Evidence of Inheritance of Intrahepatic Portosystemic Shunts in Irish Wolfhounds

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Background: The etiogenesis of congenital portosystemic shunt in dogs is not understood. In Irish Wolfhounds, intrahepatic portosystemic shunt (IHPSS) is thought to be hereditary, but the mode of inheritance is unknown.

Objectives: To document the genetic background and investigate the potential mode of inheritance of IHPSS in Irish Wolfhounds.

Animals: Three mature, privately owned, affected siblings and their progeny produced in 2 litters.

Methods: Prospective, observational study. Two test matings of 1 affected sire with 2 of his affected sisters were used to determine the inheritance pattern. Affection status was determined by measuring venous blood ammonia concentrations, detection of the shunt by ultrasonography and confirmation during surgical attenuation of the intrahepatic shunting vessel.

Results: In 1 litter of 5 pups all had an IHPSS. In the other litter 5 of 11 pups were affected. Both left- and right-sided shunts occurred in both litters. No sex predisposition was evident among affected dogs.

Conclusions and Clinical Importance: Our results show that IHPSS in Irish Wolfhounds is a familial disorder that is likely genetic. It is unlikely that the mode of inheritance is monogenic. A digenic, triallelic trait could explain the observed occurrence of IHPSS but other modes of inheritance cannot be excluded.

Key words: Dog; Liver; Patent ductus venosus; Pedigree; Test mating.

Congenital portosystemic shunts are diagnosed mainly in purebred dogs and found with high incidence in Cairn Terriers,[1] Yorkshire Terriers,[2] Dachshounds, Miniature Schnauzers, Golden Retrievers, and Labrador Retrievers.[3] Intrahepatic portosystemic shunt (IHPSS) in Irish Wolfhounds has been presumed to be hereditary, based on its overrepresentation in the breed and the familial distribution.[4] Screening of the entire Dutch Irish Wolfhound population from 1984 to 1992 showed an incidence of at least 2.1%, which increased over the years in the absence of a breeding strategy aimed at reducing the incidence of the disease.[5] However, there is no direct evidence of the inherited nature and if present, the mode of inheritance for IHPSS in Irish Wolfhounds is not known. With the present availability of high throughput genotyping platforms it becomes feasible to identify disease genes based on an established mode of inheritance. This parameter is an important part in the design of linkage studies.

The aim of this study was to document the inherited nature of IHPSS in Irish Wolfhounds and obtain insight into the mode of inheritance. We used test matings between affected dogs, which had been successfully treated to achieve these goals. An affected male was mated with 2 of his affected sisters resulting in 2 test litters. The results confirmed the familial and likely inherited nature of the disease and provide evidence of a possible mode of inheritance.

Methods

Parents

A litter of Irish Wolfhounds with a high frequency of IHPSS was identified in the course of the population screening program for the presence of IHPSS in 6 week old pups. The litter was identified using previously published methods.[1,4,5] Both parents were unaffected, but the 6 offspring included 1 affected male, 2 affected females, and 3 unaffected males. All 3 affected dogs were treated successfully by surgical attenuation of the shunt[6] at an age of 3–4 months.

The pups recovered well and were raised in private households who each kept 1 dog. The dogs remained property of the university clinic in order to follow their performance and to guarantee their well-being. Two months after surgery the patency of the shunting vessel, which was left open partially during surgery, was tested with echo-Doppler examination and ammonia tolerance tests.[6] These tests showed that there was no remaining functional portosystemic shunting in any of the 3 littermates. The dogs were fed a standard commercial dog food and were further kept as companion animals in the foster families.

Test Matings

When the 3 pups were mature (>2 years of age) the test matings were performed. The male was naturally mated with 1 sister and the other sister was artificially inseminated. Both females were kept at home, had uncomplicated pregnancy, and gave birth to their litter without any complications. A few days before delivery, 1 dog was hospitalized at the request of the foster family. None of the pups died or had evidence of disease at early age.

Phenotyping

The pups were tested at an age of 6–8 weeks. The basal blood ammonia concentration was measured after 12 hours fasting and a rectal ammonia tolerance test was performed.[7] The blood samples were collected in EDTA tubes and immediately stored on ice. Measurements were performed within 30 minutes as described previously[8] with the Ammonia Checker II (Arkray Factory Inc, Kyoto, Japan) or with the enzymatic assay[5,8] (Monotest, Boehringer, Mannheim, Germany). The diagnosis of IHPSS was confirmed

by ultrasonography, exploratory celiotomy, or postmortem examination.

