KLEIN et al., 2003). For this reason, MRI with sagittal and dorsal views in T2-weighted and FLAIR (fluid attenuated inversion recovery) images are warranted for an accurate diagnosis of DWM and concurrent malformations (KLEIN et al., 2003; SHEKDAR, 2011). A critical point of the MRI is the accurate analysis of the cerebellar vermis anatomy, with identification of the two main fissures and characterization of the vermis lobulation (BODDAERT et al., 2003). This relies on the fact that the vermis anatomy is statistically correlated with the neurological and intellectual outcome in patients with DWM (BODDAERT et al., 2003; KLEIN et al., 2003; BOLDUC & LIMPEROPOULOS, 2009).

4.5.2. Histopathology findings

Histopathology is extremely valuable to help recognize which structures are involved in DWM. Correlating them with imaging findings can help provide an accurate prognostic in patients with this malformation (ADAMSBAUM et al., 2005).

Macroscopically, a posterior fossa enlargement, with partial or complete vermian agenesis, a large membranous cyst formed by distended roof of the fourth ventricle and an upward rotation of the lateral sinuses, falx and torcula are observed (FRIEDE, 1975). In humans, a complete cerebellar vermian agenesis is seen in approximately one-fourth of the cases, and when partial vermian agenesis is observed, the posterior part of the vermis and posterior lobes of the cerebellar hemispheres, that are in contact with the cystic wall of the fourth ventricle, are usually hypoplastic (FRIEDE, 1975). The vermis remnants are rotated anteriorly or may adhere to the tentorium cerebelli osseum, and the roof of the fourth ventricle is seen as a thin translucent membrane attached dorsally to the remnants of the posterior vermis, laterally to the cerebellar hemispheres and tonsils, and posteriorly to the medulla oblongata (FRIEDE, 1975). After removing the brain, rupture of the cyst wall occurs, allowing for a direct view of the floor of the fourth ventricle (FRIEDE, 1975). Microscopically the cyst wall is composed of an outer layer of connective tissue continuous with the leptomeninges and an inner layer of glioependymal tissue (FRIEDE, 1975). Cerebellar heterotopias and/or cortical malformations may be seen, and variable patency of the cerebellar foramina is observed (FRIEDE, 1975). Postmortem findings in human patients with DWM and in animals with cerebellar hypoplasia resembling DLWM are summarized in Table 5.

Table 5. Postmortem findings in humans with DWM and in animals with cerebellar hypoplasia resembling DLWM [(1)] (PATEL & BARKOVICH, 2002); [(2)] (NOTARIDIS et al., 2006); [(3)] (KORNEGAY, 1986); [(4)] (LIM et al., 2008); [(5)] (DE LAHUNTA & GLASS, 2009); [(6)] (WONG et al., 2007)

Type of study	Macroscopic and microscopic examination
Multiple case report (16 human patients) [(1)]	Vermian and symmetric versus asymmetric hemispheres hypoplasia (n=13) and dysplasia (n=3)
Single case report (asymptomatic adult) [(2)]	Remnants of the cyst visible medially; partial vermian agenesis (posterior-inferior part visible) with upward displacement; vermian hypoplasia; two symmetrical foci of cortical dysplasia on the dorsal part of the posterior lobe of both cerebellar hemispheres; cyst's wall: exterior arachnoidal layer, middle layer of loose connective tissue, no ectopic glial or neural cells, and internal layer of ependymal cells and normal plexus choroid structure
Multiple case report (six dogs) [(3)]	Partial vermian agenesis with variable portion of the caudal vermis absent (n=6), visible fourth ventricle cyst (n=2); vermian hypoplasia with relative mild microscopic changes in the differentiated portions of the cerebellum (n=6); unilateral or bilateral hypoplasia of the hemisphere and flocculus (n=4); lateral apertures of the fourth ventricle absent (n=1); severe central chromatolysis and vacuolation in neurons of brain stem nuclei, mainly in the caudal olivary and lateral reticular nuclei; hydrocephalus (n=2)
Single case report (one dog) [(4)]	Partial vermian agenesis, caudal part absent; folial atrophy, degeneration and loss of the PC and GLC
Single case report (one dog) [(5)]	Partial vermian agenesis, caudal part until the primary fissure absent, including the medullary portion with the fastigial nucleus; symmetrical lesion with non-inflammatory pattern
Single case report (one horse) [(6)]	Complete agenesis of the cerebellar vermis; ventriculomegaly of the lateral and third ventricles, dilation of the mesencephalic aqueduct; cyst-like dilation of the fourth ventricle; corpus callosum agenesis; polymicrogyria; hemispheres dysplasia, PCL and GL hypocellularity

4.6. Prognosis and outcomes of DWM

Clinical heterogeneity is described in human patients with DWM and its variants, ranging from a completely normal intelligence and absence of neurological findings, to neuro-cognitive deficits, motor delay, hypotonia (STOLL et al., 1990; KLEIN et al., 2003; ECONOMOU & KATSETOS, 2012), speech delay, autistic features (ECONOMOU & KATSETOS, 2012), ocular signs including nystagmus and strabismus (PASCUAL-CASTROVIEJO et al., 1991; ECONOMOU & KATSETOS, 2012), cerebellar ataxia, dizziness, and seizures (NOTARIDIS et al., 2006; SPENNATO et al., 2011). Neurological abnormalities have been reported in up to 50% of survivours (BOLDUC & LIMPEROPOULOS, 2009).

These clinical inconsistencies make the prognosticating of the neurodevelopment and functional outcome of children with DWM an impossible challenge (KLEIN et al., 2003; GUIBAUD & DES PORTES, 2006; BOLDUC & LIMPEROPOULOS, 2009). The symptoms in DWM are related to the hydrocephalus grade, cerebellar and cranial nerves dysfunction and to the presence of associated anomalies (PASCUAL-CASTROVIEJO et al., 1991; PATEL & BARKOVICH, 2002; PARISI & DOBYNS, 2003; NOTARIDIS et al., 2006; SPENNATO et al., 2011). The majority of children develop symptoms early in life: 80-90% in the first year (PASCUAL-CASTROVIEJO et al., 1991; SPENNATO et al., 2011). An early control of hydrocephalus may contribute to a normal intellectual development in some cases (PATEL & BARKOVICH, 2002; BODDAERT et al., 2003; PARISI & DOBYNS, 2003; SPENNATO et al., 2011; MCCLELLAND et al., 2015). For this reason since the introduction of shunts, as surgical treatment for the reestablishment of the posterior fossa architecture, the prognosis for these patients has dramatically improved (ALEXIOU et al., 2010; SPENNATO et al., 2011; MCCLELLAND et al. 2015). In one retrospective study, the use of shunts reduced the mortality rate up to 44% (MCCLELLAND et al., 2015). Through the work of Dr. Benjamin Warf in Uganda from 2004 to 2010, a new neurosurgical method combining an endoscopic third ventriculostomy with a cauterization of the choroid plexus was developed (WARF et al., 2011). With the use of this new surgical technique, the life quality and the success rate (about 75%) improved considerably, and the need for an additional surgery has also decreased (WARF et al., 2011).

In humans factors associated with a poor prognosis in patients with DWM are: (1) the presence of seizures, which are usually associated with heterotopias of the cerebral cortex (NIESEN, 2002; SPENNATO et al., 2011); (2) hearing and visual problems, which also indicate associated CNS anomalies (NIESEN, 2002; BOLDUC & LIMPEROPOULOS, 2009; SPENNATO et al., 2011); (3) other CNS anomalies, like agenesis of the corpus callosum (NIESEN, 2002; BOLDUC & LIMPEROPOULOS, 2009; SPENNATO et al., 2011); (4) associated systemic or extra-cranial malformations (NOTARIDIS et al., 2006; SASAKI-ADAMS et al., 2008; JHA et al., 2012); (5) the degree of cerebellar hypoplasia (PARISI & DOBYNS, 2003); and (6) the lobulation of the cerebellar vermis (BODDAERT et al., 2003; KLEIN et al., 2003; BOLDUC & LIMPEROPOULOS, 2009). Mental retardation was very common in cases of severely abnormally lobulated vermis, (BODDAERT et al., 2003; KLEIN et al., 2003).

When comparing DWV with classic DWM, some differences in the outcomes are noted. Human patients with isolated DWV appear to have a better outcome then those with classic DWM (SASAKI-ADAMS et al., 2008). Dandy-Walker variant appears to be associated with ventriculomegaly, rather than with hydrocephalus (24%-27%) (SASAKI-ADAMS et al., 2008). The association with other CNS anomalies or systemic malformations appears to have a negative impact on the neurological outcome in these patients, as observed in patients with classic DWM (SASAKI-ADAMS et al., 2008).

Asymptomatic DWM was found incidentally in some human patients. The absence of associated fatal systemic anomalies increases life expectancy in such cases (NOTARIDIS et al., 2006; JHA et al., 2012). In a case series of 12 patients, hydrocephalus characterized by a mild enlargement of the lateral and third ventricles was found in five cases without clinical or radiological features of raised intracranial pressure, and agenesis of the corpus callosum was detected in two cases (JHA et al., 2012). This suggests that patients in which the posterior fossa cyst communicates freely with surrounding CSF space may remain asymptomatic, due to a normal intracranial pressure (JHA et al., 2012).

III. PUBLICATION

The following article “Inferior Cerebellar Hypoplasia Resembling a Dandy-Walker-like malformation in Purebred Eurasier Dogs with Familial Non-Progressive Ataxia: A Retrospective and a Prospective Clinical Cohort Study” was accepted for publication in the PLoS-One on 10th December 2014.

Academic Editor: Fernando de Castro, Hospital Nacional de Paraplégicos – Sescam, Spain

Received on 17th June 2014; accepted on 10th December 2014; published on 10th February 2015; 10(2): e0117670. doi:10.1371/journal.pone.0117670.

PLOS ONE

RESEARCH ARTICLE

Inferior Cerebellar Hypoplasia Resembling a Dandy-Walker-Like Malformation in Purebred Eurasier Dogs with Familial Non-Progressive Ataxia: A Retrospective and Prospective Clinical Cohort Study

Filipa Bernardino[1], Kai Rentmeister[2], Martin J. Schmidt[3], Andreas Bruehschwein[4], Kaspar Matiasek[5], Lara A. Matiasek[1], Alexander Lauda[1], Heinz A. Schoon[6], Andrea Fischer[1]*

1 Centre for Clinical Veterinary Medicine, Clinic of Small Animal Medicine, Ludwig Maximilian University, Munich, Germany, **2** Tierärztliche Praxis für Neurologie, Dettelbach, Germany, **3** Department of Veterinary Clinical Science, Small Animal Clinic, Justus-Liebig-University, Giessen, Germany, **4** Centre for Clinical Veterinary Medicine, Clinic of Small Animal Surgery and Reproduction, Ludwig Maximilian University, Munich, Germany, **5** Centre for Clinical Veterinary Medicine, Section of Clinical and Comparative Neuropathology, Ludwig Maximilian University, Munich, Germany, **6** Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

* andreafischer@lmu.de

OPEN ACCESS

Citation: Bernardino F, Rentmeister K, Schmidt MJ, Bruehschwein A, Matiasek K, Matiasek LA, et al. (2015) Inferior Cerebellar Hypoplasia Resembling a Dandy-Walker-Like Malformation in Purebred Eurasier Dogs with Familial Non-Progressive Ataxia: A Retrospective and Prospective Clinical Cohort Study. PLoS ONE 10(2): e0117670. doi:10.1371/journal.pone.0117670

Academic Editor: Fernando de Castro, Hospital Nacional de Parapléjicos—SESCAM, SPAIN

Received: June 17, 2014

Accepted: December 10, 2014

Published: February 10, 2015

Data Availability Statement: All data is available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.tn873.

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Cerebellar malformations can be inherited or caused by insults during cerebellar development. To date, only sporadic cases of cerebellar malformations have been reported in dogs, and the genetic background has remained obscure. Therefore, this study`s objective was to describe the clinical characteristics, imaging features and pedigree data of a familial cerebellar hypoplasia in purebred Eurasier dogs. A uniform cerebellar malformation characterized by consistent absence of the caudal portions of the cerebellar vermis and, to a lesser degree, the caudal portions of the cerebellar hemispheres in association with large retrocerebellar fluid accumulations was recognized in 14 closely related Eurasier dogs. Hydrocephalus was an additional feature in some dogs. All dogs displayed non-progressive ataxia, which had already been noted when the dogs were 5 – 6 weeks old. The severity of the ataxia varied between dogs, from mild truncal sway, subtle dysmetric gait, dysequilibrium and pelvic limb ataxia to severe cerebellar ataxia in puppies and episodic falling or rolling. Follow-up examinations in adult dogs showed improvement of the cerebellar ataxia and a still absent menace response. Epileptic seizures occurred in some dogs. The association of partial vermis agenesis with an enlarged fourth ventricle and an enlarged caudal (posterior) fossa resembled a Dandy-Walker-like malformation in some dogs. Pedigree analyses were consistent with autosomal recessive inheritance.

Introduction

Cerebellar malformations can be inherited or acquired from insults during cerebellar development. Evidence for inherited cerebellar hypoplasia in dogs is rare in the veterinary literature and limited to few observations in wire-haired Foxterriers, Irish setters and Chow-Chows [1–3]. The most frequently documented etiologic factor in cerebellar malformations in the veterinary literature is viral infection of the developing cerebellum [4,5], but attempts to amplify parvovirus have failed in dogs with midline malformations and vermian defects resembling a Dandy-Walker malformation (DWM) [4].

A classic Dandy-Walker malformation (DWM) consists of complete or partial agenesis of the cerebellar vermis with an upward displacement and rotation of the remnants of the vermis, cyst-like dilation of the fourth ventricle and enlargement of the posterior fossa, with upward displacement of the tentorium cerebelli osseum, transverse sinuses, and torcula. Hydrocephalus may occur in up to 80% [6–10]. In many cases, no substantial enlargement of the posterior fossa is observed, which has led to the introduction of the term "Dandy-Walker variant" [10]; however, no consensus on this terminology exists in the literature [9,11,12], and the use of this term has been discouraged [11,13–15]. Recent evidence suggests that DWM and related malformations may represent a continuum and a classification system based on embryonic development and genotype has been proposed [16,17]. With this classification system, DWM and related malformations are classified as disorders of mesenchymal-neuropithelial signaling whereas VLDLR and reelin pathway mutations are classified as malformations of neuronal migration that predominantly affect the cerebellum and brainstem [16].

Anecdotal reports have described sporadic cases of vermis hypoplasia/agenesis resembling DWM with or without associated focal or generalized hypoplasia of the cerebellar hemispheres in dogs [18–23]. Reported affected breeds include the Miniature Schnauzer [1,19], Golden Retriever [21], Boston Terrier [18,23], Cocker Spaniel [20], Labrador Retriever, Bull Terrier, Weimaraner, Dachshund, Mixed Breed [22], Beagle, Silky Terrier [24], Wire-haired Miniature Dachshund [25], Chow-Chow and Tervueren [26]. The presence of a midline malformation with some resemblance to DWM in humans has been frequently suggested. To date, evidence for inheritance has been poor and limited to observations in Boston Terriers [18,23].

Therefore, the aim of the present investigation was to describe the clinical phenotype and imaging findings of familial non-progressive ataxia and cerebellar hypoplasia resembling a Dandy-Walker-like malformation (DWLM) in purebred Eurasier dogs. We provide a comprehensive clinical picture of this cerebellar malformation, which includes assessments of the clinical course and lifespan of the dogs, and propose autosomal recessive inheritance as. A VLDLR gene mutation was recently identified based on the data provided in this investigation [27].

Materials and Methods

Retrospective and prospective case cohort studies were performed in Eurasier dogs with neurological signs.

Retrospective case review: The local Eurasier club (K.Z.G. Eurasier) provided records from 23 dogs with neurological symptoms. In individual cases, the local veterinarian was contacted for additional information.

Prospective investigations: The owners of nine purebred Eurasier dogs with neurological signs of ataxia and/or epileptic seizures were contacted by the Eurasier club and were encouraged to pursue neurological examination and advanced brain imaging by dedicated specialists. Each dog was subjected to clinical and neurological examinations by a board-certified veterinarian, and each dog underwent advanced neuroimaging. Magnetic resonance imaging (MRI) of the head (eight dogs) was performed on the anesthetized dogs using a 1.5 Tesla scanner

(Magnetom Symphony Syngo MR, Siemens AG, Erlangen, Germany; four dogs) or a 1.0 Tesla scanner (Gyroscan Intera, Philips, Hamburg, Germany; four dogs). T1-weighted (spin echo repetition time [TR], 482 ms; minimum echo time [TE], 15 ms; signal averaging [NSA], 4; slice thickness, 4 mm; interslice gap, 0.4 mm) and T2-weighted turbo-spin echo (TR, 4146 ms; TE, 108 ms; slice thickness, 2 mm; 448 × 448 matrix; FOV, 130 × 130) images were acquired in the sagittal, dorsal, and transverse planes, and fluid attenuation inversion recovery (FLAIR) and T2 gradient echo (GE) sequences were obtained in the transverse plane. A gadolinium-based contrast agent (Omniscan, 0.1 mmol/kg) was administered intravenously. In one animal, computed tomography (CT) of the head was obtained by multislice CT (Somatom Balance, Siemens, Erlangen, Germany) (120 kV; 350 mA; matrix, 512 × 512; slice thickness, 2 mm; pitch, 1). MRI was also performed on three littermates and the dam and sire of one affected dog (dog 6; litter 4), the dam of another affected dog (dog 9, litter 6), and CT was performed on the dam of two affected littermates (dogs 7, 8; litter 5) to ensure the dogs` health status prior to breeding.

