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Review

Canine congenital portosystemic shunts: Disconnections dissected

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ABSTRACT

Canine congenital portosystemic shunts (CPSS) are vascular anomalies that connect the portal vein with the systemic circulation, therefore bypassing the hepatic parenchyma. Portosystemic shunts exist in two different subtypes: extrahepatic and intrahepatic. This congenital disorder is also described in mice, cat, sheep and man. Research has been focused on pathophysiology, diagnostics and treatment of CPSS and this has resulted in increased knowledge, although the aetiology of the disease remains unclear. This review focuses on the aetiology and genetic basis of both intra- and extrahepatic shunts.

Introduction

Canine congenital portosystemic shunts (CPSS) are hereditary disorders that have a severe impact on the wellbeing of the affected dog (van den Ingh et al., 1995), although the genetic background has not been elucidated. The two different subtypes of CPSS, intrahepatic and extrahepatic, show a different epidemiology (van den Ingh et al., 1995). Intrahepatic portosystemic shunts (IHPSS) are almost exclusively diagnosed in large-sized pure-bred dogs (Hunt, 2004), whereas extrahepatic portosystemic shunts (EHPSS) occur mainly in small dog breeds (Tobias and Rohrbach, 2003; Van den Bossche et al., 2012; Fukushima et al., 2014), suggesting a hereditary basis.

For both IHPSS and EHPSS, a genetic association has been observed in the Irish Wolfhound (van Steenbeek et al., 2009) as well as in the Yorkshire terrier (Tobias, 2003), Cairn terrier (van Straten et al., 2005) and Maltese (O'Leary et al., 2014) breeds. Although both shunt types result in the same pathophysiology (as a result of nearly complete bypass of the liver by portal blood flow), a different aetiology is suspected based on the developmental processes involved and the timeframe in which the different subtypes of CPSS arise (van Steenbeek et al., 2012). This hypothesis is supported by the suggested different modes of inheritance of the two shunt subtypes (Tobias, 2003; van Straten et al., 2005; van Steenbeek et al., 2009; O'Leary et al., 2014) and has been of fundamental significance in functional studies (van Steenbeek et al., 2013b). Genetic studies will in time confirm the different backgrounds for the two disorders.

Hepatic vascular anatomy and function

The anatomical structure of the liver is unique due to its role in connecting the portal circulation with the systemic circulation (van den Ingh et al., 1995). The vascular network of the liver comprises portal veins, hepatic arteries, and hepatic veins. The portal blood flow contains blood originating from the entire gastrointestinal tract, spleen and pancreas, including the cranial and caudal mesenteric veins, the splenic vein, the gastroduodenal vein, and the left gastric vein as the major contributors of the portal vein (van den Ingh et al., 1995). The portal blood flow perfuses the liver through the liver sinusoids before entering the hepatic veins and contributes to 60–70% of the total hepatic blood flow (Cullen et al., 2006). Although the portal blood is of venous origin, it delivers 50% of the hepatic oxygen supply (Payne et al., 1990; van den Ingh et al., 1995). Two or three branches of the hepatic artery supply the liver and this blood of arterial origin increases the oxygen content of sinusoidal blood (Payne et al., 1990). The total afferent hepatic blood flow is thus the result of a complicated interaction of hepatic arterial and portal venous blood flow, regulated by local and systemic factors (Payne et al., 1990; van den Ingh et al., 1995). The efferent hepatic blood flow is provided by the hepatic veins, which enter into the caudal vena cava before crossing the diaphragm (Payne et al., 1990).

Clinical signs and histological abnormalities

Congenital portosystemic shunts cause liver atrophy and hepatic dysfunction that lead to a diversity of progressive clinical signs. As ammonia, aromatic amino acids, absorbed bacteria and endotoxins are not subjected to hepatic metabolism, the brain is exposed to toxins and metabolites causing hepatic encephalopathy (HE) in the affected animal (Rothuizen et al., 1982). The plasma ammonia concentration, used as a diagnostic method in CPSS, has also been shown to be predictive for the presence of HE in dogs, although diagnostic errors have been reported in the literature (Tivers et al., 2014).

