Canine Breed Specific Hepatopathies

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Abstract

Canine hepatopathies, both congenital and acquired, arise from an interaction between genes and environment. Many show increased breed prevalences. This article reviews the current understanding on breed predispositions for congenital portosystemic shunts; microvascular dysplasia and portal vein hypoplasia; ductal plate abnormalities (congenital hepatic fibrosis and Caroli's disease); chronic hepatitis (both copper associated and idiopathic); vacuolar hepatopathies, and gall bladder mucucoele. While all these disease can occur in many breeds and cross breeds, understanding breed predispositions helps recognition and will guide future research to improve understanding of causes and treatments.

Key Points

- Many canine liver diseases have reported breed predispositions but the genetic cause is usually poorly understood.

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- Most canine liver diseases are likely to be polygenic in inheritance and represent an interaction of genes and environment.
- Congenital portosystemic shunts and abnormalities of development of intrahepatic portal veins and ductal plates are likely inherited and probably under-recognized.
- Idiopathic chronic hepatitis in dogs shows strong breed relationships suggesting genetic causes in a number of breeds, but the reasons for this will vary between breeds.
- Vacuolar hepatopathies and gall bladder mucocoeles have also been demonstrated to have some breed relationships.

Introduction

Liver diseases, congenital and acquired, and acute and chronic, are commonly recognized in a wide variety of pedigree dog breeds and cross breeds. There are well documented breed predispositions to many liver diseases, demonstrating an inherited tendency. In many cases, these are true breed predilections, but in some cases the claims have not been substantiated by comparison with a reference population (either a biased Hospital reference population or less biased Kennel Club or insurance company data). It is important to do this before claiming increased breed prevalence because it is all too easy for 'false' data to become established fact once they are published. Recent increased understanding of disease etiologies in humans and dogs shows that many inherited diseases represent a complex interaction between genetics and environment. Many diseases are polygenic, involving more than one gene together with environmental input. Even diseases inherited in an apparently simple Mendelian manner involving one gene can be affected by environment and may not be as simple as first thought (see discussion of copper storage disease below).

It is important for clinicians to be aware of breed predispositions for liver disease: this helps with diagnosis because it increases suspicion of disease, although it is also important to remember that not all dogs of that breed with liver disease will necessarily have the 'breed typical' disease. 'Breed-spotting' is therefore not a substitute for a complete work-up: just an aid. Understanding breed predispositions will also hopefully help us further to elucidate the cause of the diseases through genetic studies and therefore help us with more effective treatment as well as informing preventative strategies, now and in the future.

Breed related congenital liver diseases

Congenital portosystemic shunts (CPSS) are one of a number of congenital liver diseases outlined in Figure 1 which encompass developmental abnormalities in hepatic vascular and ductal plate development of puppies in utero. There is some over-lap between the diseases as detailed in Figure 2, and even between isolated CPSS and ductal plate abnormalities in the liver as detailed below.

1) Congenital portosystemic shunts

Congenital portosystemic shunts (CPSS) are relatively common in dogs. A detailed discussion of PSS is beyond the scope of this article and can be found in other sources including VCNA May 2015. The focus here is to summarize the evidence for inherited PSS and the relationships between PSS and other congenital vascular diseases of the liver. Extra-hepatic PSS are most commonly diagnosed in small breed dogs and intra-hepatic PSS most commonly in large breed dogs. Strong breed associations are reported in the veterinary literature for congenital PSS but there is also a geographical variation (Table 1).

Intrahepatic CPSS are common in Irish wolfhounds throughout the world and are most commonly patent ductus venosus, which should be on the left, but not all Irish wolfhounds with CPSS have this form on shunt: Krotschek et al reported 125 dogs with intrahepatic CPSS from Australia and the USA[1]. Five of these dogs were Irish Wolfhounds of which 4 had the expected left divisional CPSS but one had an anatomically different right divisional shunt. The same study also showed that the breeds presenting with intrahepatic CPSS varied between countries. For example, Australian Cattle dogs are commonly recognized with CPSS in Australia, whereas Labrador retrievers were more commonly diagnosed in the USA. Australian cattle dogs were also more likely to have right divisional shunts than other breeds as were male dogs. However, the conclusions of this and other studies were that, although it is possible to predict that most large breed dogs will have intrahepatic as opposed to extrahepatic CPSS, the exact anatomical location of the shunt can vary and imaging is necessary to confirm this. A number of studies have investigated the genetics of CPSS in different breeds. In Irish Wolfhounds, breeding studies have shown a strong genetic basis but suggested at least 3 or 4 alleles in two loci are involved.[2] The occurrence of intrahepatic CPSS was found to be strongly inherited but not the location (right or left divisional).