All MRI and CT brain images and histological specimens were reviewed. The presence or absence of the midline cerebellar vermis structures was assessed. Special care was taken to assess the position of the tentorium cerebelli. The size of the caudal fossa was assessed visually in each dog by independent examinations of a board-certified radiologist and two board-certified neurologists and, thereafter, morphometrically as the ratio of the caudal fossa area to the total braincase area on midsagittal T2W brain images, as published previously (OsiriX; v.5.6 Pixmeo Sarl) [28].

The pedigrees were analyzed using electronic data files provided by the Eurasier club (Dog manager; Breeder Soft, Delmenhorst, Germany). The pedigrees were compiled to the first common ancestor in all dogs, using a human genealogy software program (Geno Pro 2011).

The clinical course and the life span of the dogs were assessed by information provided by the breed club and contact to the dogs` owners. Repeated neurological examinations and video documentations of the gait were performed on three dogs of the prospective cohort.

Ethics Statement

All investigations were conducted in strict compliance with the restrictions of the German Animal Protection Law. The authors declare that prior approval was obtained from the respective breed club (Kynologische Zuchtgemeinschaft Eurasier e.V.) and the Clinic of Small Animal Medicine institutional review board. All dogs (purebred Eurasier dogs) lived with their owners and the owners of the dogs gave permission for their animals to be used in this study.

Results

Inferior cerebellar hypoplasia resembling a DWLM was confirmed in 14 dogs. The main finding was absence of the caudal portions of the cerebellar vermis and the caudal aspects of the cerebellar hemispheres associated with an enlarged fourth ventricle in all dogs. An enlarged caudal (posterior) fossa consistent with a classic DWM was evident in three dogs (21%), and four dogs exhibited hydrocephalus (29%). The assessment was based on neuroimaging and morphometric measurements in eleven dogs (dogs 1–11) and on post mortem examinations in three dogs. PCR amplification of canine parvovirus type 2 nucleic acid was negative, and corpus callosum agenesis was an additional finding in these three dogs (dogs 12–14).

Other findings: Cerebellar hypoplasia with or without hydrocephalus was diagnosed during post-mortem examinations in three other dogs, but histological slides were not provided to us for secondary review. Other diagnoses were confirmed in six dogs: caudal fossa arachnoid cyst (one dog; MRI), hydrocephalus restricted to the supratentorial region (two dogs; MRI,

histopathology), and idiopathic epilepsy (three dogs; MRI). No specific diagnoses were obtained for nine dogs with ataxia which were reported to the breed club before the dogs were eight weeks old.

Signalment and neurological examination

Signalment and clinical data from 14 Eurasier dogs (6 male and 8 female dogs; 5 weeks to 4 years old at clinical presentation) with a confirmed diagnosis of inferior cerebellar hypoplasia resembling DWLM. are presented in Table 1. Non-progressive ataxia, which was noted at an early age when the dogs began to walk, was the predominant clinical sign in these 14 dogs. The frequencies of the main neurological features in each patient cohort are presented in Table 2.

Retrospective cohort (dogs 1–5, dogs 12–14, Table 1): All dogs presented with generalized ataxia. This ataxia was characterized by episodic falling and/or rolling or a hypermetric gait. Cerebellar ataxia was evident on provided video recordings (S1 Video). Additional signs described in some dogs were occasional head tremors, which resembled intention tremors and proprioceptive deficits. Three of these dogs developed epileptic seizures at one year of age (dog 1), four years of age (dog 3), or within the first year (dog 4).

Prospective cohort (dogs 6–11, Table 1): All dogs presented with non-progressive ataxia. Most notably, in one dog, ataxia was hardly visible on initial presentation and was detected as only truncal sway and subtle dysmetria to an experienced observer (dog 6, S2 Video). In the other dogs, the ataxia was specifically characterized as cerebellar ataxia with a hypermetric gait and symmetrical ataxia of the trunk and limbs (dogs 7 and 8, S3 Video), which improved in the adult dogs (S4 Video); cerebellar ataxia with episodic rolling to both sides (dog 9); a subtle dysmetric gait and pelvic limb ataxia (dog 10, S5 Video); and a subtle dysmetric gait of all limbs, mild head tilt and leaning to one side (dog 11, S6 Video). Other signs noted during the neurological examination were delayed initiation of the hopping and wheelbarrowing reactions (three dogs, S7 Video); hypermetric wheelbarrowing (two dogs); delayed postural reactions in a puppy, which were no longer evident in adulthood (one dog); absent menace reaction (six dogs); slow medial eye movements on examination of oculocephalic movements (one dog); and nystagmus (four dogs). The last was described as positional nystagmus (one dog), alternating horizontal jerk nystagmus and erratic nystagmus (one dog, S8 Video) or horizontal nystagmus (two dogs). The menace reaction remained absent or reduced in four dogs beyond 12 weeks. One dog was presented for generalized epileptic seizures at four years of age (dog 10).

Imaging

Retrospective cohort (dogs 1–5, Table 3): In all dogs, a uniform cerebellar malformation characterized by the absence of caudal portions of the cerebellar vermis and symmetrical hypoplasia of the cerebellar hemispheres was identified. Large retrocerebellar fluid accumulations associated with an enlarged fourth ventricle were identified on midsagittal views (dog 4, S1 Fig.). A butterfly shape of the cerebellum corresponding to remnants of the cranial aspects of the vermis and cerebellar hemispheres was evident on dorsal views. The reduced size of the cerebellar vermis was more accurately assessed on MR than on CT images. Cranial displacement of the tentorium cerebelli and an enlarged caudal fossa were evident in two dogs (dogs 3 and 5) of the retrospective cohort. Additional supratentorial anomalies included hydrocephalus internus (3 dogs), asymmetric lateral ventricles (1 dog), and a poorly visible corpus callosum (1 dog).

Prospective cohort (dogs 6–11, Table 3, Figs. 1–4): In all dogs, a uniform cerebellar malformation was identified that was characterized by the absence of the caudal portions of the cerebellar vermis and, to a lesser degree, the caudal aspects of the cerebellar hemispheres as well as large caudal (posterior) fossa fluid accumulations (S2–S6 Figs.). The fourth ventricle appeared

Table 1. Clinical findings in Eurasier dogs with inferior cerebellar hypoplasia resembling a Dandy-Walker-like malformation (DWLM).

Dog	Sex	Age at presentation	Presenting complaint	Neurological examination (abnormal findings)	Seizures	Clinical course and lifespan
1	F	8 w	Ataxia, circling	Circling and moderate ataxia, rolling, falling	Yes (onset 1 y)	Non-progressive, 11 y (alive)
2	M	6 w	Ataxia, circling, and head tremors	Generalized ataxia, head tremors, reduced postural reactions (all limbs)	N/A	Improved, 6 y (alive)
3	F	5 w	Ataxia, head tremors	Generalized ataxia, hypermetric limb movements, head tremors	Yes (onset 4 y)	Improved ataxia, 6 y (alive)
4	F	5 w	Ataxia	Generalized ataxia, hypermetric limb movements, head tremors, reduced postural reactions (all limbs)	Yes (onset < 1 y)	Euthanasia at 10 m of age (due to poorly controlled seizures)
5	F	6 w	Pelvic limb weakness	Severe generalized ataxia, falling to the left, reduced postural reactions (proprioceptive positioning) all limbs, cervical pain	N/A	Euthanasia at 5 m of age (for unknown reasons)
6	F	6 w	Subtle incoordination, smaller size than littermates	Very mild dysmetric limb movements, truncal sway, subtle delay in initiation of postural reactions at initial presentation (unremarkable on follow-up examinations), oculocephalic movements with a subtle delay in medial eye movements, absent menace response on follow-up examination at 1 year of age	No	Non-progressive, 5 y (alive)
7	F	8 w	Ataxia and head tremors	Moderate generalized cerebellar ataxia of trunk and limbs with hypermetric limb movements, episodic falling, head tremors, horizontal nystagmus, absent menace response beyond 12 weeks of age	No	Improved ataxia, mild dysmetria. 2 y 10 m (alive)
8	M	8 w	Ataxia and head tremors	Moderate generalized cerebellar ataxia of trunk and limbs with hypermetric limb movements and episodic falling, head tremors, horizontal nystagmus, absent menace response beyond 12 weeks of age	No	Improved ataxia, mild dysmetria, 2 y 10 m (alive)
9	F	8 w	Ataxia, episodic rolling, problems calculating distances	Moderate cerebellar ataxia, rolling to the left and right side, hypermetric limb movements, head tremors (intention tremors), hypermetric hopping reactions (thoracic limbs), absent menace response beyond 12 weeks of age	No	Improved ataxia, 3 y 10 m (alive)
10	M	4 y 9 m	Recent onset of generalized seizures, non-progressive pelvic limb ataxia since 6 w aged	Mild pelvic limb ataxia, worse following exercise, mild ataxia of thoracic limbs when going downstairs, reduced menace response, horizontal jerk nystagmus and nystagmus with alternating directions, subtle delay in initiation of postural reactions, worse in the pelvic limbs	Yes (onset < 4 y)	Non-progressive, 6 y (alive)
11	F	9 w	Ataxia and inability to climb stairs	Mild generalized ataxia, mildly dysmetric gait, mild head tilt to the left, leaning to the left, subtle positional nystagmus	No	Improved, 8 y (alive)
12	M	5 w	Ataxia	Cerebellar ataxia	No	Euthanasia at 5 w of age
13	M	5 w	Ataxia	Ataxia characterized by mild dysmetria, worse in the pelvic limbs, and truncal sway	No	Euthanasia at 5 w of age
14	M	5 w	Ataxia	Ataxia characterized by mild dysmetria, worse in the pelvic limbs, and truncal sway	No	Euthanasia at 5 w of age

M. male; F: femaley: year; m: month; w: weeks; N/A: not assessed

doi:10.1371/journal.pone.0117670.t001

Table 2. Frequencies of the main clinical features in the two patient cohorts.

Clinical and neurological findings	Retrospective cohort (n = 8)	Prospective cohort (n = 6)
Smaller size for breed standards	-	17.0%
Mild to moderate generalized ataxia	87.5%	100.0%
Severe generalized ataxia	12.5%	-
Dysmetric/hypermetric gait	50.0%	83.0%
Truncal sway	25.0%	17.0%
Circling	25.0%	-
Episodic falling and/or rolling	25.0%	50.0%
Leaning	-	17.0%
Head tilt	-	17.0%
Head tremors	37.5%	50.0%
Absent menace reaction >12 weeks of age	N/A	67.0%
Nystagmus	N/A	67.0%
Slow medial eye movements	N/A	17.0%
Proprioceptive deficits	37.5%	33.0%
Delayed initiation of hopping and wheelbarrowing reaction	N/A	50.0%
Hypermetric wheelbarrowing	N/A	33.0%
Seizures	37.5%	17.0%

doi:10.1371/journal.pone.0117670.t002

enlarged on midsagittal views and contiguous with large retrocerebellar fluid accumulations (Fig. 1, Fig. 2; Fig. 3; S2–S6 Figs.). A thin structure traversing the fluid-filled spaces in the caudal portion of the caudal fossa was frequently identified on midsagittal views (Fig. 1A-D) and less consistently on dorsal views (Fig. 2B). The tentorium cerebelli was displaced rostrodorsally, and the caudal fossa appeared enlarged in one dog (dog 11, Fig. 4). Additional supratentorial findings, such as hydrocephalus (1 dog) or ventriculomegaly (1 dog), a poorly visible or subjectively thinned corpus callosum (3 dogs), and an absent septum pellucidum (2 dogs), were identified. MRI scans of littermates and both parents of litter 4 (dog 6), the dam of litter 5 (dogs 7 and 8) and the dam of litter 6 (dog 9) failed to reveal any structural abnormalities.

Morphometric measurements (Fig. 5, S1 Table): Calculation of the ratio of the caudal fossa area to the total braincase area on midsagittal images indicated wide variation of the caudal fossa size (n = 10; range 0.191–0.441; mean ± SD 0,287±0.074) with enlargement of the caudal fossa in three dogs with cerebellar hypoplasia (dog 3: 0.441; dog no. 5: 0.344; dog no. 11: 0.354) compared with their unaffected littermates and parents (n = 9; range 0.265–0.330; mean ± SD 0,305±0.021) and with the published ratios in other dog breeds [28].

Pedigree analysis

Pedigree analysis suggested autosomal recessive inheritance. Eight litters with single or multiple affected Eurasier dogs (Fig. 6) of both sexes were reported (median 26.5%–35.5% affected) (Fig. 7). Litter 1: dog 1 belonged to a litter of seven, and non-progressive ataxia was reported in two other dogs from this litter (14%–43% affected). Litter 2: dogs 2 and 3 belonged to a litter of six, and non-progressive ataxia was reported in one additional littermate (33%–50% affected). Litter 3: dogs 4, 5, and 10 were from a litter of eight (38% affected). In this litter, the caudal fossa appeared enlarged in one affected dog (dog 5) and was unremarkable in the two other affected dogs from the same litter. Litter 4: dog 6 was the only confirmed case in a litter of five (20% affected). Litter 5: dogs 7 and 8 were from a litter of six (33% affected). Litter 6: dog 9 presented from a litter of five (20% affected). Litter 7: dog 11 was the only confirmed case in a litter

Table 3. Neuroimaging findings in Eurasier dogs with familial non-progressive ataxia and inferior cerebellar hypoplasia resembling a DWLM.

Dog	Caudal fossa imaging findings	Hydrocephalus	Size of caudal fossa
1	MRI—Absent caudal portion of the cerebellar vermis, enlarged size of the fourth ventricle, retrocerebellar fluid accumulations, absent caudal parts of the cerebellar hemispheres, normal positioned tentorium cerebelli osseum	None	Unremarkable
2	MRI—Large caudal fossa fluid accumulation dorsal to the brainstem, no visible cerebellar structure	Severe hydrocephalus internus (lateral ventricles), septum pellucidum not visible	Unremarkable
3	CT—Large hypodensity suggestive of fluid accumulation in the caudal fossa, visible tissue remnants rostrally in the caudal fossa. these present in a butterfly shape with rostrodorsal and ventral fluid accumulations dorsal to the brainstem on transverse sections, elevated tentorium cerebelli osseum (	Moderate hydrocephalus internus (lateral ventricles)	Increased
4	MRI—Absent caudal portions of the cerebellar vermis, absent caudal parts of the cerebellar hemispheres, large cyst-like appearance of the fourth ventricle, cyst extends into the retrocerebellar region, caudal border outlined by a linear structure with soft tissue density on midsagittal T2-weighted images	Moderate unilateral hydrocephalus with asymmetric lateral ventricles, corpus callosum poorly identifiable	Unremarkable
5	CT—Large hypodensity in the caudal fossa suggestive of fluid accumulation, visible tissue remnants rostrally and dorsally in the rostral third of the caudal fossa, these present in a butterfly shape with rostrodorsal and ventral fluid accumulations dorsal to the brainstem in the midline on transverse sections, elevated tentorium cerebelli osseum*	Moderate hydrocephalus internus (lateral ventricles, third ventricle), small quadrigeminal cyst	Increased
6	MRI—Absent caudal portions of the cerebellar vermis and cerebellar hemispheres with associated large retrocerebellar fluid accumulations, rostrodorsal portions of the cerebellar hemispheres preserved, fourth ventricle appears enlarged, caudally in the caudal fossa a thin band-like structure traversing the caudal fossa in a ventrodorsal direction; other findings: subtentorial flattening and thickening of the supraoccipital bone resulting in a triangular shape*	None, septum pellucidum not visible	Unremarkable
7	MRI—Absent caudal portions of the cerebellar vermis and cerebellar hemispheres, large retrocerebellar fluid accumulations, rostrodorsal portions of the cerebellar hemispheres preserved, fourth ventricle appears enlarged on midsagittal views, thin band-like structure in the caudal fossa at the level of the most caudal extend of the cerebellar hemisphere remnants; other findings: subtentorial flattening and thickening of the supraoccipital bone	None, subjectively thinned appearance of corpus callosum	Unremarkable
8	MRI—Absent caudal portions of the cerebellar vermis and cerebellar hemispheres, with associated large retrocerebellar fluid accumulations, rostrodorsal portions of the cerebellar hemispheres preserved, fourth ventricle appears enlarged on midsagittal views with thin band-like structure in the caudoventral caudal fossa; other findings: thin band-like structure in the caudoventral caudal fossa, subtentorial flattening and thickening of the supraoccipital bone	None, subjectively thinned appearance of corpus callosum	Unremarkable
9	MRI—Absent caudal portions of the cerebellar vermis and cerebellar hemispheres with associated large retrocerebellar fluid accumulations, rostrodorsal portions of the cerebellar hemispheres preserved with indiscernible foliae, sulci and fissures and ill-defined grey-white matter transition. Fourth ventricle appears enlarged on midsagittal views and continuous with retrocerebellar fluid accumulations on dorsal views. Other findings: thin lamellar structure in the caudoventral caudal fossa; subtentorial flattening and thickening of the supraoccipital bone resulting in a triangular shape and an irregular caudodorsal contour of the caudal fossa	None, subjectively thinned appearance of corpus callosum	Unremarkable

(*Continued*)

Table 3. (*Continued*)

Dog	Caudal fossa imaging findings	Hydrocephalus	Size of caudal fossa
10	MRI—Absent caudal portions of the cerebellar vermis and the cerebellar hemispheres with associated large retrocerebellar fluid accumulations, rostrodorsal portions of the cerebellar hemispheres preserved with indiscernible foliae, sulci and fissures and ill-defined grey-white matter transition. Fourth ventricle appears enlarged on midsagittal views, but continuous with retrocerebellar fluid accumulations on dorsal views. Other findings: thin lamellar structure in the caudoventral caudal fossa; subtentorial flattening and thickening of the supraoccipital bone resulting in a triangular shape and an irregular caudodorsal contour of the caudal fossa	Mild hydrocephalus internus (lateral ventricles), septum pellucidum not visible	Unremarkable
11	CT—Large hypodensity in the caudal fossa suggestive of fluid accumulation, visible tissue remnants rostrally in the caudal fossa. Tissue remnants present in a butterfly shape with rostrodorsal and ventral fluid accumulations in the midline on transverse sections; enlargement of the caudal fossa and elevated tentorium cerebelli, bilateral symmetric osseus lamina protruding into the caudal fossa from rostrodorsal creating the impression of a split tentorium	Severe hydrocephalus (lateral ventricles), quadrigeminal cyst	Increased

MRI: magnetic resonance images; CT: computed tomographic images

doi:10.1371/journal.pone.0117670.t003

of five (20% affected). Litter 8: dogs 12, 13 and 14 were from a litter of seven dogs (43% affected). All affected dogs (n = 14) were traced back to a common founder, a female dog introduced into the breeding program in 1972 (Fig. 7).