The interaction of ammonia with other factors, such as inflammation, as determined using the systemic inflammation response syndrome (SIRS) score, has been found to play a crucial role in the

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development of HE. In dogs with CPSS in which the signs of HE resolved after successful attenuation of the shunt, the plasma ammonia concentration decreased significantly. The SIRS score also decreased, although not significantly as it may not be a sensitive score to detect changes in inflammation.

CPSS can be diagnosed as early as 6 weeks of age, facilitating routine screening of litters (Kerr and van Doorn, 1999; van Straten et al., 2005). However, based on presentation due to clinical signs, dogs with CPSS are diagnosed at varying ages with the majority of affected dogs detected within the first year of life (Tisdall et al., 1994; Tobias and Rohrbach, 2003; Van den Bossche et al., 2012). Clinical signs are influenced by shunt type, anatomy, nutrition and concurrent diseases and therefore presentation is also highly variable (Van den Bossche et al., 2012; Kraun et al., 2014). An index of suspicion based on breed predisposition and owner and veterinarian recognition of early clinical signs aids the diagnosis.

Pathological findings in dogs with CPSS are the result of shunting of portal blood with all its contents. Macroscopic changes include liver atrophy and portal vein hypoplasia proximal to the shunt origin for EHPSS (van den Ingh et al., 1995; Cullen et al., 2006). Histological findings include enlarged portal fields by proliferation of small arterioles and biliary hyperplasia together with hypoplasia of the portal vein and mild to moderate fibrosis in the portal areas (Baade et al., 2006; Cullen et al., 2006; Parker et al., 2008). Sinusoidal dilatation in the periportal area has also been described (Cullen et al., 2006; Parker et al., 2008). In addition to parenchymal changes, including atrophy of hepatocytes and lipid infiltration, the presence of fatty cysts, lipogranulomas and lymphangiectasia has been reported (Baade et al., 2006; Cullen et al., 2006; Parker et al., 2008). Lipidosis, the accumulation of lipids in the hepatocytes, was compared in the livers of dogs with CPSS and control dogs using stereological point counting following Oil Red O staining (Hunt et al., 2013). The study confirmed that this technique can demonstrate lipidosis in livers of dogs with CPSS, even in the absence of lipogranulomas or large lipid vacuoles, which are necessary in a haematoxylin and eosin staining to achieve the diagnosis. Significantly more small lipid droplets have been observed in the liver tissue of dogs with CPSS compared to those of control dogs.

A more recent study demonstrated a strong association between lipogranulomas and age. Dogs < 12 months of age had significantly fewer lipogranulomas compared to dogs > 12 months. No relationship has been observed between steatosis and pre- or post-operative shunt fraction and effect on short-term outcome after shunt attenuation (Hunt et al., 2014). If a difference is observed in the amount of steatosis between healthy and CPSS dogs (Hunt et al., 2013) and shunt fraction is not correlated with steatosis (Hunt et al., 2014), it seems likely that steatosis could be a genetically determined factor. Extensive genomic research will be required to determine whether there is a genetic background for steatosis in shunt. Given the active role of specific lipids during liver regeneration (Delgado-Coello et al., 2011), it would be of great added value to determine which type(s) of lipids tend to accumulate.