In small breeds dogs, studies in Maltese terriers[3] and in Cairn terriers[4] have demonstrated what appears to be a common, partially penetrant recessive inheritance. There is also an interesting over-lap with microvascular dysplasia (see next section) with some dogs with MVD producing puppies with CPSS. This and other evidence has led to the suggestion that mutations predisposing to CPSS are in fact mutations

that predispose to a number of abnormalities in development of the liver vasculature and ductal plates and that the exact phenotype inherited depends on other genes and the environment both within and outside the uterus. A compelling theory also suggests poor nutrition and/or placental development of the fetus *in utero* as a part of this environment, which is supported by documented resorption of puppies in some affected dams.[3]

2) Microvascular dysplasia, portal vein hypoplasia, and non-cirrhotic portal hypertension

Microvascular dysplasia (MVD), portal vein hypoplasia, and non-cirrhotic portal hypertension are all congenital abnormalities in development of the intrahepatic portal veins. There is likely an overlap between these conditions and there may or may not be an overlap with other ductal plate abnormalities developmentally (see below). Microvascular dysplasia (MVD) tends to be reported mostly in smaller breed dogs and is usually not associated with portal hypertension and ascites[5-7], whereas non-cirrhotic portal hypertension is more often reported in large breed dogs and, as the name suggests, presents with portal hypertension.[8] The histological appearances of MVD, portal vein hypoplasia, and non-cirrhotic portal hypertension are also very similar with a lack of portal vein branches in smaller portal triads being the hallmark. The difference between these diseases (if there is one) is largely related to the response of the liver and the presence or absence of portal hypertension. Microvascular dysplasia is in fact physiologically unusual in that there is usually no evidence of portal hypertension and ascites. The portal vasculature is a large parallel, low resistance circulation (like a tree with multiple branches) so the portal pressure is usually low. A reduction in portal vein branches such as occurs with MVD should result in portal hypertension due to a reduction in the number of parallel vessels and thus increase in resistance. The fact that this doesn't happen argues strongly for a form of intrahepatic shunting which has been demonstrated to occur in other species via vascular shunts from the portal vein to the hepatic vein bypassing the sinusoids. In contrast, in non-cirrhotic portal hypertension, it is assumed that these shunts are nonfunctional and therefore portal hypertension develops.

The liver of a dog with CPSS also have a very similar histological appearance to these conditions – the presence of a single large shunting vessel diverting blood from the liver results in a reduction in smaller portal vein branches. The only reliable way to differentiate CPSS and MVD is to search for a large shunting vessel. Occasionally, dogs may have concurrent MVD or other microscopic vascular anomalies in addition to a gross shunting vessel and in these dogs, liver function tests fail to normalize after surgical ligation of their CPSS and there may be an increased risk of post-operative portal hypertension.

Microvascular dysplasia (MVD) has been reported as a congenital disorder in a number of breeds, but particularly Cairn terriers and Yorkshire terriers (Table 1). A recent study of histologically confirmed canine liver disease in Japan found MVD was the most common diagnosis, accounting for 29.4% of all diagnoses of liver disease in their population with Yorkshire terriers, Papillons and toy poodles being particularly affected, although the authors noted that they could not differentiate MVD and portal vein hypoplasia histologically.[5] Studies in Cairn terriers have suggested an autosomal recessive inheritance of MVD in the breed.[6] This is the only breed where genetic studies have been reported. Congenital portosystemic shunts have also been demonstrated to be inherited in cairn terriers and there has been speculation that there may be overlap in the genetic factors predisposing to MVD and CPSS, although more work needs to be done to confirm this.[4]

3) Ductal plate abnormalities

Congenital ductal plate abnormalities encompass a number of developmental abnormalities of the portal triad, including juvenile hepatic fibrosis and Caroli's disease. These particularly affect the bile ducts. Portal vein hypoplasia and microvascular dysplasia (MVD) might be separate diseases or might be related to ductal plate abnormalities: in human medicine, incomplete development of the portal vein branches is a recognized consequence of ductal plate abnormalities with an absence of portal vein branches in smaller triads (likened to "pollard willows") and embryonic development of bile ducts and portal circulation are known to be linked.[9] Figure 2 gives a summary of ductal plate development, demonstrating why abnormalities result in biliary hyperplasia with increased fibrosis. In human medicine, a variety of both

recessive and dominant genetic diseases are recognized which predispose to a variety of ductal plate abnormalities, together with a variety of concurrent diseases such as collagen disorders and polycystic kidneys. In dogs, most of theses diseases appear in the literature as small cases series and very little is understood about the underlying genetics.