Pups that were diagnosed with IHPSS were treated surgically at 3–4 months of age.

Ethical Considerations

All steps of these procedures were approved by the responsible ethical committee as regulated by Dutch legislation. The parents and all surviving pups were placed in foster families as normal companion animals, but ownership remained with the university clinic, in order to ensure that dogs could not be transferred to other foster families without consent of the clinic. This procedure also ensured that any health problem would be immediately brought to knowledge of the university clinic, where all possible experiment-related health problems would be examined and treated at the expense and responsibility of the university clinic.

Results

The first mating resulted in a litter of 5 pups (3 females, 2 males), which all were affected. Pup 3 had a right-sided shunt and died during surgery. In the other 4 pups a left-sided shunt was found. The second mating produced 11 progenies (7 males, 4 females), of which 5 dogs were affected (Fig 1). This litter was the result of artificial insemination. Pups 8 and 9 had a left-sided shunt and underwent surgery. Pup 10 died at day 23 because of pneumonia. Pups 11, 12, and 13 suffocated on day 6, when their mother lay on them. These 3 dogs had intrahepatic right-sided shunts, which did not show any sign of partial closure. All dogs with IHPSS had distinctly increased basal plasma ammonia concentrations, whereas all healthy pups had ammonia concentrations within the reference range (24–46 µmol/L). The overall prevalence of IHPSS in these test litters was 62.5% (10 cases of 16 pups). All pups were placed in private homes.

Discussion

Test matings between IHPSS affected dogs resulted in a high incidence of the disorder in the offspring. The affected pups had hyperammonemia, which is the most specific and sensitive parameter to show functional portosystemic shunting.[9] The observed overall preva-
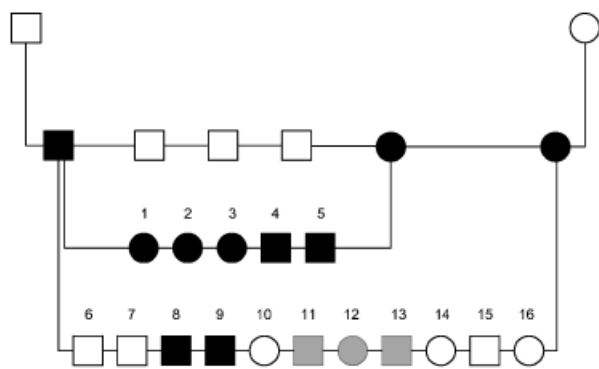

Fig 1. Pedigree of the 2 test litters. Squares represent males, circles represent females. Black symbols are affected dogs, open symbols represent unaffected pups. Pups 11, 12, and 13 are marked gray because their status is not fully certain.

lence of 62.5% in these test litters was much higher than could be expected based on the incidence in the entire Dutch population, which has been reported to be between 2.1 and 3.4%.[5,10] This overrepresentation with at least a factor of 20 most likely is because of the genetic make up of the parents indicating the likely hereditary nature of this defect in this breed. This is an independent confirmation of previous findings which were based on an epidemiologic study of Irish Wolfhounds.[4] In this study, the relatedness of IHPSS cases was assessed within a 5-generation pedigree.

Postmortem examination of 3 pups that were apparently suffocated revealed a wide-open right-sided ductus venosus. At this young age it is possible that the neonatal closure of the ductus was still in progress and not yet completed. Closure in newborn Irish Wolfhounds at day 6 is incomplete in 23% of the pups. Complete closure occurs in all pups on day 9.[11] However, it was not described whether the ductus venosus of the pups without closure were partially narrowed at day 6. A partial narrowing is to be expected because closure of the ductus venosus is a gradual process. On the basis of the wide open status of the ductus in the 3 suffocated pups it is highly likely that they were affected. If these pups would have been assigned as healthy or as unknown, this would have strengthened our conclusions from the pedigree data.

The present results are consistent with a simple monogenic trait with a reduced penetrance. These test matings then suggest a penetrance of 50%, although with marked imprecision given the small number of dogs studied. Remarkably, all 6 unaffected offspring were born in the same litter of 11. Assuming the chance of a shunt was the same for each dog in the 2 litters and considering that 10 of 16 dogs were affected, the chance that the litter of 5 would consist of affected dogs only was 0.06. We thought this was highly unlikely and conclude that the chance of a shunt was different for the 2 litters.