Clinical course and life span

Five dogs were euthanized as puppies or within their first year of life. One of these was euthanized at 10 months because of severe seizures which were refractory to treatment with antiepileptic drugs. The other nine dogs were still alive at study conclusion (age 2.8 to 11 years; median 6 years). The ataxia was non-progressive in all nine dogs and improvement was reported in six dogs (Table 1). Video documentation was obtained from two dogs which had shown severe ataxia as puppies (S3 Video) and considerable improvement of the ataxia in adulthood (S4 Video). Four dogs developed epileptic seizures. No change in behavior, attitude or mental status was appreciated by the owners in any of the dogs, and all dogs were considered completely functional pets.

Discussion

Cerebellar hypoplasia resembling DWLM was diagnosed in 14 closely related purebred Eurasier dogs with familial non-progressive ataxia. The similar imaging phenotype, the existence of multiple affected dogs in several litters, and the fact that all cases were traced to a common founder suggested an inherited developmental defect. A VLDLR receptor mutation was subsequently identified in these dogs [27].

Affected Eurasier dogs displayed non-progressive ataxia with an early onset when the dogs began to ambulate. Other clinical signs were nystagmus, an absence of the bilateral or unilateral menace reaction beyond 10–12 weeks of age, and epileptic seizures occurring later in life in some dogs. Thus the clinical phenotype of the dogs reflects VLDLR-associated cerebellar hypoplasia in humans, who display severe truncal ataxia (dysequilibrium syndrome) as the main clinical manifestation [29–35]. Similar to our dogs peripheral ataxia of the limbs, loss of smooth pursuit eye movements and epileptic seizures occur in some humans with VLDLR-associated cerebellar

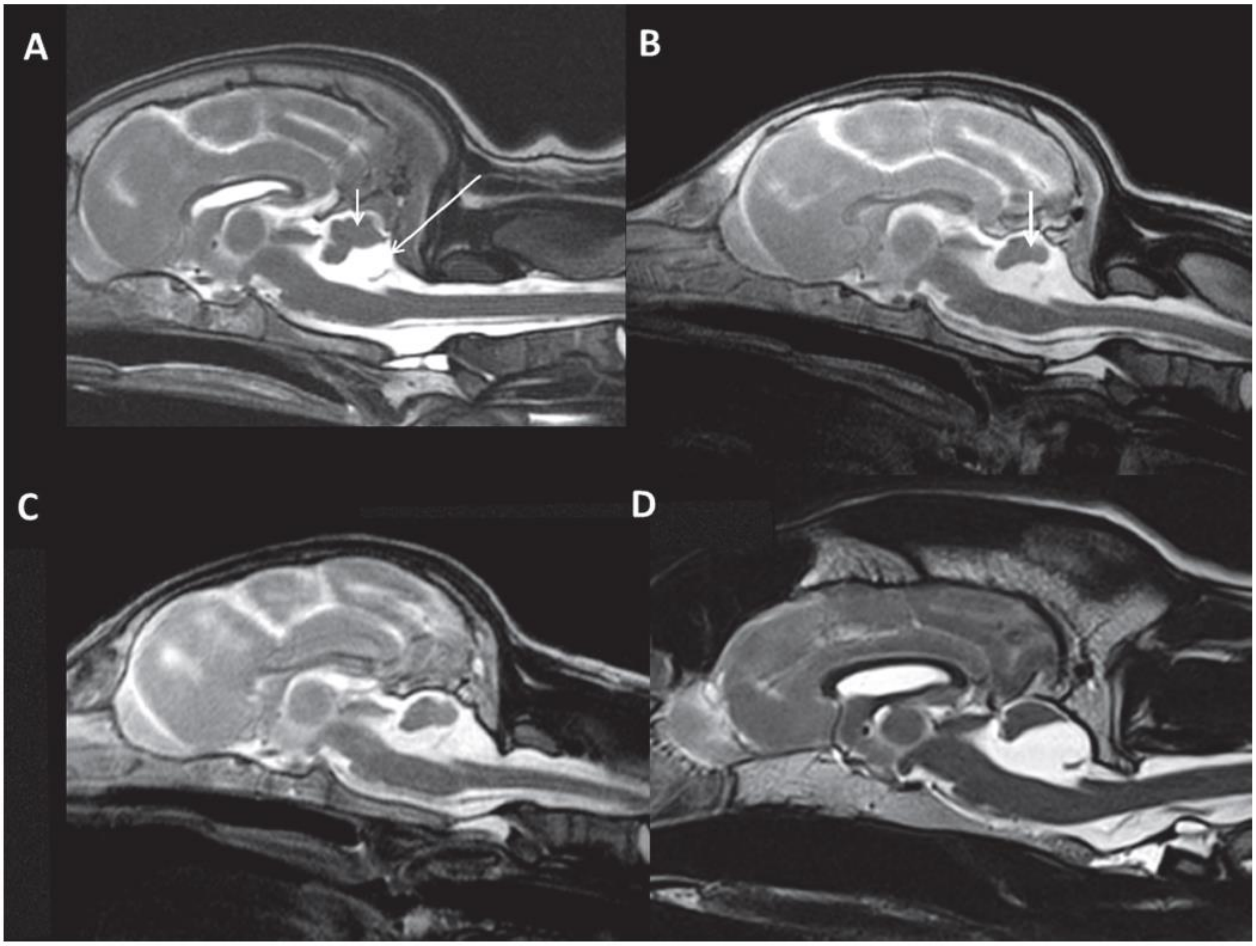

Fig 1. Midsagittal MR brain images. Midsagittal MR brain images (T2-weighted) of the affected Eurasier dogs. In all dogs, a uniform cerebellar malformation was identified, characterized by absence of the caudal portions of the cerebellar vermis and, to a lesser degree, the caudal aspects of the cerebellar hemispheres and large caudal (posterior) fossa fluid accumulations (S2 –S6 Figs.). The fourth ventricle appeared enlarged on midsagittal views and continuous with large retrocerebellar fluid accumulations (large arrow). Tissue remnants in the rostrodorsal caudal fossa correspond to the rostral portions of the cerebellar vermis (small arrow). Note the cyst-like appearance of the fourth ventricle in D. A: dog 6; B: dog 7; C: dog 9; D: dog 10.

doi:10.1371/journal.pone.0117670.g001

hypoplasia [29,33]. More clinical heterogeneity is recognized in human patients with DWM and its variants; these individuals may exhibit a variety of clinical features ranging from none to neurocognitive deficits, motor delay, hypotonia, speech delay, autistic features, ocular symptoms including nystagmus and strabismus to cerebellar ataxia, dizziness, and epileptic seizures [6,36–39] It is of interest that the dogs` carers did not recognize any relevant neurobehavioral signs or mental retardation in their dogs while neurocognitive deficits are frequently associated with VLDLR-associated cerebellar hypoplasia and also with DWM and its variants. Specifically, individuals with VLDLR-receptor associated cerebellar hypoplasia may never gain the ability to speak and remain mentally retarded [29–35]. While this is in contrast to the observations in our dogs, we cannot exclude that the dogs` carers did not appreciate mild to moderate mental retardation in their dogs or that mental retardation was missed in dogs of the retrospective study part.

In some dogs, the gait abnormalities persisted throughout life, while marked improvement was noted in others that had displayed severe cerebellar ataxia of the trunk and limbs as puppies (S3 Video) and showed only subtle gait abnormalities in adulthood (S4 Video). These

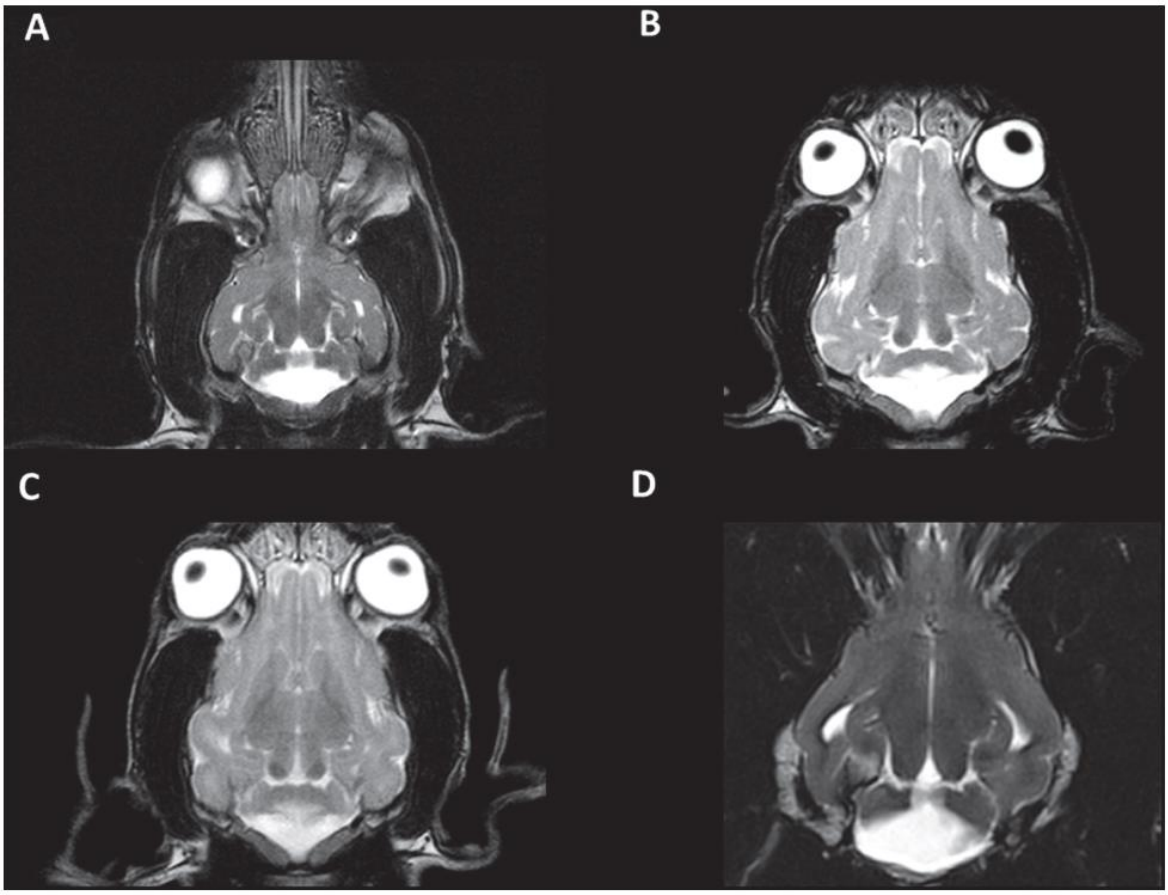

Fig 2. Dorsal MR brain images. Dorsal MR brain images (T2-weighted) of the affected Eurasier dogs reveal a prominent midline defect with absent caudal portions of the cerebellar vermis (midline) and cerebellar hemispheres (lateral) in association with a large retrocerebellar fluid accumulation. A: dog 6; B: dog 7; C: dog 9; D: dog 10. Images A and D are located more ventrally than B and C.

doi:10.1371/journal.pone.0117670.g002

findings support the presence of compensatory mechanisms or plasticity of the remaining cerebellum. The quadrupedal locomotion in some humans with VLDLR-associated cerebellar hypoplasia is explained by behavioral adaptation to the severe orthostatic instability [33,35]. As dogs walk on their four legs with a lower center of gravity, dogs may be able to adapt more easily. Repeated measurements of the degree of the ataxia should be highly valuable in future cases of cerebellar malformations in dogs and humans but were not assessed as part of this study. An absent menace reaction was considered an important indicator of cerebellar disease in affected Eurasier dogs older than twelve weeks with mild symptoms or barely visible ataxia. Thus the menace reaction should be carefully evaluated in any Eurasier dog that has a subtle gait disturbance and has not yet undergone genetic testing or MR screening for identification of a cerebellar malformation. Epileptic seizures developed later in life in some affected dogs. The seizures appeared unrelated to the presence of hydrocephalus and partial agenesis of the corpus callosum and we did not appreciate lissencephaly. Detailed assessment for other MR correlates of seizures was impaired due to lack of appropriate breed and age-matched MR controls. Epileptic seizures occur in a proportion of humans with VLDLR-associated cerebellar hypoplasia [29,33], and also with DWM and related malformations [6,36–39].

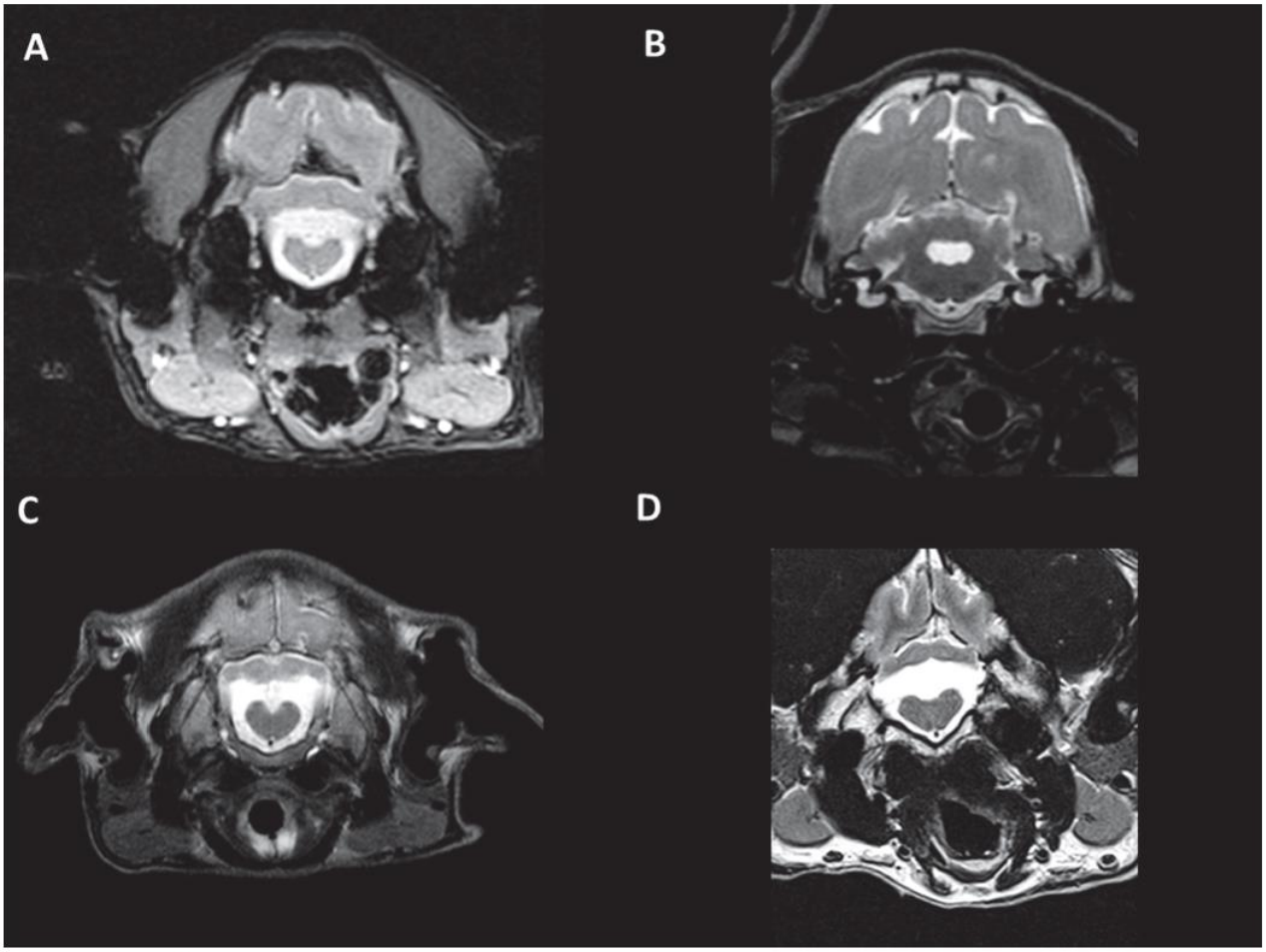

Fig 3. Transverse MR brain images. Transverse MR brain images (T2-weighted) of four affected Eurasier dogs at the level of the cerebellar peduncles (B) and medulla oblongata (A, C, D). The myelencephalon appears unremarkable. The fourth ventricle has a cyst-like appearance in the rostral sections (B) and is continuous with retrocerebellar cerebrospinal fluid accumulations in the more caudal sections (A, C, D). A: dog 6; B: dog 7; C: dog 9; D: dog 10.