4) Biliary ductal plate malformations (congenital hepatic fibrosis; Caroli's disease)

Congenital hepatic fibrosis was first reported in dogs in 2010 by Brown and others in 5 dogs of a variety of breeds.[10] Reports in the veterinary literature have been sparse since this time, but it is likely that the disease is under-recognized. The experience of this author and also of Pillai et al is that a number of affected dogs are misdiagnosed on histology as portal fibrosis; cirrhosis; chronic hepatitis, or cholangitis.[11] Increasing reporting of the disease should help improve recognition. This is important because affected dogs often do better on long term management than dogs with cirrhosis or chronic hepatitis. A key histological finding differentiating the biliary hyperplasia of congenital hepatic fibrosis from the secondary biliary hyperplasia in chronic hepatitis and cirrhosis is the lack of proliferation of the embryonic bile ducts demonstrated by negative staining with immunohistological stains such as Ki67.[10,11]

Is congenital hepatic fibrosis a breed-related disease? If it is similar to ductal plate abnormalities in humans, it should be. Pillai et al in 2016 reported 30 boxer dogs from the histology archive at Cornell with ductal plate malformations, suggesting an increased prevalence in this breed, although that has not been confirmed. Interestingly, there was a high prevalence of concurrent congenital hepatic abnormalities such as atrophy of liver lobes, gall bladder abnormalities, or vascular abnormalities. Two dogs had Caroli's disease (see below) but no dog had evidence of cysts in the pancreas or liver. Thirty-five percent of dogs in this study had a significant accumulation of copper in the liver, although the authors offered no real explanation for this apart from increased dietary copper.

Recent work at the University of Cambridge in the UK suggests that Skye terrier hepatitis may in fact be a congenital ductal plate abnormality. Skye terrier hepatitis was first reported in 1988 in nine related dogs

from the UK.[12] It was associated with hepatic copper deposition but did not show features typical of copper storage disease: copper accumulation did not accompany the initial histological features but seemed rather to be related to the severity of cholestasis suggesting a primary disorder of bile metabolism.

Histological examination of three Skye terriers recently presenting in the UK with liver disease and repeat examination of a case reported in Glasgow in 2003[13] has demonstrated features suggestive of congenital hepatic fibrosis.[14] Interestingly, there is a suggestion in the breed that some dogs also have renal dysplasia although this has yet to be confirmed. Work is continuing to further characterize the histology and genetics of the disease in Skye terriers.

Caroli's disease describes congenital dilation of the large and segmental bile ducts and appears to occur as a result of maturation arrest of medium intrahepatic bile ducts. Reports in the veterinary literature are sparse. In 2006 two golden retriever littermates from South Africa were reported to have this disease and both also had portal fibrosis and renal cysts[15]; eight affected dogs of a variety of breeds were reported from the Netherlands in 2003[16]; two out of 30 boxers with ductal plate abnormalities from Cornell in 2016 were reported to have Caroli's disease.[11]

Breed related acquired liver diseases

1) <u>Breed related idiopathic chronic hepatitis</u>

Chronic hepatitis is defined histologically by the WSAVA Liver Standardization group as a hepatic mononuclear or mixed inflammatory infiltrate with hepatocyte necrosis and/or apoptosis and varying degrees of fibrosis. It is remarkably common in dogs, with a prevalence of up to 12% at post mortem examination in old dogs. In most cases the cause of chronic hepatitis remains unknown and it remains "idiopathic".[17] Increased amounts of copper are found in the liver of some dogs with chronic hepatitis: some have true copper storage disease whereas in others, the copper build up may be secondary to the

liver disease. The interpretation of the role of copper in canine chronic hepatitis is complicated and will be discussed separately.

Chronic hepatitis is recognized in a variety of dog breeds and cross breeds. However, there are strong breed predilections reported in the literature and these appear to have altered with time and geographical location. Breed predilections are summarized in Table 2. There are also reported gender and age biases in some breeds in the studies referenced: Andersson and Sevelius noted a male predisposition in American and English cocker spaniels which were a mean of 5 years old and a female predisposition in Labradors which were a mean of 6.9 years old at presentation. Bexfield et al reported a median age for all dogs of 8 years with an overall sex ratio of 1.5 females to one male. However, Dalmatians, Dobermans and English springer spaniels presented at a significantly younger age than Labrador retrievers, Cairn terriers and English Cocker spaniels. There was an over-representation of females in the Dalmatians; Dobermans; English cocker spaniels; English springer spaniels and Labrador retrievers, and an over-representation of males in the American cocker spaniels. Hirose at al also reported a female predisposition in Labradors and Dobermans with chronic hepatitis in Japan and a strong male predisposition in miniature dachshunds with cholangiohepatitis.