Reduced penetrance could be caused by environmental factors. An effect of environmental factors seems to be rather unlikely because the phenotype is established before weaning. Another explanation for reduced penetrance could be epistasis between multiple loci. The simplest model to explain the observed results would then be an oligogenic disorder with 2 interacting genes. The mode of inheritance for IHPSS could very well be recessive with a modifier of penetrance.[12]

In the 2 litters, a persistent ductus venosus occurred in both the left and the right liver lobes. We suggest that these phenotypes both are caused by the same genetic defect, because closure of the ductus venosus is probably regulated by a single pathway. The mixture of left- and right-sided shunts in the 2 litters confirms this theory. Our findings are in agreement with previously reported epidemiologic findings that breed (other than Australian Cattle Dog) is no predictor for the location of intrahepatic shunts.[13]

There is only one known genetic mouse model causing intrahepatic shunts, which are phenotypically identical to IHPSS in large dog breeds. This knockout mouse strain has a homozygous deletion in the aryl hydrocarbon receptor (AHR).[14] More recently it was demonstrated that AHR signaling in endothelial/hematopoietic cells is nec-

essary for developmental closure of the ductus venosus, whereas AHR signaling in hepatocytes is necessary to generate adaptive and toxic responses of the liver in response to dioxin exposure.[15] This is certainly a strong candidate gene; however, in mice all homozygous knock outs have IHPSS. Additionally, this model has a quite complex phenotype with multiorgan lesions, which do not occur in dogs with IHPSS.

References

1. van Straten G, Leegwater PA, de Vries M, et al. Inherited congenital extrahepatic portosystemic shunts in Cairn Terriers. J Vet Intern Med 2005;19:321–324.

2. Tobias KM, Rohrbach BW. Association of breed with the diagnosis of congenital portosystemic shunts in dogs: 2,400 cases (1980–2002). J Am Vet Med Assoc 2003;223:1636–1639.

3. Van den Ingh TSGAM, Rothuizen J, Meyer HP. Circulatory disorders of the liver in dogs and cats. Vet Q 1995;17:70–76.

4. Ubbink GJ, van de Broek J, Meyer HP, et al. Prediction of inherited portosystemic shunts in Irish Wolfhounds on the basis of pedigree analysis. Am J Vet Res 1998;59:1553–1556.

5. Meyer HP, Rothuizen J, Ubbink GJ, et al. Increasing incidence of hereditary intrahepatic portosystemic shunts in Irish Wolfhounds in The Netherlands (1984 to 1992). Vet Rec 1995;136:13–16.

6. Wolschrijn CF, Mahapokai W, Rothuizen J, et al. Gauged attenuation of congenital portosystemic shunts: Results in 160 dogs and 15 cats. Vet Q 2000;22:94–98.

7. Rothuizen J, Van den Ingh TSGAM. Rectal ammonia tolerance test in the evaluation of portal circulation in dogs with liver disease. Res Vet Sci 1982;33:22–25.

8. Sterczer A, Meyer HP, Boswijk HC, et al. Evaluation of ammonia measurements in dogs with two analysers for use in veterinary practice. Vet Rec 1999;144:523–526.

9. Gerritzen-Bruning MJ, Van den Ingh TSGAM, Rothuizen J. Diagnostic value of fasting plasma ammonia and bile acid concentrations in the identification of portosystemic shunting in dogs. J Vet Intern Med 2006;20:13–19.

10. Kerr MG, van Doorn T. Mass screening of Irish Wolfhound puppies for portosystemic shunts by the dynamic bile acid test. Vet Rec 1999;144:693–696.

11. Lamb CR, Burton CA. Doppler ultrasonographic assessment of closure of the ductus venosus in neonatal Irish Wolfhounds. Vet Rec 2004;155:699–701.

12. Burghes AH, Vaessin HE, de La Chapelle A. Genetics. The land between Mendelian and multifactorial inheritance. Science 2001;293:2213–2214.

13. Krotscheck U, Adin CA, Hunt GB, et al. Epidemiologic factors associated with the anatomic location of intrahepatic portosystemic shunts in dogs. Vet Surg 2007;36:31–36.

14. Lahvis GP, Lindell SL, Thomas RS, et al. Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. Proc Natl Acad Sci USA 2000;97:10442–10447.

15. Walisser JA, Glover E, Pande K, et al. Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types. Proc Natl Acad Sci USA 2005;102:17858–17863.