Treatment and prognosis

The treatment of choice in dogs with CPSS that is designed to achieve long-term improvement consists of surgical attenuation of the shunt vessel (Rothuizen et al., 1982; Greenhalgh et al., 2014). However, complete ligation can be fatal due to a sudden increase in portal blood flow resulting in portal hypertension and shock when hypoplasia or aplasia of the portal venous circulation cranial to the shunt is present. The technique is therefore not applicable in the majority of dogs. Gradual shunt attenuation is often implemented as an alternative, using partial ligation with silk ligatures in which the shunt is closed to the maximum tolerated level so portal pressure does not reach critical values (Winkler et al., 2003; Kummeling et al., 2004). Ameroid ring constrictor placement (Falls et al., 2013), cellophane banding (Hunt et al., 2004) and thrombogenic intravascular coils (Gonzalo-Orden et al., 2000; Weisse et al., 2014) have also been used to achieve progressive ligation. Post-operative outcomes remain variable (Winkler et al., 2003; Kummeling et al., 2004), which makes each surgical intervention a challenge. Therefore (and due to the expense of surgery) medical management provides an alternative therapy for reducing the clinical signs such as hepatic encephalopathy and urinary tract disease in dogs with CPSS (Watson and Herrtage, 1998; Winkler et al., 2003; Greenhalgh et al., 2014).

Medical therapeutics include dietary adjustments (high-quality, easily digestible low-protein diets) in combination with antimicrobials (i.e. ampicillin or metronidazole) and/or a synthetic disaccharide, like lactulose (Center, 1998; Proot et al., 2009; Greenhalgh et al., 2010, 2014). Research has been performed on the influence of soy protein isolate vs. meat-based protein source in a low-protein diet on hepatic encephalopathy (Proot et al., 2009). Both diets had a long-term positive effect on HE-scores in dogs with CPSS. Although no difference in HE-scores was observed between the two diets, the soy protein based diet reduced plasma ammonia levels and decreased prothrombin time in dogs with CPSS compared to the control diet, thereby decreasing the risk for HE and supporting liver function.

As this management approach offers purely supportive therapy to reduce the clinical signs, it does not resolve the underlying disease, neither does it reduce the frequency of ongoing clinical signs and it does not appear to have improved survival time over the long term compared to surgical treatment (Greenhalgh et al., 2014). In cases with high surgical risk, or where the owner has declined surgery, this therapy could be recommended for long-term support as an alternative to attenuation and, in addition, it may be provided in preparation for surgery or in dogs with insufficient clinical improvement after surgical attenuation (Proot et al., 2009; Greenhalgh et al., 2014).

Aetiology vs. physiology

The veins located in the abdominal cavity are derived from the umbilical, vitelline and caudal cardinal veins of the embryo. The portal vein originates from the umbilical and vitelline veins, whereas the non-portal venous drainage of the abdominal organs is derived from the cardinal venous system of the fetus. No functional vascular connections exist between the cardinal veins and the umbilical–vitelline veins. In contrast, numerous non-functional vascular portocaval and portoazygos communications are present and may become functional due to portal hypertension (Payne et al., 1990). The vitelline veins include a left and a right vitelline vein, connected by three separate anastomoses defined as cranial, middle and caudal. The ductus venosus connects the cranial anastomosis and the left umbilical vein. The ductus venosus is responsible for the flow of nutrient and oxygen rich blood derived from the placenta directly to vital organs, bypassing the liver sinusoids. In dogs this vessel is functionally closed within 2–9 days after birth, establishing the normal hepatic circulation (Lamb and Burton, 2004).

Congenital portosystemic shunts are vascular anomalies that directly connect the portal venous system with the systemic venous circulation, thereby bypassing the liver sinusoids. EHPSS represent abnormal developmental functional communications between the embryonic vitelline veins, responsible for the entire extrahepatic portal system and the cardinal venous system that contributes only to all non-portal abdominal veins (Payne et al., 1990). Distinctions have been reported between left, central and right divisional intrahepatic portosystemic shunts but the majority of the IHPSS are left divisional shunts that are classified as a patent ductus venosus, compatible with the normal embryology of the dog (White et al., 1998).