doi:10.1371/journal.pone.0117670.g003

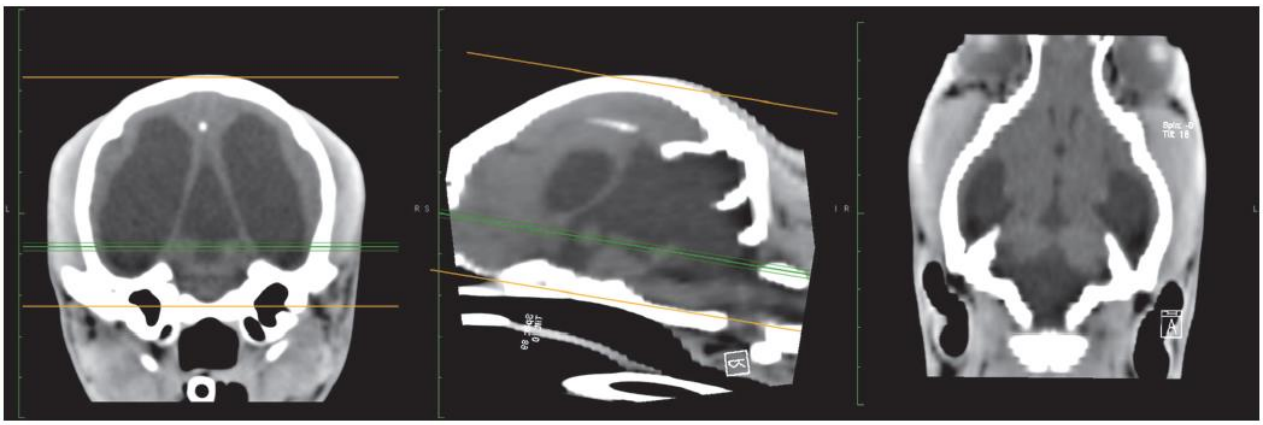

Fig 4. CT brain images. Computed tomographic images of dog 11, featuring transverse views of the brain at the level of the tympanic bullae, midsagittal and dorsal views. Images show hydrocephalus of the lateral ventricles, supracollicular fluid accumulation ("quadrigeminal cyst") and large fluid accumulations in the caudal fossa dorsal to the brainstem. The cerebellar remnant tissue in the caudal fossa has a butterfly shape consistent with the loss of midline cerebellar vermis structures. The caudal fossa appears enlarged.

doi:10.1371/journal.pone.0117670.g004

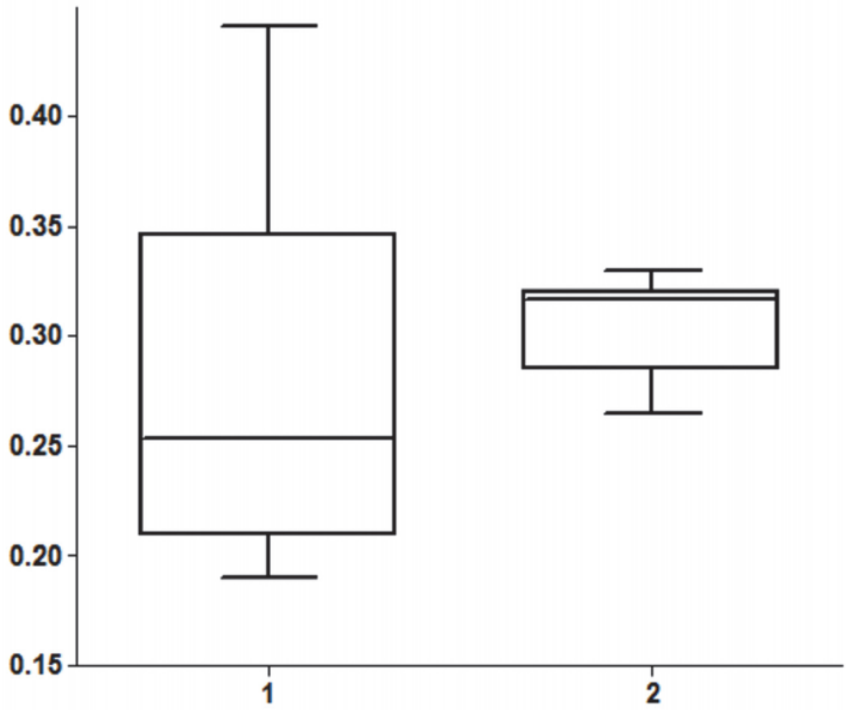

Fig 5. Midline caudal fossa ratio in Eurasier dogs with (1) and without (2) cerebellar hypoplasia. Boxplots demonstrate wide variations in midline caudal fossa ratio in Eurasier dogs with inferior cerebellar hypoplasia resembling DWLM (1: range 0.191–0.441; n = 9) compared to Eurasier dogs with unremarkable brain images (2: range 0.2645–0.3300; n = 10). Midline caudal fossa ratio was increased in three dogs with inferior cerebellar hypoplasia resembling DWLM (S1 Table).

doi:10.1371/journal.pone.0117670.g005

Abscence of the caudal portions of the cerebellar vermis and the caudal portions of the cerebellar hemispheres in association with large caudal fossa fluid accumulation were the main imaging finding in each Eurasier dog. There was close correlation between the specific malformation and the clinical signs because all affected dogs showed signs of non-progressive cerebellar disease, mainly ataxia and dysmetria. Additional vestibular signs like head tilt in one dog and nystagmus may be explained by flocculondular lobe involvement. The menace reaction may be abolished by diffuse cerebellar disorders [1,22]. Thus the cerebellar hypoplasia in our dogs mirrors closely the inferior cerebellar hypoplasia described in humans in association with VLDLR mutations [29–35]. The very low-density lipoprotein receptor is part of the reelin pathway which modulates neuronal migration in the cerebral cortex and cerebellum [1,40]. The different types of VLDLR mutations in humans share a unique pathologic picture characterized by inferior cerebellar hypoplasia with absence of the caudal (posterior) portions of the cerebellar vermis and the cerebellar hemispheres. Other pathological features are a small pons and a simplified cortical sulcation pattern in humans [33]. Yet additional pathologic features with resemblance to classic DWM were present in a proportion of dogs. We observed wide variation in caudal fossa size with an enlarged caudal fossa supportive of classic DWM in three dogs, a normal sized caudal fossa in others and both phenotypes within the same litter (Fig. 5, S1 Table). Enlargement of the posterior fossa is required for the diagnosis of classic DWM in humans and differentiates classic DWM from isolated vermis agenesis/hypoplasia and generalized hypoplasia of the cerebellar vermis and hemispheres [14,15]. In humans, controversy exists regarding when the posterior fossa should be considered large enough to qualify as DWM

Fig 6. Eurasier dog breed.

doi:10.1371/journal.pone.0117670.g006

rather than vermian hypoplasia [14], and some authors have recommended abandonment of the term "DW variant" [10–12,15,41,42]. Furthermore, genetic studies have demonstrated that the loss of a specific gene or interstitial deletion of a chromosome can lead to variable phenotypes, ranging from mild cerebellar vermis hypoplasia to classic DWM [43,44], and their classification within the group of mesenchymal-neuroepithelial signaling defects is suggested [16,17]. Concurrent supratentorial anomalies were identified in a proportion of our dogs. Among these, hydrocephalus, which was severe in some cases, was the most common finding, followed by a subjectively thinned corpus callosum or an incomplete septum pellucidum. Enlarged lateral ventricles attributed to persistence of the fetal ventricular system rather than true hydrocephalus were reported in human reelin mutations [40], but neither hydrocephalus nor agenesis of the corpus callosum have been described in humans with VLDLR-associated cerebellar hypoplasia, while ventriculomegaly and agenesis of the corpus callosum are the most commonly identified supratentorial malformations in humans with DWM [15,42], Other malformations, such as inter-hemispheric cysts or encephaloceles, gray matter heterotopias, malformations of the dentate nucleus and brainstem, hamartomas, and lissencephaly are also described with DWM [10,15,37,38,45,46]. In the past, hydrocephalus has frequently been

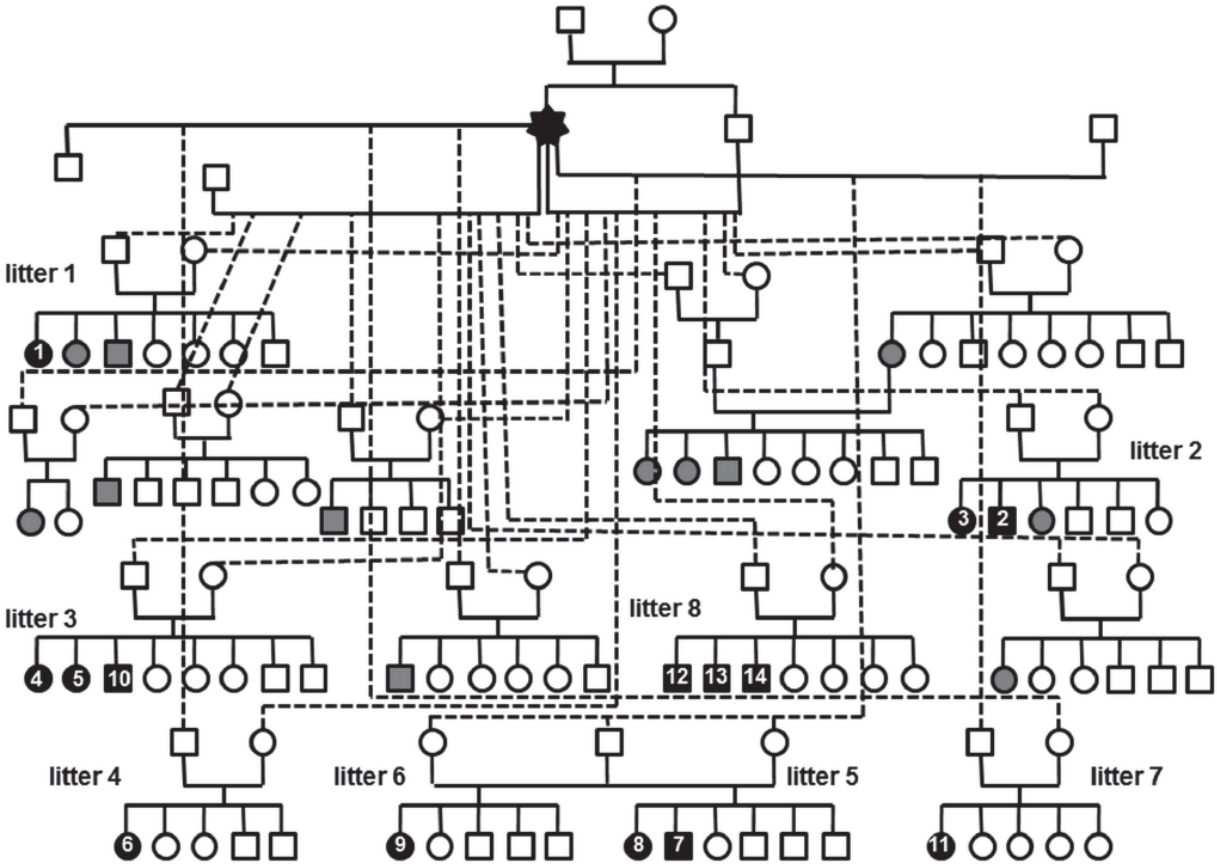

Fig 7. Pedigree. Pedigree of Eurasier dogs with familial non-progressive ataxia and cerebellar hypoplasia resembling a Dandy-Walker like malformation (DWLM). Female dog, ○; male dog, □; black, confirmed cases; the numbers refer to the dog numbers in Tables 1 and 2; gray, suspected cases, based on clinical signs, not confirmed by imaging. All cases could be traced to a common female founder.

doi:10.1371/journal.pone.0117670.g007

recognized in dogs in association with agenesis of the cerebellar vermis and a Dandy-Walker malformation was frequently suggested [18–20,22,23,24].

The data presented in our dog families suggested autosomal recessive inheritance and excluded X-linked or dominant inheritance because both sexes were affected, and both parents of one affected dog were healthy as assessed by MRI. The percentage of affected littermates was most compatible with autosomal recessive inheritance and with the observed high frequency of the VLDLR gene mutation in the population [27]. Up to now, seven different VLDLR mutations are recognized in humans. These are phenotypically and neuroanatomically indistinguishable and inherited in an autosomal recessive fashion population [33,35]. Mutations in WDR81 and the CA8 cause a similar phenotype in humans [35,47]. In contrast, the genetic basis of DWM in humans has not been completely clarified. DWM can occur as an isolated syndrome or together with clinically recognizable genetic syndromes (Meckel-Gruber and Walker-Warburg Syndrome); alternatively, DWM can have a multifactorial origin [15,48–50]. Familial cases of DWM have been described and several candidate genes for DWM have been recently uncovered. These include the cerebellar genes ZIC1 and ZIC4, FOXC1, a de novo 2.3-Mb deletion of chromosome 8p21.2-p21.3 associated with down regulation of the FGF17 gene, and ZIC2 and ZIC5 on the long arm of chromosome 13 [51–57]. In addition to these findings, DWM has been reported in a wide variety of other chromosomal anomalies

[15,49,57–59]. The etiological heterogeneity and yet undefined genetic basis of DWM impair current prenatal and genetic counselling [60,61].

The outcomes of the dogs with cerebellar hypoplasia resembling DWLM varied in the present investigation. Some dogs were euthanatized as puppies presumably due to severe ataxia, but in those that continued to live with their carers the ataxia was non-progressive and did not interfere with the dogs` normal activity and behavior. Many of these affected dogs appeared to develop normally and showed a learning capacity and behavior that were normal for the breed. One dog was euthanized because of epileptic seizures that were refractory to standard antiepileptic therapy. The functional outcome of children with DWM is still poorly defined because of disease heterogeneity [15,37]. DWM usually presents as an isolated case of hydrocephalus in pediatric patients but occasionally presents clinically in adults [14,56]. Mental retardation is common in cases of a severely abnormal lobulated vermis [37]. The introduction of shunts as a surgical treatment to reestablish the posterior fossa architecture has dramatically improved the prognosis of DWM [10,14,15,56].

In summary, an inferior cerebellar hypoplasia resembling DWLM was diagnosed in purebred Eurasier dogs with early-onset, non-progressive ataxia. Pedigree data suggested autosomal recessive inheritance. Most recently, we identified a deletion in the VLDLR receptor gene based on the information provided by the dogs from the present study [27]. Eurasier dogs with cerebellar hypoplasia resembling DWLM provide a spontaneous animal model that may help to investigate the long-term consequences and cogniti-ve outcomes of this disease and provide insights into the mechanisms of cerebellar development.