It is very important to realize that a number of different insults to the liver may produce histologically very similar findings: for example, chronic infectious causes, toxic causes, and autoimmune causes may appear identical on histology. This increases the challenge for clinicians to decide appropriate treatment in affected dogs because in most cases, the cause is not confirmed. In some cases, breed predilections might help understand the cause but in many cases it doesn't because of our currently limited understanding of etiopathogenesis. The hope is that ongoing research in specific dog breeds will help elucidate causes. Table 3 lists possible reasons for increased prevalence of chronic hepatitis in a particular dog breed. The current state of knowledge of potential causes in individual dog breeds is summarized below. It is clear that there is still much work to be done (Figure 3).

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- Dobermans

Chronic hepatitis was first reported in Doberman Pinschers in the 1980s. Since that time, they have been consistently identified in studies all over the world as having an increased risk of disease, but there has been debate about the cause. Most reports show a strong female predominance and work from Scandinavia suggests an autoimmune cause with a strong association to DLA class II alleles and haplotypes[18] and increased expression of DLA class II on hepatocytes in affected dogs.[19]

In contrast, studies published from Utrecht have provided strong evidence for a unique form of copper storage disease in Dobermans (see section on copper storage disease). Which is correct? It is very likely that these studies show two different sub-groups of Doberman Pinschers, a proportion of which have autoimmune hepatitis and a proportion of which have copper storage disease. It is known that the Dobermans with copper storage disease respond well to copper chelation whereas the Dobermans with autoimmune disease do not need this but may respond to immunosuppression: there is no recent direct evidence of a response to steroids in Dobermans with chronic hepatitis, but the original paper demonstrating the efficacy of steroids in canine chronic hepatitis included a large number of Dobermans.[20] It is therefore very important to take a liver biopsy from all affected Dobermans and assess for copper before considering treatment. If copper is not deemed to be the cause, assessing the severity and type of inflammatory cell infiltrate in the liver and checking the dog's DLA Class II haplotype may help direct treatment for autoimmune disease.

- Cocker spaniels

English and American Cocker spaniels have consistently appeared as having increased risk of disease in surveys of dogs with chronic hepatitis (see Table 2). One early study showed a strong male predominance in both American and English Cockers[21], whereas a more recent UK study showed a male predominance in American cockers but a female predominance in English cockers [22] and a recent Japanese study of American Cocker spaniels showed no sex predisposition.[23] The median age at diagnosis was reported to

be 8 years 9 months in English cockers and 5 years 6 months in American cockers in one study[22] and 4 years 6 months in American cockers in another study.[23]

The cause of the disease in cockers remains a matter of debate and it is unknown whether American and English cocker spaniels have the same or different diseases. Early investigations suggested that some English and American Cocker spaniels might suffer from a condition similar to alpha-1 antitrypsin (AAT) deficiency in humans with accumulation of an unusual form of the molecule in hepatocytes. However, this has never been convincingly confirmed. Unlike the very well described AAT deficiency in humans, affected dogs did not have deficient circulating AAT and it was unclear if the abnormal form in the hepatocytes was the cause or an effect of the disease.[24] Thirteen American cocker spaniels reported more recently with chronic hepatitis in Japan all had severe fibrosis and cirrhosis with minimal inflammation suggesting that their disease had been subclinical until end stage. The original cause was unknown and copper staining ruled out copper storage disease.[23] English cocker spaniels in the UK have been reported as suffering from a multi-organ duct-centered autoimmune disease similar to a human disease called IgG4-related disease.[25,26] Affected dogs and humans typically suffer from chronic pancreatitis; glomerulonephritis; dry eye and dry mouth. Humans also often have immune attack on the biliary tract.[27] It is plausible this might also occur in a subset of cocker spaniels, although this has not yet been convincingly demonstrated. Interestingly, and unlike many other autoimmune diseases, human IgG4-related disease is more common in older males and is suggested to be a disease predisposed by immune senescence. This might explain the male predominance in cockers with chronic hepatitis in some canine studies. It is also interesting to note that Hirose et al reported five America cocker spaniels with cholangiohepatitis, stressing involvement of the bile duct.