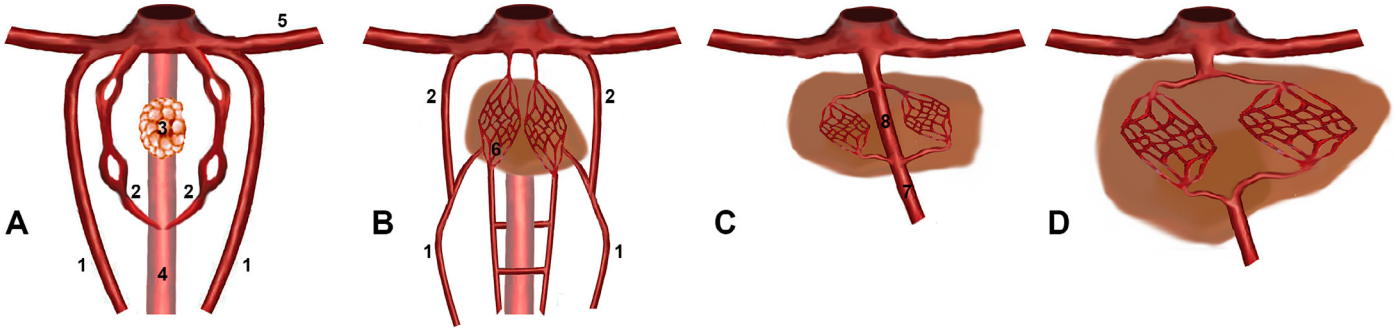

Fig. 1. Schematic overview of the development of the liver and the hepatic portal vascular system. The umbilical and vitelline veins existing in early embryonic phase (A) will eventually give rise to the portal vein. No functional vascular connection exists between the umbilical–vitelline and cardinal veins in the normal healthy post-natal animal (D). The vitelline veins will regress to stimulate vascularization of the liver (B) and failure of this process causes EHPSS. Once the liver is fully developed the ductus venosus (C) should close after birth. Patency of the ductus venosus results in IHPSS. 1, umbilical veins; 2, vitelline veins; 3, liver buds; 4, duodenum; 5, common cardinal veins; 6, liver sinusoids; 7, portal vein; 8, ductus venosus; EHPSS, extrahepatic portosystemic shunts; IHPSS, intrahepatic portosystemic shunts.

A recent publication using twice as many samples showed a more equal distribution between left, right and central divisional (Weisse et al., 2014). Right-sided shunts are classified as a patent right ductus venosus (White et al., 1998) but are also classified as a persistent right omphalomesenteric vein or a hepatic sinusoid malformation (Lamb and White, 1998). A clear classification of these types is lacking, but with the presence of an intrahepatic portosystemic shunt observed in both the left and the right liver lobes in one single litter (van Steenbeek et al., 2009), it appears that both of these phenotypes may be caused by the same genetic defect, suggesting that closure of the ductus venosus is most likely regulated by a single pathway.

These findings are in agreement with epidemiological findings reporting that breed is not a predictor for the location of intrahepatic shunts (Krotscheck et al., 2007). Whereas the physiological consequences of both CPSS types are identical, the process of the closure of the ductus venosus differs hugely from the formation of a vessel. Both processes take place during entirely different phases of development (Fig. 1). When comparing both processes based on their Gene Ontology terms (Ashburner et al., 2000), a total of 455 genes were annotated for vascular development, whereas for vasoconstriction 80 different genes are known to be involved in total. Merely 14 genes have been reported to be involved in both processes (Table 1).

An elegant micro-array experiment was performed on liver tissue making use of the shared physiology but differing aetiology between EHPSS and IHPSS (van Steenbeek et al., 2013b). The beneficiary effect of using both types of shunt (comparing them with healthy liver tissue) was shown in the small list of only 25 genes aberrantly expressed for one of the two subtypes. Differences in the hepatic expression of genes in dogs with either IHPSS or EPHSS compared to the control group were interpreted as specific characteristics of each subtype, whereas differences shared by both IHPSS and EHPSS compared with controls dogs are most likely due to secondary effects, such as the absence of normal portal vein perfusion of the liver.