Supporting Information

S1 Fig. MRI of dog 4. Midsagittal, dorsal and transverse T2W MR brain images of the caudal fossa. Corresponding levels are outlined by green lines.
(TIF)

S2 Fig. MRI of dog 6. Midsagittal, dorsal and transverse T2W MR brain images of the caudal fossa. Corresponding levels are outlined by green lines.
(TIF)

S3 Fig. MRI of dog 7. Midsagittal, dorsal and transverse T2W MR brain images of the caudal fossa. Corresponding levels are outlined by green lines.
(TIF)

S4 Fig. MRI of dog 8. Midsagittal T2W MR brain image (littermate of dog 7; dorsal and transverse MR views are unavailable from this dog).
(TIF)

S5 Fig. MRI of dog 9. Midsagittal, dorsal and transverse T2W MR brain images of the caudal fossa. Corresponding levels are outlined by green lines.
(TIF)

S6 Fig. MRI of dog 10. Midsagittal, dorsal and transverse T2W MR brain images at the level of the caudal fossa. Corresponding levels are outlined by green lines.
(TIF)

S1 Table. Morphometric measurements.
(DOCX)

S1 Video. Cerebellar ataxia in the puppies (dogs 12–14).
(MP4)

S2 Video. Dog 6—Subtle dysmetria and truncal sway. Dog 6 displayed only a subtle dysmetric gait, which was barely detectable. The neurologic examination revealed an absent menace response and a subtle delay in medial eye movements on examination of oculocephalic movements. This dog was followed for two years. Repeat examinations showed a still absent menace response.
(MP4)

S3 Video. Dogs 7 and 8—Cerebellar ataxia in the puppies. Dogs 7 and 8 showed cerebellar ataxia of the trunk and limbs with hypermetria as puppies on initial examination.
(MP4)

S4 Video. Dogs 7 and 8—Improved ataxia in the adult dogs. Reevaluation of dogs 7 and 8 at one year of age: The ataxia was barely visible when the dogs were running or walking. The dogs still had problems calculating distance and easily lost balance when jumping. Some difficulty ascending and descending stairs was observed (shown at the end of the video sequence). The menace reaction was still absent, and an intermittent spontaneous horizontal nystagmus was recorded during the neurologic examination.
(MP4)

S5 Video. Dog 10—Subtle incoordination while navigating stairs. Dog 10 was presented because of a recent onset of epileptic seizures at four years of age. The owner complained about non-progressive pelvic limb ataxia since the dog was a puppy. The dog exhibited subtle thoracic limb incoordination when walking downstairs and "bunny-hopping" of the pelvic limbs when walking upstairs.
(MP4)

S6 Video. Dog 11—Mild ataxia and head tilt. Dog 11 showed a mild dysmetric gait with leaning to the left and a head tilt to the left. Positional nystagmus was evident in this dog during the neurologic examination.
(MP4)

S7 Video. Dog 10—Postural reactions. Dog 10 exhibited delayed initiation of movements (hemiwalk and wheelbarrowing). The pelvic limbs appeared more impaired.
(MP4)

S8 Video. Dog 10—Nystagmus. Dog 10 displayed episodes with alternating directions of nystagmus and horizontal jerk nystagmus.
(MP4)

Acknowledgments

The authors thank the breed club Kynologische Zuchtgemeinschaft Eurasier e.V. for initiating this investigation and for their outstanding efforts and support throughout this study. We wish that all breed clubs would show the type of responsibility that you do. Special appreciation is also extended to Susanne Lindsteding, the dog owners and their veterinarians who participated in the data acquisition.

Author Contributions

Conceived and designed the experiments: FB KR AL AF. Performed the experiments: FB KR AB MS KM AL HAS AF. Analyzed the data: FB KR AB MS KM AL HAS AF. Contributed reagents/materials/analysis tools: KR HAS MS. Wrote the paper: FB KR MS AB KM LM AL AF.

References

1. DeLahunta A, Glass E (2009) Cerebellum. Veterinary Neuroanatomy and Clinical Neurology. St. Louis: Saunders Elsevier. pp. 348–388. doi: 10.1002/wsbm.50 PMID: 20930952
2. Palmer AC, Payne JE, Wallace ME (1973) Hereditary quadriplegia and amblyopia in the Irish Setter. J Small Anim Pract 14: 343–352. PMID: 4803922
3. Knecht CD, Lama CH, Schaible R, Pflum K (1979) Cerebellar hypoplasia in Chow-Chows. J Am Anim Hosp Assoc 15: 51–53.
4. Résibois A, Coppens A, Poncelet L (2007) Naturally occurring parvovirus-associated feline hypogranular cerebellar hypoplasia—a comparison to experimentally-induced lesions using immunohistology. Vet Pathol 44: 831–841. PMID: 18039896
5. Schatzberg SJ, Haley NJ, Barr SC, Parrish C, Steingold S, et al. (2003) Polymerase chain reaction amplification of parvoviral DNA from the brains of dogs and cats with cerebellar hypoplasia. J Vet Intern Med 17: 538–544. PMID: 12892305
6. Dandy WE, Blackfan KD (1914) Internal hydrocephalus. An experimental, clinical and pathological study. Am J Dis Child 8: 406–482.
7. Taggart JK Jr, Walker AE (1942) Congenital atresia of the foramens Luschka and Magendie. Arch Neurol 48: 583–612.
8. Benda CE (1954). The Dandy-Walker syndrome or the so-called atresia of the foramen of Magendie. J Neuropathol Exp Neurol 13: 14–39. PMID: 13118372
9. Shekdar K (2011) Posterior fossa malformations. Semin Ultrasound CT MRI 32: 228–241. doi: 10.1053/j.sult.2011.02.003 PMID: 21596278
10. Spennato P, Mirone G, Nastro A, Buonocore MC, Ruggiero C, et al. (2011) Hydrocephalus in Dandy-Walker malformation. Childs Nerv Syst 27: 1665–1681. doi: 10.1007/s00381-011-1544-4 PMID: 21928031
11. Niesen CE (2002) Malformations of the posterior fossa: current perspectives. Semin Pediatr Neurol 9: 320–334. PMID: 12523556
12. Malinger G, Lev D, Lerman-Sagie T (2009) The fetal cerebellum. Pitfalls in diagnosis and management. Prenat Diagn 29: 372–380. doi: 10.1002/pd.2196 PMID: 19194867
13. Barkovich JA, Kjos BO, Norman D, Edwards MS (1989) A revised classification of posterior fossa cysts and cystlike malformations based on the results of multiplanar MR imaging. Am J Neuroradiol 10: 977–988.
14. Patel S, Barkovich AJ (2002) Analysis and classification of cerebellar malformations. Am J Neuroradiol 23: 1074–1087. PMID: 12169461
15. Parisi MA, Dobyns WB (2003) Human malformations of the midbrain and hindbrain: review and proposed classification scheme. Mol Genet Metab 80: 36–53. PMID: 14567956
16. Barkovich AJ, Millen KJ, Dobyns WB (2009). A developmental classification for midbrain-hindbrain malformations. Brain 132: 3199–3230. doi: 10.1093/brain/awp247 PMID: 19933510
17. Barkovich AJ (2012) Developmental disorders of the midbrain and hindbrain. Frontiers Neuroanat 6: 7
18. Noureddine C, Harder R, Olby NJ, Spaulding K, Brown T (2004) Ultrasonographic appearance of Dandy-Walker-like syndrome in a Boston Terrier. Vet Radiol Ultrasound 45: 336–339. PMID: 15373261
19. Choi H, Sangkyu K, Seongmok J, Sungwhan C, Kichang L, et al. (2007) Imaging diagnosis—cerebellar vermis hypoplasia in a miniature Schnauzer. Vet Radiol Ultrasound 48: 129–131. PMID: 17385369
20. Lim JH, Kim DY, Yoon JH, Kim WH, Kweon OK (2008) Cerebellar vermian hypoplasia in a Cocker Spaniel. J Vet Sci 9: 215–217. PMID: 18487946
21. Schmidt MJ, Jawinski S, Wigger A, Kramer M (2008) Imaging diagnosis—Dandy-Walker malformation. Vet Radiol Ultrasound 49: 264–266. PMID: 18546782
22. Kornegay JN (1986) Cerebellar vermian hypoplasia in dogs. Vet Pathol 23: 374–379. PMID: 3750731
23. Dow RS (1940) Partial agenesis of the cerebellum in dogs. J Comp Neurol 72: 569–586.
24. Pass DA, Howell McC, Thompson RR (1981) Cerebellar malformation in two dogs and a sheep. Vet Pathol 18: 405–407. PMID: 7257084
25. Kobatake Y, Miyabayashi T, Yada N, Kachi S, Ohta G, et al. (2013) Magnetic resonance imaging diagnosis of Dandy-Walker-like syndrome in a Wire-haired Miniature Dachshund J Vet Med Sci 75: 1379–1381. PMID: 23719692
26. Schmid V, Lang J, Wolf M (1992) Dandy-Walker-like syndrome in four dogs: cisternography as a diagnostic aid. J Am Anim Hosp Assoc 28: 355–360.

27. Gerber M, Fischer A, Jagannathan V, Drögemüller M, Drögemüller C, et al. (2015) A deletion in the *VLDLR* gene in Eurasier dogs with cerebellar hypoplasia resembling a Dandy-Walker-like malformation (DWLM). PLoS ONE 10(2): e0108917.

28. Carrera I, Dennis R, Mellor DJ, Penderis J, Sullivan M (2009) Use of magnetic resonance imaging for morphometric analysis of the caudal cranial fossa in Cavalier King Charles Spaniels. Am J Vet Res 70: 340–345. doi: 10.2460/ajvr.70.3.340 PMID: 19254145

29. Glass H, Boycott K, Adams C, Barlow K, Scott JN et al. (2005) Autosomal recessive cerebellar hypoplasia in the Hutterite population: a syndrome of non-progressive cerebellar ataxia with mental retardation. Dev Med Child Neurol 47: 691–695. PMID: 16174313

30. Oczelik T, Akarsu N, Uz E, Caglayan S, Gulsuner S et al. (2008) Mutations in the very low-density lipoprotein receptor cause cerebellar hypoplasia and quadrupedal locomotion in humans. Proc Natl Acad Sci 105: 4032–4036. doi: 10.1073/pnas.0800376105 PMID: 18322013

31. Moheb LA. Tzschach A, Garshasbi M, Kahrizi K, Darvish H et al. (2008) Identification of a nonsense mutation in the very low-density lipoprotein receptor gene (VLDLR) in an Iranian family with dysequilibrium syndrome. Eur J Hum Gen 16: 270–273. PMID: 18043714

32. Türkmen S, Hoffmann K, Demirhan O, Aruoba D, Humphrey N, et al. (2008) Cerebellar hypoplasia with quadrupedal locomotion caused by mutations in the very low-density lipoprotein receptor gene. Eur J Hum Genet 16: 1070–1074. doi: 10.1038/ejhg.2008.73 PMID: 18364738

33. Boycott KM, Bonnemann C, Herz J, Neuert S, Beaulieu C, et al. (2009) Mutations in VLDLR as cause for autosomal recessive cerebellar ataxia with mental retardation.J Child Neurol 24: 1310–1315. doi: 10.1177/0883073809332696 PMID: 19332571

34. Kolb LE, Arlier Z, Yalcinkaya C, Ozturk KA, Moliterno JA, et al. (2010) Novel VLDLR microdeletion identified in two Turkish siblings with pachygyria and pontocerebellar atrophy. Neurogenetics 11: 319–325. doi: 10.1007/s10048-009-0232-y PMID: 20082205

35. Ali BR, Silhavy JL, Gleeson MJ, Gleeson JG, Al-Gazai L (2012) A missense founder mutation in VLDLR is associated with dysequilibrium syndrome without quadrupedal locomotion. BMC Medical Genetics 13:80. doi: 10.1186/1471-2350-13-80 PMID: 22973972

36. Stoll C, Huber C, Alembik Y, Terrade E, Maitrot D (1990) Dandy-Walker variant malformation, spastic paraplegia, and mental retardation in two sibs. Am J Med Genet 37: 124–127. PMID: 2240029

37. Klein O, Pierre-Kahn A, Boddaert N, Parisot D, Brunelle F (2003) Dandy-Walker malformation: prenatal diagnosis and prognosis. Childs Nerv Syst 19: 484–489. PMID: 12879343

38. Weimer J, Cohen M, Wiedemann U, Heinrich U, Jonat W, et al. (2006) Proof of partial imbalances 6q and 11q due to maternal complex balanced translocation analyzed by microdissection of multicolor labeled chromosomes (FISH-MD) in a patient with Dandy-Walker variant. Cytogenet Genome Res 114: 235–239. PMID: 16954659

39. Economou A, Katsetos CD (2012) Patterns of cognitive and fine motor deficits in a case of Dandy-Walker Continuum. J Child Neurol 27: 930–937. doi: 10.1177/0883073811429500 PMID: 22241712

40. Hong SE, Shugart YY, Huang DT, Shawan SA, Grant PE, et al. (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat Genet 26:93–96. PMID: 10973257

41. Garel C, Fallet-Bianco C, Guibaud L (2011) The fetal cerebellum: development and common malformations. J Child Neurol 26: 1483–1492. doi: 10.1177/0883073811420148 PMID: 21954430

42. Bolduc ME, Limperopoulos C (2009) Neurodevelopmental outcomes in children with cerebellar malformations: a systematic review. Dev Med Child Neurol 51: 256–267. doi: 10.1111/j.1469-8749.2008.03224.x PMID: 19191827

43. Aldinger KA, Lehmann OJ, Hudgins L, Chizhikov VV, Bassuk AG, et al. (2009) FOXC1 is required for normal cerebellar development and is a major contributor to chromosome 6p25–3 Dandy-Walker malformation. Nat Genet 41: 1037–1044. doi: 10.1038/ng.422 PMID: 19668217

44. Millen KJ, Gleeson JG (2008) Cerebellar development and disease. Curr Opin Neurobiol 18: 12–19. doi: 10.1016/j.conb.2008.05.010 PMID: 18513948

45. Alexiou GA, Sfakianos G, Prodromou N (2010) Dandy-Walker malformation: analysis of 19 cases. J Child Neurol 25: 188–191. doi: 10.1177/0883073809338410 PMID: 19833975

46. Notaridis G, Ebbing K, Giannakopoulos P, Bouras C, Kövari E (2006) Neuropathological analysis of an asymptomatic adult case with Dandy-Walker variant. Neuropathol Appl Neurobiol 32: 344–350. PMID: 16640653

47. Sarac O, Gulsuner S, Yildiz-Tasci Y, Oczelik T, Kansu T (2012). Neuro-ophthalmologic findings in humans with quadrupedal locomotion. Ophthalmic Genet 33: 259–252.

48. Murray JC, Johnson JA, Bird TD (1985) Dandy-Walker malformation: etiologic heterogeneity and empiric recurrence risks. Clin Genet 28: 272–283. PMID: 4064366

49. Imataka G, Yamanouchi H, Arisaka O (2007) Dandy-Walker syndrome and chromosomal abnormalities. Congenit Anom 47: 113–118. PMID: 17988252

50. Abdel-Salam GMH, Shehab M, Zaki MS (2006) Isolated Dandy-Walker malformation associated with brain stem dysgenesis in male sibs. Brain Dev 28: 529–533. PMID: 16564660

51. Grinberg I, Northrup H, Ardinger H, Prasad C, Dobyns WB, et al. (2004) Heterozygous deletion of the linked genes ZIC1 and ZIC4 is involved in Dandy-Walker malformation. Nat Genet 36: 1053–1055. PMID: 15338008

52. Blank MC, Grinberg I, Aryee E, Laliberte C, Chizhikov VV, et al. (2011) Multiple developmental programs are altered by loss of Zic1 and Zic4 to cause Dandy-Walker malformation cerebellar pathogenesis. Development 138:1207–1216. doi: 10.1242/dev.054114 PMID: 21307096

53. Lim BC, Park WY, Seo EJ, Kim KJ, Hwang YS, et al. (2011) De novo interstitial deletion of 3q22.3-q25.2 encompassing FOXL2, ATR, ZIC1, and ZIC4 in a patient with Blepharophimosis/Ptosis/Epicantus inversus syndrome, Dandy-Walker malformation, and global developmental delay. J Child Neurol 26: 615–618. doi: 10.1177/0883073810384996 PMID: 21471554

54. Ferraris A, Bernardini L, Avramovska VS, Zanni G, Loddo S, et al. (2013) Dandy-Walker malformation and Wisconsin syndrome: novel cases add further insight into the genotype-phenotype correlations of 3q23q25 deletions. Orphanet J Rare Dis 8: 75. doi: 10.1186/1750-1172-8-75 PMID: 23679990

55. Zanni G, Barresi S, Travaglini L, Bernardini L, Rizza T, et al. (2011) FGF17, a gene involved in cerebellar development, is downregulated in a patient with Dandy-Walker malformation carrying a de novo 8p deletion. Neurogenetics 12: 241–245. doi: 10.1007/s10048-011-0283-8 PMID: 21484435

56. McCormack WM Jr, Shen JJ, Curry SM, Berend SA, Kashork C, et al. (2002) Partial deletions of the long arm of chromosome 13 associated with holoprosencephaly and the Dandy-Walker malformation. Am J Med Genet A 112: 384–389.