- West Highland White terriers

West Highland White terriers were reported as having an increased risk of chronic hepatitis by Andersson and Sevelius in 1991 and the disease was further investigated by Thornburg et al in 1996 who reported two subgroups of affected dogs: one with apparently classic copper storage disease and one with no

copper involvement and idiopathic chronic hepatitis.[28] West Highland White terriers were also reported to have an increased risk of hepatitis in a recent retrospective study from Utrecht in which 2 out of 4 dogs with chronic hepatitis had copper associated disease and two out of four dogs had idiopathic chronic hepatitis. No further studies have been published on hepatitis in the breed and a recent epidemiological study of canine chronic hepatitis in dogs in the UK did not identify West highland white terriers as having increased risk of disease.[22]

- English Springer Spaniels

English springer spaniels (ESS) have been identified in the UK and Norway as having a significantly increased risk of chronic hepatitis, which is more common in dogs from show lines than working lines.[22] A recently published study documents the clinical and histological signs in 68 affected dogs.[29] There was a marked female predominance and the median age of affected dogs was younger than most other cases of chronic hepatitis at 3 years 7 months (range 7 months to 8 years 5 months). Clinical signs tend to be severe and prognosis poor; half of the dogs were icteric at presentation and median survival time was only 189 days, although 12 dogs survived more than a year from diagnosis. None of the dogs had evidence of excessive copper accumulation on histology. The cause remains unclear but a recent study demonstrated an association with a DLA class II haplotype, suggesting the disease might be infectious or autoimmune.[30] Searches for an infectious cause of disease have so far been fruitless. However, anecdotally, affected ESS respond well to steroid treatment, suggesting the disease may be autoimmune, although more work will be required to confirm this.

To the author's knowledge hepatitis has not yet appeared with increased prevalence in ESS in the USA. However, anecdotally, there is also an increased prevalence of hepatitis in ESS in Norway. Interestingly, Norwegian ESS show dogs are more closely related to UK dogs than North American dogs. There have also been sporadic reports of hepatitis in ESS in Australia, another country which has used largely UK blood lines. Initial pedigree analysis of affected UK dogs failed to find a common ancestor within 6 generations. All this evidence argues for a disease with some genetic involvement which emerged relatively recently,

probably appearing in UK dogs longer ago than the 6 generation pedigree but after the founder dogs went to North America.

- Dalmatians (see copper section below)
- Labrador retrievers (see copper section below)
- Skye terriers (see ductal plate section above)

2) Breed related copper storage disease

Copper storage disease should be considered separately from idiopathic CH although there is obvious overlap. This is another disease where there is a clear interaction of genes and environment (Figure 4). Breeds such as the Bedlington terrier with a strong genetic tendency to copper storage disease will develop clinical disease with 'normal' amounts of dietary copper and have to be fed on a low copper diet lifelong to avoid hepatitis. Other breeds, such as the Labrador retriever are less susceptible to copper overload but may develop copper storage disease if their diet is high in copper and their genetic susceptibility to this appears to vary between countries and continents. A retrospective study of acute and chronic hepatitis in dogs in Utrecht suggested up to 36% of dogs with chronic hepatitis had copper associated disease.[31] Any dog of any breed or cross breed can develop copper-associated hepatitis either with or without dietary copper loading and it is important to rule this out on all liver biopsies specimens, at least semi-quantitatively by staining for copper. Clinicians should also remember the potential for ingestion of toxic quantities of copper by any dog for example through inadvertent access to high copper calf food or supplements. There is evidence for increased environmental exposure to copper in dogs since the 1980s leading to increased accumulation in the liver, although it is unclear whether this is in the diet itself, dietary supplements used by owners or both.[32] There is also some evidence that copper in manufactured dog food has become more bioavailable in recent years, potentially increasing the risk of copper storage disease in susceptible dogs.[33]

The traditional definition of copper storage disease relies on number of classic histopathological and clinical findings as detailed in Table 4. A more fluid interpretation is presented in Figure 4. In either case, two important points are worth noting:

1) Dogs are much more resistant than humans to the build up of periportal copper secondary to cholestasis. In an experimental study in mixed breed dogs, the bile ducts were ligated from 21-93 days. Liver copper concentrations in the liver only increased significantly if the diet was copper loaded, suggesting that dogs are resistant to copper build up secondary to cholestasis unless they have increased dietary concentrations or a problem with copper excretion. The dogs showed histological changes typical of chronic cholestasis and early biliary cirrhosis but no significant liver injury in spite of copper concentrations which were 8 times normal suggesting that they needed an additional insult such as oxidant damage for increased hepatic copper to lead to hepatitis.[34]
2) Serious consideration should be given to copper chelation and a low copper diet in dogs with more than a small amount of copper identified on histopathology – even if this is believed to be secondary – because of the risk that the copper will result in hepatocyte necrosis in response to an additional insult in the future. Therefore to an extent it is academic whether the copper deposition is primary or secondary – the most important question is whether it is present in significant amounts.