This micro-array strategy has however some key limitations. It is expected that genes involved in EHPSS are most likely actively transcribed during the embryonic phase and genes involved in IHPSS are most likely expressed during the first days after birth. If any genetic defects are present, impaired expression of those genes during adulthood may still be detected. A further concern is the disadvantage of using a micro-array, where only half of the known canine genes are annotated. Both drawbacks will result in false negative rather than false positive data. Compared with their expression in healthy liver samples, van Steenbeek et al. (2013b) found that 19 and 6 annotated genes were specific to liver samples from dogs with either IHPSS or EHPSS, respectively. Follow-up experiments using qPCR, immunohistochemistry and Western blots confirmed up-regulation of WEE1 homologue (*Schizosaccharomyces pombe*) (WEE1) in IHPSS and down-regulation of Vascular cell adhesion molecule 1 (VCAM1) in EHPSS.

Table 1
Genes involved in vascular development and vasoconstriction based on Gene Ontology classification.

Ensembl (www.ensembl.org) gene ID	Gene name	Description
ENSG00000006210	*CX3CL1*	Chemokine (C–X3–C motif) ligand 1
ENSG00000073756	*PTGS2*	Prostaglandin-endoperoxide synthase 2
ENSG00000078401	*EDN1*	Endothelin 1
ENSG00000100345	*MYH9*	Myosin, heavy chain 9, non-muscle
ENSG00000105974	*CAV1*	Caveolin 1, caveolae protein, 22 kDa
ENSG00000107796	*ACTA2*	Actin, α 2, smooth muscle, aorta
ENSG00000135744	*AGT*	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
ENSG00000142208	*AKT1*	v-akt murine thymoma viral oncogene homolog 1
ENSG00000148926	*ADM*	Adrenomedullin
ENSG00000151617	*EDNRA*	Endothelin receptor type A
ENSG00000160691	*SHC1*	SHC (Src homology 2 domain containing) transforming protein 1
ENSG00000169032	*MAP2K1*	Mitogen-activated protein kinase 1
ENSG00000204217	*BMPR2*	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
ENSG00000222040	*ADRA2B*	Adrenoceptor α2B

WEE1 seems to play an essential role in hypoxia-induced pathological processes in endothelial cells, given that it is up-regulated in endothelial cells under hypoxic conditions and ensures cell viability (Hong et al., 2011). These hypoxic conditions are considered to be the trigger in the postnatal closure of a comparable structure, the ductus arteriosus (Starling and Elliott, 1974). A comparable mechanism involving oxygen tension might be essential in closure of the ductus venosus as well.

VCAM1 was found to be down-regulated in EHPSS liver tissue, and VCAM1, after binding with integrin α4β1, is involved in angiogenesis. *VCAM1* is mainly expressed by proliferating vascular smooth muscle cells, whereas integrin α4β1 is expressed by proliferating endothelial cells. The binding of integrin α4β1 and VCAM1 facilitates the adhesion of endothelial cells to vascular smooth muscle-like pericytes. This process is essential for the survival of endothelial and mural cells during neovascularization.

Antagonists of this integrin–ligand pair induce endothelial cell and pericyte apoptosis, thereby inhibiting angiogenesis (Garmy-Susini et al., 2005). The expression of cysteine conjugate beta-lyase 1 (CCBL1) protein was significantly lower, measured immunohistochemically, in samples from dogs with IHPSS or EHPSS

than in samples from control dogs, whereas at the mRNA level there was only an indication for a down-regulation in IHPSS liver. Based on these results, it was concluded that *CCBL1* expression was a secondary effect of portosystemic shunting. CCBL1 plays a role in metabolizing cysteine conjugates of halogenated alkenes and alkanes leading to the formation of reactive metabolites that can be responsible for nephro- and neuro-toxicity (Perry et al., 1995). *CCBL1* could therefore be an interesting candidate to study aspects not yet elucidated in the pathophysiology of hepatic encephalopathy.