57. Mademont-Soler I, Morales C, Armengol L, Soler A, Sánchez A (2010) Description of the smallest critical region for Dandy-Walker malformation in chromosome 13 in a girl with a cryptic deletion related to t (6;13)(q23;q32). Am J Med Genet A 152A: 2308–2312. doi: 10.1002/ajmg.a.33550 PMID: 20683983

58. Sasaki-Adams D, Elbabaa SK, Jewells V, Carter L, Campbell JW, et al. (2008) The Dandy-Walker variant: a case series of 24 pediatric patients and evaluation of associated anomalies, incidence of hydrocephalus, and developmental outcomes. J Neurosurg Pediatr 2: 194–199. doi: 10.3171/PED/2008/2/9/194 PMID: 18759601

59. Wakeling EM, Jolly M, Fisk NM, Gannon C, Holder SE (2002) X-linked inheritance of Dandy-Walker variant. Clin Dysmorphol 11: 15–18. PMID: 11822699

60. Vasudevan C, McKechnie L, Levene M (2012) Long-term outcome of antenatally diagnosed agenesis of corpus callosum and cerebellar malformations. Semin Fetal Neonatal Med 17: 295–300. doi: 10.1016/j.siny.2012.07.001 PMID: 22840681

61. Guibaud L, Larroque Ville D, Sanlaville D, Till M, Gaucherand P, et al. (2012) Prenatal diagnosis of 'isolated' Dandy–Walker malformation: imaging findings and prenatal counselling. Prenat Diagn 32: 185–193. doi: 10.1002/pd.3828 PMID: 22418964

IV. DISCUSSION

Cerebellar vermian hypoplasia resembling DWLM was confirmed in 14 Eurasier dogs presented with non progressive ataxia. Multiple affected dogs in each litter and a close relationship between litters suggested an inherited disorder in the Eurasier dog breed. To our knowledge, this study was the first confirming the existence of an inherited congenital cerebellar hypoplasia in dogs. To date, confirmed genetic etiology was limited to the inherited progressive cerebellar ataxias, also known as cerebellar cortical abiotrophy or cerebellar cortical degeneration (O'BRIEN, 1993; CHIETTO et al., 1994; STEINBERG et al., 2000; DE LAHUNTA & GLASS, 2009; URKASEMSIN et al., 2010; SHEARMAN et al., 2011; FORMAN, et al., 2012; KYÖSTILÄ et al., 2012; AGLER et al., 2014; URKASEMSIN & OLBY, 2014). Evidence for inheritance of cerebellar hypoplasia resembling DWLM in dogs has been limited to descriptions in three Boston Terriers (DOW, 1940; NOUREDDINE et al., 2004). Subsequently based on the results of this study and blood collected from the affected Eurasier dogs of the present study, a mutation in the VLDLR gene was identified in these dogs (GERBER et al., 2015). Thus, an autosomal recessive mode of inheritance, which was already suspected by the median percentage of affected littermates, and the fact that an X-linked or dominant inheritance could be excluded because both sexes were affected, and both parents of one affected dog were healthy as assessed by MRI, was confirmed.

Clinical presentation

Affected dogs presented clinically variable degrees of non-progressive cerebellar ataxia when the dogs began to ambulate. The severity of the cerebellar ataxia ranged from barely visible, to moderate cerebellar ataxia characterized by a hypermetric gait or severe cerebellar ataxia with rolling and falling. In some cases, a symmetrical truncal ataxia was observed. Interestingly, cerebellar ataxia considerably improved with time and was barely visible in some of the adult dogs or only evident when walking stairs. Nystagmus, absence of the bilateral or unilateral menace reaction beyond 10^{th} to 12^{th} week of age, intention tremors, and epileptic seizures were also observed. Thus phenotype as some similarities with the autosomal recessive VLDLR-associated cerebellar hypoplasia (VLDLR-CH)

in humans, also known as dysequilibrium syndrome (DES), displaying severe truncal ataxia, peripheral ataxia of the limbs, strabismus, intentional tremors, and epileptic seizures (BOYCOTT et al., 2005; GLASS et al., 2005; MOHEB et al., 2008; OZCELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; SARAC et al., 2012). It also shares some of the clinical features, such as the nystagmus, the cerebellar ataxia, and epileptic seizures, of DWM in humans (STOLL et al., 1990; KLEIN et al., 2003; ECONOMOU & KATSETOS, 2012). In humans, the phenotype (clinical and neuroimaging) of VLDLR-CH patients is highly specific (ALI et al., 2012), whereas DWM and its variants are characterized by more clinical and neuroimaging heterogeneity (STOLL et al., 1990; KLEIN et al., 2003; ECONOMOU & KATSETOS, 2012). Human patients with DWM display extreme clinical heterogeneity, varying from completely asymptomatic with normal cognition and motor development, to severe mental retardation and impaired motor development characterized by motor delay, cerebellar ataxia and hypotonia, speech dysarthria, nystagmus, strabismus, dizziness and epileptic seizures (STOLL et al., 1990; KLEIN et al., 2003; ECONOMOU & KATSETOS, 2012). The neurocognitive deficits (moderate to severe mental retardation and dysarthric speech) observed in all VLDLR-CH patients (GLASS et al., 2005; OZCELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; SARAC et al., 2012), as well as in some DWM human patients (several degrees of mental retardation, speech delay, autistic features) (STOLL et al., 1990; KLEIN et al., 2003; ECONOMOU & KATSETOS, 2012), were not observed in our dogs. According to the owners affected dogs showed unchanged learning capacity, as well as normal social behaviour for the breed standards. However, a limitation is that no specific assessment was performed through questionaires about cognition and life quality of the affected dogs. Therefore, it is possible that mild to moderate mental retardation may have been missed with the subjective evaluation made by the dogs' owners. This finding is in accordance with the observation in the VLDLR-deficient mouse model, that appear neurologically normal (BOYCOTT et al., 2005; OZCELIK et al., 2008; TÜRKMEN et al., 2008), and as well with some human patients with DWM, who present with normal neurocognitive development (NOTARIDIS et al., 2006; JHA et al., 2012). Careful evaluation of the menace reaction beyond 12 weeks of age should be considered an important indicator of cerebellar disease in this breed. Some dogs of this study improved considerably, and in the adulthood the main

neurological deficit observed was the absence of the menace reaction and a barely visible ataxia, which sometimes was only evident when walking stairs. The epileptic seizures observed in some affected dogs were not associated with any concurrent supratentorial anomalies, such as hydrocephalus or partial agenesis of the corpus callosum. Since idiopathic epilepsy was diagnosed in three dogs that present with neurological symtoms in the prospective investigation, it remains unclear if the epileptic seizures are associated with supratentorial malformations, which were not identified due to the use of CT images in some, or MRI of low resolution in others, or the absence of protocols with age-matched MRI, or if the dogs in this study may, additionally, suffer from idiopathic epilepsy. Other diagnoses, such as isolated hydrocephalus and caudal fossa arachnoid cyst were also observed in this breed. Dogs with these diagnosis were not included in further genetic investigations. Thus, it remains unclear if these different pathologies are related to the identified VLDLR mutation. Further investigation is required.

Diagnostic procedure methods: magnetic resonance imaging versus computer tomography

Imaging was proposed for dogs presenting with any neurological signs, mainly cerebellar ataxia, and any previous imaging data were collected and reviewed. Various imaging protocols were applied in this study because of the partial retrospective design. The main neuroimaging findings in each Eurasier dog were an absence of the caudal portions of the cerebellar vermis and the caudal portions of the cerebellar hemispheres and a large caudal fossa fluid accumulation. Magnetic resonance imaging was far superior to CT for detailed assessment of the caudal fossa and identification of the anatomical structures of the cerebellar vermis and hemispheres, as well as any concurrent supratentorial malformation. In the dorsal view, computed tomography identified the partial or complete absence of the cerebellar vermis, as well as of the cerebellar hemispheres, although without anatomical accuracy. In the midsagittal view, the reduced size of the cerebellar hemispheres, cyst-like dilation of the fourth ventricle, caudal fossa size, position of the tentorium cerebelli osseum, and presence or absence of hydrocephalus were preferably evaluated. Computed tomography was able to confirm the diagnosis of cerebellar hypoplasia associated with large caudal fossa fluid accumulation. In humans, CT is not precise enough to evaluate the cerebellar vermis or identify concurrent malformations, such as partial or complete corpus

callosum agenesis (SPENNATO ET AL., 2011). Even CT scans with sagittal reconstruction were inadequate because of insufficient definition. Therefore, T2-weighted and FLAIR MR imaging with sagittal and dorsal views are necessary to accurately diagnose DWM and concurrent malformations in human patients (KLEIN et al., 2003; SHEKDAR, 2011). Furthermore, evaluation of the vermian anatomy, with identification of the two main fissures and characterization of the vermis lobulation, is critical in human patients (BODDAERT et al., 2003), because vermis anatomy is statistically correlated with neurological and intellectual outcome (KLEIN et al., 2003; BOLDUC & LIMPEROULOS, 2009).

Neuroimaging findings

The neuroimaging findings, mainly the absence of the caudal portions of the cerebellar vermis and hemispheres in our dogs, resembled the inferior cerebellar hypoplasia described in humans in association with VLDLR mutations (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; MOHEB et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; KOLB et al., 2010; ALI et al., 2012). However, a wide variation in the caudal fossa size with an enlarged caudal fossa supportive of classic DWLM in three dogs and a normal-sized caudal fossa in others were also observed. Interestingly, both phenotypes were identified within the same litter. In some of our dogs, hydrocephalus, which was severe in some cases and was the most common supratentorial anomaly, subjectively thinned corpus callosum or incomplete septum pellucidum, were concurrently observed. Neither hydrocephalus nor agenesis of the corpus callosum nor enlargement of the posterior fossa have been described as in humans with VLDLR-CH (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; KOLB et al., 2010; ALI et al., 2012), while in humans with DWM, these are commonly identified (PARISI & DOBYNS, 2003; BOLDUC & LIMPEROPOULOS, 2009). Simplified cortical gyration (lissencephaly), as well as a small brainstem, mainly in the area of the pons, are part of the characteristic imaging features of VLDLR-CH in humans (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; KOLB et al., 2010; ALI et al., 2012), but these features were not observed in our dogs. Identification of lissencephaly may have been impaired in our dogs, due to lack of age-matched controls. In the past, hydrocephalus has frequently been identified in dogs in

association with partial or complete agenesis of the cerebellar vermis and a DWLM has been suggested (Dow, 1940; PASS et al., 1981; KORNEGAY, 1986; NOUREDDINE et al., 2004; CHOI et al., 2007; LIM et al., 2008; SCHMIDT et al., 2008). Nevertheless, the size of the caudal fossa was never considered or investigated ih these dogs, which is an essential criterion in humans.

Differential Diagnosis

Enlargement of the posterior fossa, which is required for the diagnosis of classic DWM in humans, has been described with other cyst like lesions of the posterior fossa including arachnoid cysts, choroid plexus cysts in the fourth ventricle (described in two dogs) (MACKILLOP, 2011; SHEKDAR, 2011), persistent Blake's pouch characterized by tetra-ventricular hydrocephalus, mega-cisterna magna (PARISI & DOBYNS, 2003; ADAMSBAUM et al., 2005; GUIBAUD & DES PORTES, 2006; BOLDUC & LIMPEROULOS, 2009; SHEKDAR, 2011) and epidermoid/dermoid cyst (STEINBERG et al., 2007; SHEKDAR, 2011); conversely, differential diagnosis for cases without enlargement of the posterior fossa include non-cystic malformations such as, inferior vermian hypoplasia, Joubert syndrome, rhombencephalosynapsis, (PATEL & BARKOVICH, 2002; PARISI & DOBYNS, 2003; ADAMSBAUM et al., 2005; MALINGER et al., 2009; SHEKDAR, 2011; SPENNATO et al., 2011) and tectocerebellar dysraphism with occipital encephalocele (SPENNATO et al., 2011). Using conventional MRI, arachnoid cysts, mega cisterna magna (lined by arachnoid), and Blake's pouch (lined by ependyma) cannot be distinguished because their signals are similar to the signal of the CSF (SHEKDAR, 2011; PARISI & DOBYNS, 2003). However, these cysts and epidermoid/dermoid cysts (lined by epidermal tissue) can be distinguished by FLAIR imaging (PARISI & DOBYNS, 2003). Although not applied in this study, CT cisternography can be used to distinguish between DWM and arachnoid cysts (SHEKDAR, 2011). An arachnoid cyst should not produce enhancement or should only produce enhancement after a delay, because no communication exists with the surrounding subarachnoid space or fourth ventricle (SHEKDAR, 2011). Cysts that fill immediately with contrast are regarded as diverticula of the subarachnoid space (SHEKDAR, 2011). Modern MRI software permits not only morphological evaluation but also the study of CSF flow dynamics (SPENNATO et al., 2011). The use of this software may be warranted.

Controversy about the classification

In human medicine specific size criteria to measure posterior fossa size have not yet been clearly established in the literature. Rather, differentiation between classic DWM, DWV and IVH is based on to the experience of the neuroradiologists (BOLDUC & LIMPEROPOULOS, 2009). In reviewing the literature, particularly when referring to prenatal diagnosis of cerebellar malformations, the term DWV was often used synonymously with isolated vermian hypoplasia, inferior vermian hypoplasia and even with vermian dysgenesis/agenesis as part of the molar tooth syndromes (ADAMSBAUM et al., 2004; MALINGER et al., 2009). The use of this inaccurate terminology led to the question of, when the posterior fossa in humans should be considered large enough to qualify as classic DWM (NIESEN, 2002; MALINGER et al., 2009; MACKILLOP, 2011; SPENNATO et al., 2011). Consequently recomendation to abandon the term DWV appeared in the literature with increasing frequency (NIESEN, 2002; PARISI & DOBYNS, 2003; BOLDUC & LIMPEROPOULOS, 2009; MALINGER et al., 2009; GAREL et al., 2011; SPENNATO et al., 2011). Genetic investigation demonstrated that these different phenotypes may potentially share a common genotype, e.g. loss of a gene or deletion of a chromosome, and thus may also share a common pathogenesis (MILLEN & GLEESON, 2008; ALDINGER et al., 2009). Consequently they have been recently classified within the same group of mesenchymal-neuroepithelial signaling defects (BARKOVICH et al., 2009; BARKOVICH, 2012). We observed the two different phenotypes in the same litter sharing the same genotype, a mutation in the VLDLR gene. This supports the hypothesis that IVH, DWV and DWM share the same pathogenesis in at least some affected individuals. Instead of being different entities, they represent different phenotypes of the same mutation.

Etiology of Dandy-Walker malformation and very low density lipoprotein receptor associated cerebellar hypoplasia

In this study we propose autosomal recessive inheritance. Subsequently, a VLDLR mutation was identified (GERBER, 2015) based on the results and the blood samples of affected and control dogs collected by the author of this study. The etiology of VLDLR-CH in humans is very well characterized with an autosomal mode of inheritance and seven different mutations identified (BOYCOTT et al., 2009; ALI et al., 2012). These multiple mutations share a unique phenotype and neuroanatomically features (BOYCOTT et al., 2009; ALI et al., 2012). Mutations in WDR81 and the CA8 which also belong to the CAMRQ group, also cause a similar phenotype in humans (TÜRKMEN et al., 2009; GULSUNER et al., 2011). The three types of CAMRQ belong to reelin pathway, responsible for the modulation of neuronal migration in the cerebral cortex and cerebellum (HONG et al., 200; DE LAHUNTA & GLASS, 2009). On the other hand, the etiology of DWM in humans is characterized by a complex heterogeneity, involving environmental factors (e.g. rubella, toxoplasmose, cytomegalovirus) (NIESEN, 2002; PARISI & DOBYNS, 2003; SPENNATO et al., 2011), Mendelian disorders or chromosomal anomalies (STOLL et al., 1990; NIESEN, 2002; WAKELING et al., 2002; PARISI & DOBYNS, 2003; WEIMER et al., 2006; IMATAKA et al., 2007; SPENNATO et al., 2011). An autosomal recessive (STOLL et al., 1990; ABDEL-SALAM et al., 2006), X-linked recessive, multifactorial (WAKELING et al., 2002), and autosomal dominant inheritance have been described (JALALI et al., 2008). However it remains to be determined which genes are responsible for this malformation (PATEL & BARKOVICH, 2002). Several candidate genes for DWM have been recently identified. These include the genes ZIC1 and ZIC4, FOXC1 gene, FGF17 gene, and ZIC2 and ZIC5 genes (MCCORMACK ET AL., 2012; GRINBERG et al., 2004; MADEMONT-SOLER et al., 2010; BLANK et al., 2011; ZANNI et al., 2011; LIM et al, 2012; FERRARIS et al., 2013). Dandy-Walker malformation has also been reported in a wide variety of other chromosomal anomalies (NIESEN, 2002; PARISI & DOBYNS, 2003; WEIMER et al., 2006; IMATAKA et al., 2007; SASAKI-ADAMS et al., 2008), and the most common chromosomal defects observed are trisomy 18, triploidy and trisomy 13 (IMATAKA et al., 2007).