- <u>Bedlington terrier</u>

Bedlington terriers have the most well documented and investigated copper storage disease of any dog breed. Reports demonstrate a clear primary copper storage disease with centrilobular distribution and clear association between the degree of copper build up and severity of disease. The disease is confined to the liver but dogs with marked copper build up can suffer from acute hepatocyte damage and hemolysis. The disease appears to be inherited as an autosomal recessive trait and had a high prevalence in the breed previously, although this has been reducing with selective breeding. Studies identified a genetic defect associated with the disease which was initially assumed to be the only cause, a deletion in the COMMD1 gene (previously MURR1)[35] and this has been used for screening for breeding. However,

Bedlington Terriers with copper storage disease but without a COMMD1 deletion have been reported in the United States, United Kingdom, and Australia[36-38] (Coronado et al, 2003; Haywood, 2006; Hyun et al, 2004), suggesting that there are additional mutations involved in the breed. One of these has recently been identified in homozygous COMMD1 negative Bedlington terriers with copper storage disease in the UK where a mutation has been found in the *ABCA12* gene, which encodes for an ATP-binding cassette - a divalent metal transporter protein.[39] Hepatic copper excretion is a complex process and it seems likely that multiple genes are involved such that, as Bedlingtons with COMMD1 deletions have been selectively removed from the population, other genetic defects contributing to copper storage disease have become more prevalent.

Dalmatians

Copper associated hepatitis has been reported in a number of young Dalmatian dogs in the USA and Canada.[40,41] Dogs usually presented as young adults (mean 6 years in one study) with acute onset gastrointestinal signs and marked elevation in liver enzymes. Hepatic histology was very supportive of primary copper storage disease with centrilobular distribution of copper and very high concentrations. Prognosis was poor with a mean time from diagnosis to death of 80 days suggesting dogs present in end stage disease. There have been no published reports of copper storage disease in Dalmatians in the UK, but the breed was reported as having an increased prevalence of chronic hepatitis in one UK study[22] and the author has anecdotally heard reports of cases in the UK.

Doberman Pinschers

A number of Dutch studies have demonstrated without doubt that a proportion of Doberman Pinschers suffer from a form of copper storage disease. The copper appears to build up in zone 3 in most cases and there is clinical response to copper chelators.[42,43] A recent study in Dutch Dobermans showed reduced expression of genes associated with copper efflux and reduce glutathione in affected dogs.[44] However, as discussed in the previous section, not all Doberman Pinschers with chronic hepatitis have copper storage disease so it is very important to take a liver biopsy from affected dogs prior to initiating treatment.

Labrador retrievers

Published reports from Utrecht and the USA suggested that copper storage disease was the predominant cause of chronic hepatitis in this breed.[45-48] However, until recently, copper storage disease was not recognized in Labradors with chronic hepatitis in the UK, even though Labradors are predisposed to disease in this country.[22] More recently, the author has identified individual Labradors with high concentrations of hepatic copper but this remains uncommon. This is interesting because if does suggest a difference in genetics between UK Labradors and those in the USA or the Netherlands and underlines the fact that chronic hepatitis is a diverse disease with a number of potential etiologies and potential reasons for increased breed susceptibilities. There may be a change in prevalence with time: more recently, Poldervaart et al reported 16 Labradors in their study of hepatitis in dogs in the Netherlands, of which 11 were idiopathic and only 5 associated with copper.

Affected dogs tend to be middle-aged at presentation and histological findings are supportive of primary copper storage disease with a centrilobular pattern and colocalization with inflammatory cells, together with high concentrations of copper (Table 4).[45,46] A genetic predisposition is further supported by finding evidence of increased copper in the livers of clinically normal relatives.[48] Affected dogs respond to treatment with copper chelators and low copper diet, although the long term response to diet seems to vary depending on the severity of the phenotype.[48,49]

3) Breed related vacuolar hepatopathies

The term 'vacuolar hepatopathy' is used to describe a variety of causes of vacuolated hepatocytes, which are generally considered to be secondary to other diseases, particularly endocrine disease. Vacuoles may result from hepatocytes being full of fat (steatosis) or glycogen or water (hepatocellular swelling, or cloudy swelling). Hyperadrenocorticism typically results in glycogen vacuolation and diabetes mellitus and hypothyroidism typically result in fat deposition. However, there are many other causes of vacuolation including hepatocyte injury with toxins, which may be primary rather than secondary hepatopathies. The

different types of vacuolation can to some extent be distinguished by their patterns on light microscopy but this can be challenging in some cases and special stains may be necessary.