In humans, CPSS is reported to be rare (Stringer, 2008), whereas in dogs the disorder is diagnosed frequently (van Steenbeek et al., 2012). The total prevalence of CPSS in dogs is 0.06–0.2% (Center, 1996), where purebred dogs seem to be more affected compared to mixed-breed dogs (Wolschrijn et al., 2000; Tobias and Rohrbach, 2003). In dogs, IHPSS particularly affect large breeds dogs including the Irish Wolfhound (Meyer et al., 1995; van den Ingh et al., 1995), the Golden retriever and the Labrador retriever (van den Ingh et al., 1995), whereas EHPSS are mostly diagnosed in small breed dogs such as the Yorkshire terrier (van den Ingh et al., 1995; Tobias, 2003), Cairn terrier (van den Ingh et al., 1995; van Straten et al., 2005), Dachshund (van den Ingh et al., 1995), Maltese (O'Leary et al., 2014) and Miniature Schnauzer (van den Ingh et al., 1995), indicating a hereditary basis in these predisposed breeds.

Although no sex predisposition is usually noted (Rothuizen et al., 1982; Tobias and Rohrbach, 2003; Hunt, 2004), an overrepresentation of females with EHPSS has been described in the literature (Van den Bossche et al., 2012). Breed predisposition appears to vary with geographical location, possibly due to the lack of awareness of CPSS in a particular breed (Tisdall et al., 1994; Hunt et al., 2000; Tobias and Rohrbach, 2003; Hunt, 2004). Another explanation would simply be the population difference, which is nicely illustrated with dilated cardiomyopathy in Doberman Pinchers from the USA (Meurs et al., 2012) and Europe (Owczarek-Lipska et al., 2013) where no overlap is shown in their genetic causal background.

Canine intrahepatic portosystemic shunt

IHPSS are mainly observed in large sized purebred dogs and a familial distribution was first described in Irish Wolfhounds indicating a genetic basis (Meyer et al., 1995; Ubbink et al., 1998). Test matings in Irish Wolfhounds (between an affected sire and two affected sisters) resulted in a fully affected litter and a partially affected litter, demonstrating a hereditary basis with a possible di-genic mode of inheritance.

Only a few genes have been reported to cause IHPSS. The aryl hydrocarbon receptor (*AHR*) was the first gene documented to cause patency of the ductus venosus in knockout mice (Lahvis et al., 2000, 2005). Comparable results were obtained when evaluating knockout mice for aryl hydrocarbon receptor interacting protein (*AIP*) (Lin et al., 2008) and aryl hydrocarbon receptor nuclear translocator (*ARNT*) (Walisser et al., 2004). It is not surprising that all of these genes are involved in one specific pathway, although the fact that this is a cascade of genes involved in toxicological response is striking. The mechanism connecting the toxin induced pathway and the physiological processes of closure of the ductus venosus remain unclear. The finding that all of these genes originate from one and the same pathway would however suggest a polygenic inheritance. One disadvantage of mouse models is that a disease is often induced by manipulation of a single gene, whereas diseases in target species are generally spontaneous and often polygenic. Moreover, genetic manipulations in mice focus on one single major gene, whereas complex human and dog diseases are invariably polygenic.

We investigated the murine candidate genes reported in IHPSS and genes in the pathways in Irish Wolfhounds (van Steenbeek et al., 2013a). Because the pathways include multiple genes known to be involved in the patency of the ductus venosus in knock-out mice, as many genes as possible from these pathways were included in analysis. A retrotransposon (LINE-1) insertion was found in intron 2 of Irish Wolfhounds using DNA sequencing.

Using a microarray analysis combined with qPCR experiments, we confirmed down-regulation of AIP, aryl hydrocarbon receptor nuclear translocator 2, cytochromes P450 1A2 and 1B1 and heat shock protein 90AA1 (*HSP90AA1*) expression, whereas the expression of hypoxia inducible factor 1α (*HIF1A*) was up-regulated (van Steenbeek et al., 2013a). Immunohistochemistry was performed to confirm the observed differences and we found reduced levels of AHR, HIF1A, and vascular endothelial growth factor A protein in the nucleus and lower levels of ARNT and HSP90AA1 protein in the cytoplasm of the hepatocytes of Irish Wolfhounds. The impaired expression of *HSP90AA1* seems to have been the key finding in this experiment, since it could trigger the observed differences in mRNA and protein levels and therefore explain the link between two very different functions of AHR, namely, regulation of the closure of the ductus venosus and the response to toxins. Additionally, it has been found in mice that *HSP90α* seems to be repressing retrotransposon activity (Ichiyanagi et al., 2014), strongly pointing towards an explanation for the LINE-1 insertion in the Irish Wolfhound.