Outcome

The outcomes of the dogs in the study varied considerably. Three dogs were euthanized as puppies presumably due to severe ataxia; one because of epileptic seizures refractory to standard antiepileptic drugs, and another for unknown reasons. From the surviving dogs, some presented subtle cerebellar ataxia which persisted throughout life, while in others a marked improvement was noted. The physiological quadrupedal walk of the canine specie, which decreases the gravity center, may be the reason for the efficient and fast adaptation of our dogs. These findings support the presence of compensatory mechanisms or plasticity of the remaining cerebellum. The quadrupedal gait in some humans with VLDLR-CH may also be explained by behavioural adaptation to the severe orthostatic instability during the transition from crawling to bipedal walking (OZCELIK et al., 2008; TÜRKMEN et al., 2008; SARAC et al., 2012), or even the adaptation to environmental conditions, like uneven surfaces in rural areas and lack of adaptive devices, which would promote walking in four legs with a lower gravity centre, thus increasing stability (TÜRKMEN et al., 2008; BOYCOTT et al., 2009). Although, some agree with the theory that the quadrupedal gait results from special environmental influences (TÜRKMEN et al., 2008; BOYCOTT et al., 2009; SARAC et al., 2012), others consider extremely unlikely that social or environmental influences play any role in this adaptation (OZCELIK et al., 2008). The obligatory bipedal gait is a unique characteristic feature of primates, however the molecular and genetic pathways involved in this evolution mechanism are still unclear (OZCELIK et al., 2008). As referred previously, the cognition of the affected dogs appeared unchanged. Conversely, the functional outcome of children with DWM is still poorly defined because of disease heterogeneity (KLEIN et al., 2003; PARISI & DOLBYNS, 2003), but several cases of asymptomatic patients have been described (NOTARIDIS et al., 2006; JHA et al., 2012). When comparing DWV with classic DWM, some differences in the outcomes were noted, with isolated DWV patients appearing to have a better outcome when compared to those with classic DWM (SASAKI-ADAMS et al., 2008). Mental retardation is common in cases of a severely abnormal lobulated vermis in DWM patients (BODDAERT et al., 2003; KLEIN et al., 2003), as well as in VLDLR-CH patients (BOYCOTT et al., 2005; GLASS et al., 2005; OCZELIK et al., 2008; TÜRKMEN et al., 2008; BOYCOTT et al., 2009; KOLB

et al., 2010; ALI et al., 2012). The association with other CNS anomalies or systemic malformations was a negative factor for the outcome in patients with classic DWM or DWV patients (BODDAERT et al., 2003; KLEIN et al., 2003; SASAKI-ADAMS et al., 2008). The introduction of shunts (SPENNATO et al., 2011; MCCLELLAND et al. 2015) and more recently the use of endoscopic third ventriculostomy with or without cauterization of the choroid plexus, as surgical treatment to re-establish the posterior fossa architecture, has dramatically improved the prognosis in patients with hydrocephalus associated with DWM (WARF et al., 2011).

Limitations of the sutdy

This study has several potential limitations, mainly because of its retrospective part. The dogs of the retrospective part were not adequately evaluated neurologically by a board certified neurologist. Consequently, some important information may have been missed, which would have been helpful in a more accurate phenotypically characterization of the disease, as well as recognition of important indicators of this condition in this dog breed (e.g. absent menace reaction beyond twelve weeks of age). The lack of standardized imaging due to the multicentre nature of the study was a limitation of the study. The use of CT in some dogs and MRI in others, sometimes with lower image resolution, may have been responsible for missing some important information, such as associated malformations. It would have also been important that age matched-MR images have been used, to identify subtle alterations, such as lissencephaly, which is a feature of VLDLR-CH in humans. Detailed longitudinal studies on threadmills to accurately assess the degree, progression and regression of cerebellar ataxia, as well as the use of standardized questionaries to assess cognition, would have been important. Even more blood samples from healthy or even suspected dogs could have been collected, but there were some restrictions related to owner compliance and sometimes difficulty in reaching some of the patients living very far away from the points of blood collection.

Perspectives

A DNA test has been developed allowing for the identification of affected Eurasier and is available at www.generatio.de. These tests are particularly useful in the breeding programs to improve the genetic health of the breed

(MELLERSCH, 2014). However a judicious use of DNA tests is critical and veterinarians should be aware, that phenotypically similar conditions can be caused by different mutations, even when dogs belong to the same breed, because genetically distinct forms of a disease can segregate within the same breed. (MELLERSCH, 2014). DNA testing only confirms a single, specific mutation and absence of the mutation is not a guarantee that the dog will never have a clinically similar disease, although dogs with a negative result for a specific autosomal recessive mutations, can be considered at low risk for disease development (MELLERSCH, 2014). These tests play an important role in the control and eventual elimination of inherited diseases - mainly recessive diseases - which can be extremely difficult for the breeder to eliminate because of the clinically healthy carriers (MELLERSCH, 2014). Autosomal recessive inheritance and clinically healthy carriers were found in the Eurasier breed dogs presented in our study. As a consequence genetic testing prior to breeding will identify heterozygous animals and avoid breeding between them. Another very important role of these DNA tests, is to help the veterinarian diagnose a specific disease (MELLERSCH, 2014). However, one additional factor that should always be considered alongside the DNA test result is the age of onset. Since inherited disorders always have a specific age of onset (MELLERSCH, 2014). Congenital cerebellar malformations are usually evident when the puppy starts to walk, while cerebellar cortical degenerations present specific age of onset, characteristic for the disorder. In conclusion, DNA tests are definetely a valuable tool for diagnosis and like all test results, they should always be consider as a part of a complete clinical history of the patient. They are also particularly useful to breeders to improve the genetic health of the breed (MELLERSCH, 2014). Hopeffully genetic tests will be cautiously and judiciously implemented in the breeding programs, allowing the genotype of each individual to be considered in first place, instead of the “ideal” phenotype, which has led to a decreasing genetic pool and the accumulation of variable genetic diseases within dog breeds.

V. SUMMARY

Cerebellar malformations result from insults during embryogenesis or are inherited. Inherited cerebellar abiotrophies are frequently recognized in dogs and usually result in progressive ataxia. Evidence for inherited cerebellar hypoplasia is rare in the veterinary literature. Therefore the aim of this study was to describe the clinical and neuroimaging phenotype of a potentially inherited cerebellar malformation in Eurasier dogs. Clinical and imaging data of 32 Eurasier dogs with neurological signs were collected retrospectively and prospectively. An uniform cerebellar malformation characterized by absence of the caudal parts of the cerebellar vermis and the caudal parts of the cerebellar hemispheres in association with large retrocerebellar fluid accumulations was identified in 14 closely related purebred Eurasier dogs. Diagnosis was based on MRI (n = 8), CT (n = 3), or post-mortem examination (n = 3). A wide variation in caudal fossa size as well as hydrocephalus and thinning of the corpus callosum were observed in some dogs. Clinically the dogs showed non-progressive cerebellar ataxia, which was observed for the first time when the puppies began to walk. Variable degrees of ataxia were observed, varying from mild truncal sway, mild dysmetria, dysequilibrium and pelvic limb ataxia to severe cerebellar ataxia with episodic falling or rolling. Other clinical signs observed in some dogs were nystagmus, an absence of the bilateral or unilateral menace reaction beyond 10-12 weeks of age, head tremors and epileptic seizures with an age of onset between 5 months and 4 years of age. Follow-up examinations showed a pronounced improvement of the cerebellar ataxia in adult dogs. Detailed pedigree analyses, which included confirmation of the phenotype by MRI of littermates and parents of multiple litters suggested an autosomal recessive inheritance.

In conclusion, the neuroimaging phenotype observed in the Eurasier breed dogs is characterized by an autosomal recessive inherited inferior cerebellar hypoplasia with some resemblance to a Dandy-Walker-like malformation.

VI. ZUSAMMENFASSUNG

Missbildungen des Kleinhirns können durch die Einwirkung von Noxen in den verschiedenen Entwicklungsstadien des Kleinhirns oder genetisch bedingt sein. Genetisch bedingte Kleinhirndegenerationen wurden häufig beim Hund beschrieben. Diese zeigen sich mit progressive Ataxie und Kleinhirnatrophie. Es gibt in der Literatur jedoch nur wenige gesicherte Hinweise auf das Vorkommen einer erblichen Kleinhirnmissbildung beim Hund. Das Ziel dieser Studie war daher, den klinischen Phänotyp und die Befunde der bildgebenden Diagnostik einer potentiell vererbten Kleinhirnmissbildung beim Eurasier im Detail zu beschreiben. Klinische Befunde, Videoaufzeichnungen des Gangs und die Befunde der bildgebenden Diagnostik mit CT und MR von 32 Eurasiern mit neurologischen Symptomen wurden retrospektiv und prospektiv erhoben. Bei 14 Hunden wurde eine uniforme Kleinhirnmissbildung nachgewiesen, die durch Fehlen der kaudalen Anteile des cerebellären Vemis und der kaudalen Anteile der Kleinhirnhemisphären sowie groβe retrocerebelläre Flüssigkeitsansammlungen charakterisiert war. Die Diagnose basierte auf den Befunden im MR (n = 8), CT (n = 3) und histopathologischen Befunden (n = 3). Zusätzlich fiel eine erhebliche Variabilität in der Größe der hinteren Schädelgrube sowie bei einigen Hunden auch Hydrocephalus und eine unterschiedliche Ausprägung des Corpus callosum auf.

Klinisch zeigten die betroffenen Hunde eine nicht-progressive cerebelläre Ataxie, die sich erstmals zeigte wenn die Welpen zu laufen begannen. Der Grad der Ataxie variierte von mildem Schwanken des Rumpfes, milder Dysmetrie, Dysequilibrium und Ataxie der Hintergliedmaßen bis zu hochgradiger cerebellärer Ataxie mit episodischem Fallen oder Rollen. Andere klinische Symptome waren Nystagmus, persistierend fehlende Drohreaktion eines Auges oder beider Augen bei Hunden, die älter als 10-12 Wochen waren, Kopftremor und epileptische Anfälle, die erstmalig in einem Alter von 5 Monaten bis zu 4 Jahren auftraten. Folgeuntersuchungen zeigten eine deutliche Verbesserung der cerebellären Ataxie. Detaillierte Untersuchungen der Pedigrees und die eindeutige Erfassung des Phänotyps von Geschwistern und Eltern betroffener Hunde aus mehreren Würfen auf der Basis von MR Untersuchungen wiesen auf das Vorliegen einer autosomal rezessiv vererbte Kleinhirnmissbildung hin.

Zusammenfassend wurde in dieser Arbeit eine autosomal rezessiv vererbte Kleinhirnhypoplasie bei der Hunderasse Eurasier nachgewiesen, die auch einzelne Aspekte einer Dandy-Walker Malformation aufwies.

VII. REFERENCES

Abdel-Salam GMH, Shehab M, Zaki MS. Isolated Dandy-Walker malformation associated with brain stem dysgenesis in male sibs. Brain Dev 2006; 28: 529-533

Adamsbaum C, Moutard ML, André C, Merzoug V, Ferey S, Quéré MP, Lewin F, Fallet-Bianco C. MRI of the fetal posterior fossa. Pediatr Radiol 2005; 35: 124-140

Agler C, Nielsen DM, Urkasemsin G, Singleton A, Tonomura N, Sigurdsson S, Tang R, Linder K, Arepalli S, Hernandez D, Lindblad-Toh K, van de Leemput J, Motsinger-Reif A, O'Brien DP, Bell J, Harris T, Steinberg S, Olby NJ. Canine hereditary ataxia in old English sheepdogs and Gordon setters is associated with a defect in the autophagy gene encoding RAB24. PLoS Genet 2014; 10: e1003991

Aldinger KA, Lehmann OJ, Hudgins L, Chizhikov VV, Bassuk AG, Ades LC, Krantz ID, Dobyns WB, Millen KJ. FOXC1 is required for a normal cerebellar development and is a major contributor to chromosome 6p25.3 Dandy-Walker malformation. Nat Genet 2009; 41: 1037-1044

Alexiou GA, Sfakianos G, Prodromou N. Dandy-Walker malformation: analysis of 19 cases. J Child Neurol 2010; 25: 188-191

Ali BR, Silhavy JL, Gleeson MJ, Gleeson JG, Al-Gazali L. A missense founder mutation in VLDLR is associated with Dysequilibrium Syndrome without quadrupedal locomotion. BMC Med Genet 2012; 13: 80

Benda CE. The Dandy-Walker syndrome or the so-called atresia of the foramen Magendie. J Neuropathol Exp Neurol 1954; 13: 14-39

Biran V, Verney C, Ferriero DM. Perinatal cerebellar injury in human and animal models. Neurol Res Int. 2012; 2012: 1-9

Boddaert N, Klein O, Ferguson N, Sonigo P, Parisot D, Hertz-Pannier L, Baraton J, Emond S, Simon I, Chigot V, Schmit P, Pierre-Kahn A, Brunelle F. Intellectual prognosis of the Dandy-Walker malformation in children: the importance of vermian lobulation. Neuroradiology 2003; 45: 320-324

Bolduc ME, Limperopoulos C. Neurodevelopmental outcomes in children with

cerebellar malformations: a systematic review. Dev Med Child Neurol 2009; 51: 256-267

Boycott KM, Flavelle S, Bureau A, Glass HC, Fujiwara TM, Wirrell E, Davey K, Chudley AE, Scott JN, McLeod DR, Parboosingh JS. Homozygous deletion of the very low density lipoprotein receptor gene causes autosomal recessive cerebellar hypoplasia with cerebral gyral simplification. Am J Hum Genet 2005; 77: 477-483

Boycott KM, Bonnemann C, Herz J, Neuert S, Beaulieu C, Scott JN, Venkatasubramanian A, Parboosingh JS. Mutations in VLDLR as a cause for autosomal recessive cerebellar ataxia with mental retardation (dysequilibrium syndrome). J Child Neurol 2009; 24: 1310-1315

Bragg TWH, St. George EJ, Wynne-Jones GA, Hockley A, Morton JEV. Familial Dandy-Walker syndrome: a case report supporting an autosomal inheritance. Chids Nerv Syst 2006; 22: 539-541

Can SS, Karakaş Uğurlu G, Cakmak S. Dandy walker variant and bipolar I disorder with graphomania. Psychiatry Investig 2014; 11: 336-339

Charles HV, Johnny RC. Correlating magnetic resonance findings with neuropathology and clinical signs in dogs and cats. Vet Radiol Ultrasound 2011; 52, Supp. 1: S23-S31

Chieffo C, Stalis IH, Van Winkle TJ, Haskins ME, Patterson DF. Cerebellar Purkinje's cell degeneration and coat color dilution in a family of Rhodesian ridgeback dogs. J Vet Intern Med 1994; 8: 112-116

Choi H, Sangkyu K, Seongmok J, Sungwhan C, Kichang L, Kidono E, Lee H, Chang D, Yoon J, Lee Y. Imaging Diagnosis – cerebellar vermis hypoplasia in miniature Schnauzer. Vet Radiol Ultrasound 2006; 48: 129-131

Dandy WE: Internal hydrocephalus: An experimental, clinical and pathological study. Am J Dis Child 1914; 8: 406-482

De Lahunta A, Fenner WR, Indrieri RJ, Mellick PW, Gardner S, Bell JS. Hereditary cerebellar cortical abiotrophy in the Gordon setter. J Am Vet Med Assoc 1980; 177: 538-541

De Lahunta A, Glass E. Veterinary neuroanotomy and clinical neurology, 3rd

Edition. Philadelphia: Saunders Elsevier; 2009: 348-388

De Smet HJ, Paquier P, Verhoeven J, Mariën P. The cerebellum: its role in language and related cognitive and affective functions. Brain Lang 2013; 127: 334-342

Dow RS. Partial agenesis of the cerebellum in dogs. J Comp Neurol 1940; 72: 569-586

Economou A, Katsetos CD. Patterns of cognitive and fine motor deficits in a case of Dandy-Walker Continuum. J Child Neurol 2012; 27: 930-937

Feder A. Eurasier Heute, 2. Auflage. Mürlenbach/Eifel: Kynos Verlag; 2004: 3-10

Ferraris A, Bernardini L, Sabolic Avramovska V, Zanni G, Loddo S, Sukarova-Angelovska E, Parisi V, Capalbo A, Tumini S, Travaglini L, Mancini F, Duma F, Barresi S, Novelli A, Mercuri E, Tarani L, Italian CBCD Study Group, Bertini E, Dallapiccola B, Valente EM. Dandy-Walker malformation and Wisconsin syndrome: novel cases add further insight into the genotype-phenotype correlations of 3q23q25 deletions. Orphanet J Rare Dis 2013; 8: 75

Forman OP, De Risio L, Stewart J, Mellersh CS, Beltran E. Genome-wide mRNA sequencing of a single canine cerebellar cortical degeneration case leads to the identification of a disease associated SPTBN2 mutation. BMC Genet 2012; 13: 55

Friede RL. Developmental neuropathology, 1st Edition. Wien: Springer-Verlag; 1975: 314-326

Garel C, Fallet-Bianco C, Guibaud L. The fetal cerebellum: development and common malformations. J Child Neurol 2011; 26: 1483-1492

Gerber M, Fischer A, Jagannathan V, Drögemüller M, Drögemüller C, Schmidt MJ, Bernardino F, Manz E, Matiasek K, Rentmeister K, Leeb T. A Deletion in the VLDLR Gene in Eurasier Dogs with Cerebellar Hypoplasia Resembling a Dandy-Walker-Like Malformation (DWLM). PLoS One 2015; 10: e0108917

Glass HC, Boycott KM, Adams C, Barlow K, Scott JN, Chudley AE, Fujiwara TM, Morgan K, Wirrell E, McLeod DR. Autosomal recessive cerebellar hypoplasia in the Hutterite population. Dev Med Child Neurol 2005; 47: 691-695

Grinberg I, Northrup H, Ardinger H, Prasad C, Dobyns WB, Millen KJ.