Vacuolar hepatopathies are very common and generally are not considered to be breed related, but with two notable exceptions which will be discussed here.

Vacuolar hepatopathy in Scottish Terriers

An apparently breed-related glycogen vacuolar hepatopathy has been reported in Scottish Terriers in the USA and France.[50-52] Affected dogs present with a marked increase in serum alkaline phosphatase and an apparently increased risk of developing hepatocellular carcinoma. The median age at presentation was 8 years for hepatopathy and older for carcinoma. The pathogenesis remains poorly understood. Some affected dogs show clinical features suggestive of hyperadrenocorticism but results of adrenal function testing are inconsistent and in one study 5 out of 7 dogs tested had normal urine cortisol:creatinine ratios.[50] Gall bladder mucocele has been reported in 16% of cases. Dogs show a poor response to treatments for hyperadrenocorticism and management is currently supportive.

Familial hypertriglyceridemia in Miniature Schnauzers

Familial hypertryglyceridemia (FHTG) is very common in Miniature Schnauzers and the prevalence increases with age. In a recent study of 192 miniature schnauzers in the USA, 32.8% showed increased serum triglycerides and more than 75% of dogs over 9 years of age.[53] Pancreatitis is the most clinically significant clinical consequence of FHTG but liver disease is also very common in affected dogs, with vacuolar hepatopathy and gall bladder mucocele being the commonest pathologies.

In human medicine, FHTG is known to be a polygenic disease, involving the interaction of genes with environmental factors, such as fat concentration of the diet; concurrent endocrine disease and liver disease.[54] Individuals with FHTG have delayed clearance of dietary fats by the liver, which becomes more clinically significant with age because of hepatocyte aging. A recent study in dogs demonstrated reduction

in expression of genes involved in hepatic cholesterol trafficking with age in dogs, supporting this theory in dogs as well as humans.[55]

Candidate gene studies in miniature schnauzers for mutations in single genes involved in fat metabolism (lipoprotein lipase and apo c II) have failed to find a difference between affected and unaffected dogs.[56]

4) Gall bladder mucocele

A gall bladder mucocele is cystic mucinous hyperplasia of the gall bladder wall with accumulation of thick mucus. A mucocele can be an incidental finding on diagnostic imaging or post mortem examination but can cause secondary obstruction of the biliary tract or even gall bladder wall pressure necrosis and rupture. The cause is poorly understood but there does appear to be a relationship with hyperlipidemia. There is a suggestion in the literature of breed relationships, with an increased incidence in small breed dogs. Aquirre et al 2007 reported gall bladder mucocele in 38 Shetland sheepdogs[57] and Malek et al 2013 report gall bladder mucocele in 43 dogs including 10 cocker spaniels; 5 Shetland sheepdogs and 4 miniature schnauzers.[58] A Japanese case-control study of gall bladder mucocele showed a significant association with increased cholesterol or triglycerides and miniature schnauzers were one of the breeds predisposed to mucocele.[59] There is conflicting evidence that mutation of the gene *ABCB4*, which encodes a membrane transporter protein, is associated with gall bladder mucoceles in Shetland sheep dogs. An initial study found such an association[60] but in a subsequent study this result was not reproduced in this or other breeds of dog.[61] The authors of the latter study concluded their findings do not rule out the possibility that ABCB4 dysfunction may be one of many contributors in a multifactorial etiology.[61]

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Figure 1: Congenital liver diseases in dogs.
Note that as well as overlapping syndromes, it is possible to have concurrent diseases, for example, congenital portosystemic shunt and microvascular dysplasia (see text for details).

Figure 2: Diagrammatic demonstration of development of ductal plate in liver.
Data from Shorbagi A. Experience of a single center with congenital hepatic fibrosis: A review of the literature. WJG. 2010;16(6):683.

Comment [RE3]: <<AU>>Please verify credit line in Figure 2.

Figure 3: Breeds predispositions for copper and autoimmune hepatitis.

Figure 4: Dynamic model of copper storage disease demonstrating interaction of genes and environment.