Unfortunately, no obvious association was observed between the pathway we investigated and IHPSS in Irish Wolfhounds, underlining the difference between induced and naturally occurring disorders. Remarkably, both *HSP90* as well as *WEE1* are known to interact and are essential in cell cycle control (Aligue et al., 1994). The WEE1 kinase is capable of phosphorylating a conserved tyrosine residue in HSP90. Currently a combined *WEE1*/*HSP90* inhibition is indicated as novel therapeutic strategy in treating cancer (Iwai et al., 2012). Whether the combination of both genes might play a role in the physiological process of ductus venosus closure needs to be investigated.

The relevance of the sphincter and its response to a unique cytochrome P-450 system in closure of the ductus venosus has been described in lambs (Adeagbo et al., 1990). The physiologically comparable process of closure of the ductus arteriosus is associated with endothelin-1 (Baragatti et al., 2011), which also is found to play a role in the contractile tone by its function in smooth muscle cells (Rapoport and Zuccarello, 2012). Mutations causing patency of the ductus arteriosus in humans have been found in myosin heavy chain 11 (Harakalova et al., 2013), which also plays a role in smooth muscle cells. In dogs both right and left divisional IHPSS were presented, suggesting that location is regulated by one major genetic pathway. Nevertheless, IHPSS is most likely to be polygenic, possibly digenic, or tri-allelic (van Steenbeek et al., 2009).

Canine extrahepatic portosystemic shunt

The first report of a human EHPSS case was documented by John Abernethy in 1793. In this case report a post-mortem examination of a female infant with multiple congenital anomalies, including an insertion of the portal vein into the inferior vena cava, was described (Abernethy and Banks, 1793). In dogs EHPSS were previously classified into two subtypes based on the vessels they connect, namely, portocaval and portoazygos shunts (Payne et al., 1990). Computed tomographic angiography images revealed that, in addition, a third shunt type can be classified based on the contribution of a phrenic vein entering the shunting vessel before its insertion into the caudal vena cava (Nelson and Nelson, 2011).

Combined with the anatomical definitions of the veins associated with the shunts and shunt origin, six general shunt types were identified, namely, splenocaval, splenoazygos, splenophrenic, right gastric-caval, right gastric-caval with a caudal shunt loop, and right gastric-azygos with a caudal shunt loop (Nelson and Nelson, 2011). In addition, other variations have been described since the initial publication (White and Parry, 2013; Fukushima et al., 2014). The

portoazygos type described in previous studies most probably contains either portoazygos or phrenic vein insertions (Kraun et al., 2014). Remarkably, in the Japanese dog population, the splenophrenic variation is the most frequently observed shunt type (Fukushima et al., 2014), whereas in the study performed in North America the spleno-azygos shunts are found (Nelson and Nelson, 2011). Fukushima et al. (2014) suggested that this discrepancy can probably be explained by the geographical breed distribution because of the popularity of Miniature Dachshunds and Toy Poodles that contribute to the high incidence of spleno-phrenic variation observed in Japan (Fukushima et al., 2014). In our opinion genetic bias might also be responsible for this discrepancy.