Heterozygous deletion of the linked genes ZIC1 and ZIC4 is involved in Dandy-Walker malformation. Nat Genet 2004; 36: 1053-1055

Guibaud L, des Portes V. Plea for an anatomical approach to abnormalities of the posterior fossa in prenatal diagnosis. Ultrasound Obstet Gynecol 2006; 27: 477-481

Gulsuner S, Tekinay AB, Doerschner K, Boyaci H, Bilguvar K, Unal H, Ors A, Onat OE, Atalar E, Basak AN, Topaloglu H, Kansu T, Tan M, Tan U, Gunel M, Ozcelik T. Homozygosity mapping and targeted genomic sequencing reveal the gene responsible for cerebellar hypoplasia and quadrupedal locomotion in a consanguineous kindred. Genome Res 2011; 21: 1995-2003

Haldipur P, Gillies GS, Janson OK, Chizhikov VV, Mithal DS, Miller RJ, Millen KJ. Foxc1 dependent mesenchymal signalling drives embryonic cerebellar growth. Elife 2014; 3: e03962

Hong SE, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat Genet 2000; 26: 93-96

Imataka G, Yamanouchi H, Arisaka O. Dandy-Walker syndrome and chromosomal abnormalities. Congenit Anom 2007; 47: 113-118

Jalali A, Aldinger KA, Chary A, McLone DG, Bowman RM, Le LC, Jardine P, Newbury-Ecob R, Mallick A, Jafari N, Russell EJ, Curran J, Nguyen P, Ouahchi K, Lee C, Dobyns WB, Millen KJ, Pina-Neto JM, Kessler JA, Bassuk AG. Linkage to chromosome 2q36.1 in autosomal dominant Dandy-Walker malformation with occipital cephalocele and evidence for genetic heterogeneity. Hum Genet. 2008; 123: 237-245

Jha VC, Kumar R, Srivastav AK, Mehrotra A, Sahu RN. A case series of 12 patients with incidental asymptomatic Dandy-Walker syndrome and management. Childs Nerv Syst 2012; 28(6): 861-867

Klein O, Pierre-Kahn A, Boddaert N, Parisot D, Brunelle F. Dandy-Walker malformation: prenatal diagnosis and prognosis. Child Nerv Syst 2003; 19: 484-489

Knecht CD, Lama CH, Schaible R, Pflum K. Cerebellar hypoplasia in Chow-

Chows. J Am Anim Hosp Assoc 1979; 15: 51-53

Kobatake Y, Miyabayashi T, Yada N, Kachi S, Ohta G, Sakai H, Maeda S, Kamishina H. Magnetic resonance imaging diagnosis of Dandy-Walker-like syndrome in a wire-haired miniature dachshund. J Vet Med Sci 2013; 75: 1379-1381

Kolb LE, Arlier Z, Yalcinkaya C, Ozturk AK, Moliterno JA, Erturk O, Bayrakli F, Korkmaz B, DiLuna ML, Yasuno K, Bilguvar K, Ozcelik T, Tuysuz B, State MW, Gunel M. Novel VLDLR microdeletion identified in two Turkish siblings with pachygyria and pontocerebellar atrophy. Neurogenetics 2010; 11: 319-325

Kornegay JN. Cerebellar vermian hypoplasia in dogs. Vet Pathol 1986; 23: 374-379

Kyöstilä K, Cizinauskas S, Seppälä EH, Suhonen E, Jeserevics J, Sukura A, Syrjä P, Lohi HA. SEL1L mutation links a canine progressive early-onset cerebellar ataxia to the endoplasmic reticulum-associated protein degradation (ERAD) machinery. PLoS Genet 2012; 8: e1002759

Lim BC, Park WY, Seo EJ, Kim KJ, Hwang YS, Chae JH. De novo interstitial deletion of 3q22.3-q25.2 encompassing FOXL2, ATR, ZIC1, and ZIC4 in a patient with Blepharophimosis/Ptosis/Epicantus inversus syndrome, Dandy-Walker malformation, and global developmental delay. J Child Neurol 2011; 26: 615-618

Lim JH, Kim DY, Yoon JH, Kim WH, Kweon OK. Cerebellar vermis hypoplasia in a Cocker Spaniel. J Vet Sci 2008; 9: 215-217

Limperopoulos C, Robertson RL Jr, Khwaja OS, Robson CD, Estroff JA, Barnewolt C, Levine D, Morash D, Nemes L, Zaccagnini L, du Plessis AJ. How accurately does current fetal imaging identify posterior fossa anomalies? AJR Am J Roentgenol 2008; 190: 1637-1643

Limperopoulos C, Robertson RL, Estroff JA, Barnewolt C, Levine D, Bassan H, du Plessis AJ. Diagnosis of inferior vermian hypoplasia by fetal magnetic resonance imaging: potential pitfalls and neurodevelopmental outcome. Am J Obstet Gynecol 2006; 194: 1070-1076

MacKillop E. Magnetic resonance imaging of intracranial malformation in dogs

and cats. Vet Radiol Ultrasound 2011; 51, Supp. 1: 336-339

Mademont-Soler I, Morales C, Armengol L, Soler A, Sánchez A. Description of the smallest critical region for Dandy-Walker malformation in chromosome 13 in a girl with a cryptic deletion related to t(6;13)(q23;q32). Am J Med Genet A 2010 152A: 2308-2312

Malinger G, Lev D, Lerman-Sagie T. The fetal cerebellum. Pitfalls in diagnosis and management. Prenat Diagn 2009; 29: 372-380

Mancuso M, Orsucci D, Siciliano G, Bonuccelli U. The genetics of ataxia: through the labyrinth of Minotaur, looking for Ariadne's thread. J Neuro 2014; 261: S528-S541

McClelland S, Ukwuoma OI, Lunos S, Okuyemi KS. The natural history of Dandy-Walker syndrome in the United States: A population-based analysis. J Neurosci Rural Pract 2015; 6: 23-26

McCormack WM Jr, Shen JJ, Curry SM, Berend SA, Kashork C, Pinar H, Potocki L, Bejjani BA. Partial deletions of the long arm of chromosome 13 associated with holoprosencephaly and the Dandy-Walker malformation. Am J Med Genet A 2003; 112: 384-389

Mellersch C. Inherited neurological disorders in the dog. The science behind the solutions. Vet Clin North Am Small Anim Pract 2014; 44: 1222-1234

Millen KJ, Gleeson JG. Cerebellar development and disease. Curr Opin Neurobiol 2008; 18: 12-29

Moheb LA, Tzschach A, Garshasbi M, Kahrizi K, Darvish H, Heshmati Y, Kordi A, Najmabadi H, Ropers HH, Kuss AW. Identification of a nonsense mutation in the very low-density lipoprotein receptor gene (VLDLR) in an Iranian family with dysequilibrium syndrome. Eur J Hum Genet 2008; 16: 270-273

Murray JC, Johnson JA, Bird TD. Dandy-Walker malformation: etiologic heterogeneity and empiric recurrence risks. Clin Genet 1985; 28: 272-283

Nigri F, Cabral IF, da Silva RT, Pereira HV, Ribeiro CR. Dandy-walker malformation and Down syndrome association: good developmental outcome and successful endoscopic treatment of hydrocephalus. Case Rep Neurol 2014; 6: 156-160

Niesen CE. Malformations of the posterior fossa: current perspectives. Semin Pediatr Neurol 2002; 9: 320-334

Notaridis G, Ebbing K, Giannakopoulos P, Bouras C, Kövari E. Neuropathological analysis of an asymptomatic adult case with Dandy-Walker variant. Neuropathol Appl Neurobiol 2006; 32: 344-350

Noureddine C, Harder R, Olby NJ, Spaulding K, Brown T. Ultrasonograpic appearance of Dandy Walker-like syndrome in Boston Terrier. Vet Radiol Ultrasound 2004; 45: 336-339

O'Brien D. Hereditary cerebellar ataxia. From the Proceedings 11th ACVIM Forum. Washington, DC 1993; 546-549

Ozcelik T, Akarsu N, Uz E, Caglayan S, Gulsuner S, Onat OE, Tan M, Tan U. Mutations in the very low-density lipoprotein receptor VLDLR cause cerebellar hypoplasia and quadrupedal locomotion in humans. Proc Natl Acad Sci USA 2008; 105: 4232-4236

Palau F, Espinós C. Autosomal recessive cerebellar ataxia. Orphanet J Rare Dis 2006; 1: 47

Palmer AC, Payne JE, Wallace ME. Hereditary quadriplegia and amblyopia in the Irish setter. J Small Anim Pract 1973; 14: 343-352

Parisi MA, Dobyns WB. Human malformations of the midbrain and hindbrain: review and proposed classification scheme. Mol Genet Metab 2003; 80: 36-53

Pascual-Castroviejo I, Velez A, Pascual-Pascual SI, Roche MC, Villarejo F. Dandy-Walker malformation: analysis of 38 cases. Childs Nerv Syst 1991; 1: 88-97

Pass DA, Howell McC, Thompson RR. Cerebellar malformations in two dogs and a sheep. Vet Pathol 1981; 18: 405-407

Patel S, Barkovich AJ. Analysis and classification of cerebellar fossa malformations. Am J Neuroradiol 2002; 23: 1074-1087

Percy DH, Carmichael LE, Albert DM, King JM, Jonas AM. Lesions in puppies surviving infection with canine herpesvirus. Vet Pathol 1971; 8: 37-53

Raybaud C. Cystic malformations of the posterior fossa. Abnormalities associated

with the development of the roof of the 4th ventricle and adjacent meningeal structures. J Neuroradiol 1982; 9: 103-133

Rusbridge C, Knowler P, Rouleau GA, Minassian BA, Rothuizen J. Inherited occipital hypoplasia/syringomyelia in the cavalier King Charles spaniel: experiences in setting up a worldwide DNA collection. J Hered 2005; 96: 745-749

Sarac O, Gulsuner S, Yildiz-Tasci Y, Ozcelik T, Kansu T. Neuro-ophthalmologic findings in humans with quadrupedal locomotion. Ophthalmic Genet 2012; 33: 249-252

Sasaki-Adams D, Elbabaa SK, Jewells V, Carter L, Campbell JW, Ritter AM. The Dandy-Walker variant: a cases series of 24 pediatric patients and evaluation of associated anomalies, incidence of hydrocephalus, and developmental outcomes. J Neurosurg Pediatrics 2008; 2: 194-199

Schatzberg SJ, Haley NJ, Barr SC, Parrish C, Steingold S, Summers BA, deLahunta A, Kornegay JN, Sharp NJH. Polymerase chain reaction (PCR) amplification of parvoviral DNA from the brains of dogs and cats with cerebellar hypoplasia. J Vet Intern Med 2003; 17: 538-544

Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. Brain 1998; 121: 561-579

Schmahmann JD, Caplan D. Cognition, emotion and the cerebellum. Brain 2006; 129: 288-292

Schmidt MJ, Jawinski S, Wigger A, Kramer M. Imaging diagnosis – Dandy-Walker malformation. Vet Radiol Ultrasound 2007; 49: 264-266

Shearman JR, Cook RW, McCowan C, Fletcher JL, Taylor RM, Wilton AN. Mapping cerebellar abiotrophy in Australian Kelpies. Anim Genet 2011; 42: 675-678

Shekdar K. Posterior fossa malformations. Semin Ultrasound CT MRI 2011; 32: 228-241

Spennato P, Mirone G, Nastro A, Buonocore MC, Ruggiero C, Trischitta V, Aliberti F, Cinalli G. Hydrocephalus in Dandy-Walker malformation. Childs Nerv Syst 2011; 27: 1665-1681

Steinberg HS, Van Winkle T, Bell JS, de Lahunta A. Cerebellar degeneration in old English sheepdogs. J Am Vet Med Assoc 2000; 217: 1162-1165

Steinberg T, Matiasek K, Brühschwein A, Fischer A. Imaging diagnosis - intracranial epidermoid cyst in a Doberman Pinscher. Vet Radiol Ultrasound 2007; 48: 250-253

Stoll C, Huber C, Alembik Y, Terrade E, Maitrot D. Dandy-Walker variant malformation, spastic paraplegia, and mental retardation in two sibs. Am J Med Genet 1990; 37: 124-127

Türkmen S, Hoffmann K, Demirhan O, Aruoba D, Humphrey N, Mundlos S. Cerebellar hypoplasia, with quadrupedal locomotion, caused by mutations in the very low-density lipoprotein receptor gene. Eur J Hum Genet 2008; 16: 1070-1074

Türkmen S, Guo G, Garshasbi M, Hoffmann K, Alshalah AJ, Mischung C, Kuss A, Humphrey N, Mundlos S, Robinson PN. CA8 mutations cause a novel syndrome characterized by ataxia and mild mental retardation with predisposition to quadrupedal gait. PLoS Genet 2009; 5: e1000487

Urkasemsin G, Linder KE, Bell JS, de Lahunta A, Olby NJ. Hereditary cerebellar degeneration in Scottish terriers. J Vet Intern Med 2010; 24(3): 565-570

Urkasemsin G, Olby NJ. Canine hereditary ataxia. Vet Clin North Am Small Anim Pract 2014; 44: 1075-1089

Vasudevan C, McKechnie L, Levene M. Long-term outcome of antenatally diagnosed agenesis of corpus callosum and cerebellar malformations. Semin Fetal Neonatal Med 2012; 17: 295-300

Wakeling EL, Jolly M, Fisk NM, Gannon C, Holder SE. X-linked inheritance of Dandy-Walker variant. Clin Dysmorphol 2002; 11: 15-18

Wang VY, Zoghbi HY. Genetic regulation of the cerebellar development. Nat Rev Neurosci 2001; 2: 484-491

Warf BC, Dewan M, Mugamba J. Management of Dandy-Walker complex-associated infant hydrocephalus by combined endoscopic third ventriculostomy and choroid plexus cauterization. J Neurosurg Pediatr 2011; 8: 377-383

Weimer J, Cohen M, Wiedemann U, Heinrich U, Jonat W, Arnold N. Proof of partial imbalances 6q and 11q due to maternal complex balanced translocation analyzed by microdissection of multicolor labeled chromosomes (FISH-MD) in a patient with Dandy-Walker variant. Cytogenet Genome Res 2006; 114: 235-239

Wong D, Winter M, Haynes J, Sponseller B, Schleining J. Dandy-Walker-Like syndrome in a quarter horse colt. J Vet Intern Med 2007; 21: 1130-1134

Zanni G, Barresi S, Travaglini L, Bernardini L, Rizza T, Digilio MC, Mercuri E, Cianfarani S, Valeriani M, Ferraris A, Da Sacco L, Novelli A, Valente EM, Dallapiccola B, Bertini ES. FGF17, a gene involved in cerebellar development, is downregulated in a patient with Dandy-Walker malformation carrying a de novo 8p deletion. Neurogenetetics 2011; 12: 241-245

VIII. ACKNOWLEDGEMENT

First I would like to thank my supervising tutor apl. Prof. Dr. Andrea Fischer for all the time, attention, support, trust and freedom during our journey. It was a huge pleasure to work with you.

Prof. Dr. Katrin Hartmann I would also like to thank you for making all of this possible and for giving me the chance to work with such an amazing group of persons.

Special thanks to Dr. Kai Rentmeister, the pioneer of the project and always a great support.

Thanks to all the co-workers involved in this project for the great work together, for always being there for me when I needed; for all the constructive criticism: Prof. Dr. Kaspar Matiasek, Dr. Lara Matiasek, Ass. Prof. Dr. Martin Schmidt. Dr. Andreas Brühschwein, Alexander Lauda and Prof. Dr. Schoon.

Thanks to Prof. Dr. Tosso Leeb and Martina Gerber for making the genetic a reality in our project.

A very special thanks to Frau Ulrike Müller-Windus and her lovely dogs Aruna, Alyssa and Alessa, without your help, dedication, patience, trust and friendship nothing would have been possible.

A special thanks to the breeding club Zuchtgemeinschaft für Eurasier e. V. mainly Mr. and Mrs. Vorbeck, Dr. Susanne Lindsteding, Mrs. Andrea Sauer-Löw and Dr. Thomas Post.

Thanks to all the Eurasier owners and dogs for all your support, dedication, time and patience.

Thanks to the best team I have ever had, the neurology team: Velia, Katrin, Vivi, Barbara, Stephi, Elisabeth, Tina and Cyril, for supporting me any time I needed, for the knowledge, for the funny moments, for helping me write the reports in German!

Also many thanks to all the team in the Clinic of Small Animal Medicine, especially to the anesthesiology team, for being always supportive and helpful.

A special thanks to my very good friend Mariachen, for always being there for me whenever I needed it.

A huge thanks to my all family, my parents and my sister, for the unconditional love and the emotional (and financial) support throughout this long journey, for understanding how important this was for me, and for showing me every day how proud you are. I know it was not easy with the distance, but we made it!!

A special thanks to my dearest and unconditional supporter, David, for loving me and believing in me, for dealing with my sometimes difficult moods and for helping me whenever I needed.

At last, the most special thanks to my sunshine and love of my life Mia!