Table 1: Some published breed relationships for congenital portosystemic shunts in dogs

Intrahepatic shunt	**Extrahepatic shunt**	**Microvascular dysplasia**
Irish Wolfhounds	Cairn terrier	Cairn terrier
Deerhounds	Yorkshire terrier	Yorkshire terrier
Australian Cattle dog	Maltese terrier	Papillon
Golden Retriever	Jack Russell Terrier	Toy Poodle
Labrador retriever	Dachshunds	
Old English Sheepdogs	Havanese	
	Dandy dinmont	
	Miniature Schnauzer	
	West highland white terrier	
	Shi Tzu	
	Pug	

Table 2: Reported breed predilections for canine chronic hepatitis

Andersson and Sevelius 1991*	**Bexfield et al 2012 $**	**Hirose et al 2014£**	**Poldevaart et al 2009α**	**Other single breed reports**
Labrador Retrievers	Labrador retriever	Labrador retriever	Labrador retriever and Golden retriever	
American Cocker spaniel	American Cocker Spaniel	American Cocker Spaniel^	American Cocker Spaniel	American Cocker Spaniel⌘
English Cocker spaniel	English Cocker Spaniel		English Cocker Spaniel	
Doberman pinscher	Doberman pinscher			
West Highland White terrier			West Highland White terrier	West Highland White terrier&
Scottish Terrier				
	Dalmatian			Dalmatians@
	English Springer spaniel			
		Miniature Schanuzer^		
		Pomeranian^		
		Miniature Dachshund^		
				Skye terriers ¢
			German Pointer	

- *250 cases of histopathologically confirmed chronic hepatitis one diagnostic laboratory in Sweden + 49 dogs with histopathically confirmed chronic hepatitis at the Animal Hospital of Helsingborg 1984 – 1989. Control population: Swedish Kennel Club registrations (representing 60-70% of all dogs in Sweden)
- $ 551 cases histopathologically confirmed chronic hepatitis from 6 histopathology labs in the UK 2001-2008. Control population: microchip data from one company from 2001 and 2008; 175,442 and 311,085 dogs respectively
- 463 canine liver biopsies at Veterinary Medical Center of University of Tokyo 2006-2012. Odds ratios compared with all cases with chronic hepatitis. Dogs with ^ were classified as cholangiohepatitis

- Retrospective study of histologically confirmed hepatitis at University of Utrecht 2002-2006. 21 acute hepatitis; 67 chronic hepatitis. Control population: total clinic population. Breeds shown had increased risk of chronic hepatitis. Jack Russell terrier also had increased risk of acute hepatitis
- ⌘Kanemoto et al. May be some overlap of cases with Hirose et al
- &Thornburg et al
- £A proportion are probably copper storage disease – see text
- @ Probably copper storage disease – see relevant section
- ¢Probably ductal plate abnormality – see text

Table 3: Potential reasons for breed-related chronic hepatitis in dogs and comparison to human medicine

Increased susceptibility to infectious causes of CH and/or to chronicity of infection rather than recovery: • No reports for hepatitis in dogs, but known breed predispositions to other diseases (e.g. rottweilers and parvovirus) • In humans, known genetic variations in susceptibility to chronic hepatitis B and other viruses
Susceptibility to autoimmune disease • Suspected in some Dobermans (see text) and also possibly English Springer Spaniels and Cocker spaniels. • In humans, known genetic variations in susceptibility to autoimmune hepatitis
Mutation of gene coding for protein involved in metal transport/storage/excretion • Copper storage disease in Bedlington terriers and other breeds (see text) • In human, Wilson's disease (equivalent to copper storage disease) and also hemochromatosis due to excess iron
Gene mutations resulting in hepatic accumulation of glycoprotein protease inhibitor • Alpha-1 anti-trypsin inhibitor deficiency suspected but never proved in cocker spaniels (see text) • Alpha-1 anti-trypsin inhibitor deficiency well described in humans
Increased susceptibility to chronic hepatic damage with toxic causes • None described for chronic hepatitis but known susceptibility of Dobermans to acute liver insult from potentiated sulphonamides and suspected susceptibility of Labrador retrievers to carprofen toxicity • In humans, there are known genetic predispositions to alcohol-induced chronic hepatitis and cirrhosis

Table 4: Traditional criteria for diagnosis of primary copper storage disease

Primary Copper Storage disease	Copper deposition secondary to cholestasis
Copper accumulates around the central vein (zone 3)	Copper accumulates around the portal triad (zone 1)
Copper accumulation pre-dates inflammation (clear cause and effect)	Copper accumulation comes after inflammation
Copper co-locates with inflammation	Copper does not co-locate with inflammation
Degree of copper accumulation correlates with degree of liver disease	Copper accumulation is much less than the severity of liver disease
Hepatic copper concentration < 400 μg/g dry weight[32]	Hepatic copper concentration > 1,800 μg/g dry weight[32]

Note comments in text about dogs being relatively resistant to secondary copper accumulation