In order to determine if a common genetic basis for both extrahepatic types is plausible, we analyzed the distribution of portocaval and portoazygos shunts in several dog breeds (Van den Bossche et al., 2012). Data from 135 dogs with a single EHPSS were retrospectively investigated. Our main observation was the finding of both subtypes in all dog breeds (apart from the Pug). In general, a predisposition for EHPSS is observed in a variety of small dog breeds (Tobias and Rohrbach, 2003; Van den Bossche et al., 2012; Fukushima et al., 2014). In Yorkshire terriers (Tobias, 2003), Cairn Terriers (van Straten et al., 2005) and Maltese (O'Leary et al., 2014) breeds a genetic background for this shunt type is determined. In Yorkshire terriers the mode of inheritance of EHPSS is not currently thought to be sex-linked, simple autosomal dominant or simple autosomal recessive (Tobias, 2003). A complex mode of inheritance, probably polygenic, is also suspected in the Cairn terrier (van Straten et al., 2005). Pedigree analysis performed in 299 Maltese dogs with 164 dogs of uncertain phenotype support, at least to a certain degree, a complex genetic basis for EHPSS, and a partially penetrant recessive mode of inheritance was suggested by O'Leary et al. (2014).

In dog breeds with a predisposition for EHPSS, the two shunt types (portocaval and portoazygos) coexist in nearly all of those breeds. This has led to the idea that the two subtypes of EHPSS are commonly determined by a small number of major genes, and that a minor gene or non-genetic factor determines the site of insertion (Van den Bossche et al., 2012). Presumably the partial penetrance suggested in the Maltese dogs (O'Leary et al., 2014) is caused by these minor genetic components as well. When conducting a genetic study it seems therefore reasonable to use both types of shunt in fine-mapping.

Future genetic research

The genetic background for IHPSS and EHPSS is expected to be different considering the physiological processes involved. We would expect IHPSS to be caused by differences in the composition of the ductus venosus, whereas the occurrence of EHPSS is susceptible to specific events during development. During early development, blood vessels are initially formed by vasculogenesis, representing the differentiation of endothelial cells from the mesoderm into primary vessels. The primary vessels within this embryonic circulation are remodelled into arteries and veins to develop a functional adult vascular circulation by angiogenesis, which involves capillary sprouting, splitting and remodelling (Risau, 1997). The mature character of these vessels after arterial-venous differentiation is maintained through the interaction between ephrin-B2 and ephrin type-B receptor 4 (le Noble et al., 2004). For EHPSS, it has been postulated that the dysfunctional regression of the vitelline veins is causative (Payne et al., 1990).

Given the specific time point, vessels and the knowledge that EHPSS normally does not relate to additional vascular problems, it is most likely that EHPSS is not caused by general angiogenic factors; instead, epigenetic variation may play a major role. The annotated canine genome is still under development and much is unknown about the epigenetic landscape in the dog. The first steps towards an improved canine genome have been made by RNA-sequencing data sets from 10 different canine tissues resulting in the annotation of novel protein coding genes and additional alternative isoforms per gene (Hoeppner et al., 2014). Despite the fact that this will be an invaluable tool in canine genetic research, the canine genome requires further development comparable to the Human ENCODE (Pennisi, 2012; Skipper et al., 2012) to solve disorders caused by noncoding variants as has been found, for example, in osteosarcoma (Karlsson et al., 2013) and obsessive compulsory disorders (Tang et al., 2014).

Conclusions

The physiological consequences of IHPSS and EHPSS are identical. Portal blood bypasses the liver either through the patent ductus venosus or through the extrahepatic vascular anomaly. Despite the shared physiology, the aetiology of the different CPSS types is not identical as the process of the closure of the ductus venosus differs hugely from angiogenesis and these processes take place during different phases of development. The severity of both IHPSS and EHPSS and the high prevalence of these conditions in numerous breeds indicate that there is a degree of urgency to unravel the genetic background to the disease and to provide breeders with a reliable genetic test. Genotyping both affected and unaffected dogs from a predisposed population, then performing fine mapping using cases from other dog breeds as a follow up experiment, is likely to be the most successful strategy. In addition, the canine genome requires improvement with regard to the non-coding genetic landscape as shown in man and mice. Solving a complex disorder like CPSS could benefit greatly from such a CANCODE approach.

Conflict of interest statement

Neither of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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