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Canine Imerslund-Gräsbeck Syndrome: A literature review

BY

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List of abbreviations

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AMN - Amionless

B_{12} – Vitamin B_{12}

BCS – Body Condition Score

BW – Body Weight

CN-Cbl – CyanoCobalamin

Cbl – Cobalamin

CoA – Co-Enzyme A

CUBN – Cubulin

GI – Gastrointestinal

IF – Intrinsic Factor

I-GS – Imerslund Grasbeck Syndrome

MMA – MethylMalonic Acis

OH-Cbl – HydroxyCobalamin

PO – Per Os

SC – Subcutaneous

TC-II – Transcobalamin II

tHCY – Total Homocysteine

UK – United Kingdom

Introduction

Cobalamin (Vitamin B_{12}) is an essential micronutrient for mammals synthesized only by certain microorganisms, obtained by monogastric species from animal derived foods via a complex receptor-mediated mechanism of the GI tract. Cobalamin deficiency is most often caused by GI malabsorption rather than dietary deficiency. Successful cobalamin absorption is a sequence of protein-binding events that each depend on the longitudinal secretory and absorptive organisation if the GI tract. (Nielsen, et al., 2012).

When metabolised cobalamin forms 5`-adenosyl cobalamin and methyl-cobalamin, serving as a co-factor for the enzyme, methlymalonyl-CoA mutase and methionine synthase, respectively. Deficiency at a cellular level inhibits the respective enzymatic activities resulting in an accumulation of unprocessed substrates observed as methylmalonic academia/-uria and homocysteimia. Secondary metabolites disrupt ammonia elimination, glucose homeostasis and nucleotide synthesis. Thus, clinical signs of severe cobalamin deficiency and its metabolic effects are far reaching and include dyshematopoiesis, gastrointestinal disturbances, post-natal development delay and life-threatening metabolic derangements (Stabler, 2013).

Hereditary selective malabsorption is caused mainly by defects that interrupt secretion or function of intrinsic factor, a protein product of gastric parietal cells in humans and pancreatic duct cells in dogs, or of cubam, the highly specific, IF-cobalmin receptor on the apical, brush-border membrane in the distal small intestine (Tanner, et al., 2012). In the ileum, cubam selectively mediates absorption of the IF-cobalamin complex from food, and absorbed cobalamin bind transcobalamin, a plasma transport protein for delivery of the vitamin to cells (Nielsen, et al., 2012). *Figure 1.*

The Imerslund-Grasbeck(I-GS) syndrome is a rare autosomal recessive disorder characterised by cobalamin deficiency due to selective malabsorption of this vitamin. In humans it results in megaloblastic anaemia appearing in childhood (Scheibel, et al., 1981). The canine disorder resembles the I-GS in humans. In dogs with I-GS genetic mutations of the ileal cubam receptor lead to cobalamin deficiency, with subsequent nonregenerative anaemia, erythrocyte anisocytosis, megaloblastic bone marrow, leukopenia, neutrophil hypersegementation and thrombocytopenia (Fyfe, et al., 1990). The precise mutations of the cubam receptor will be discussed later and each known breed affected.

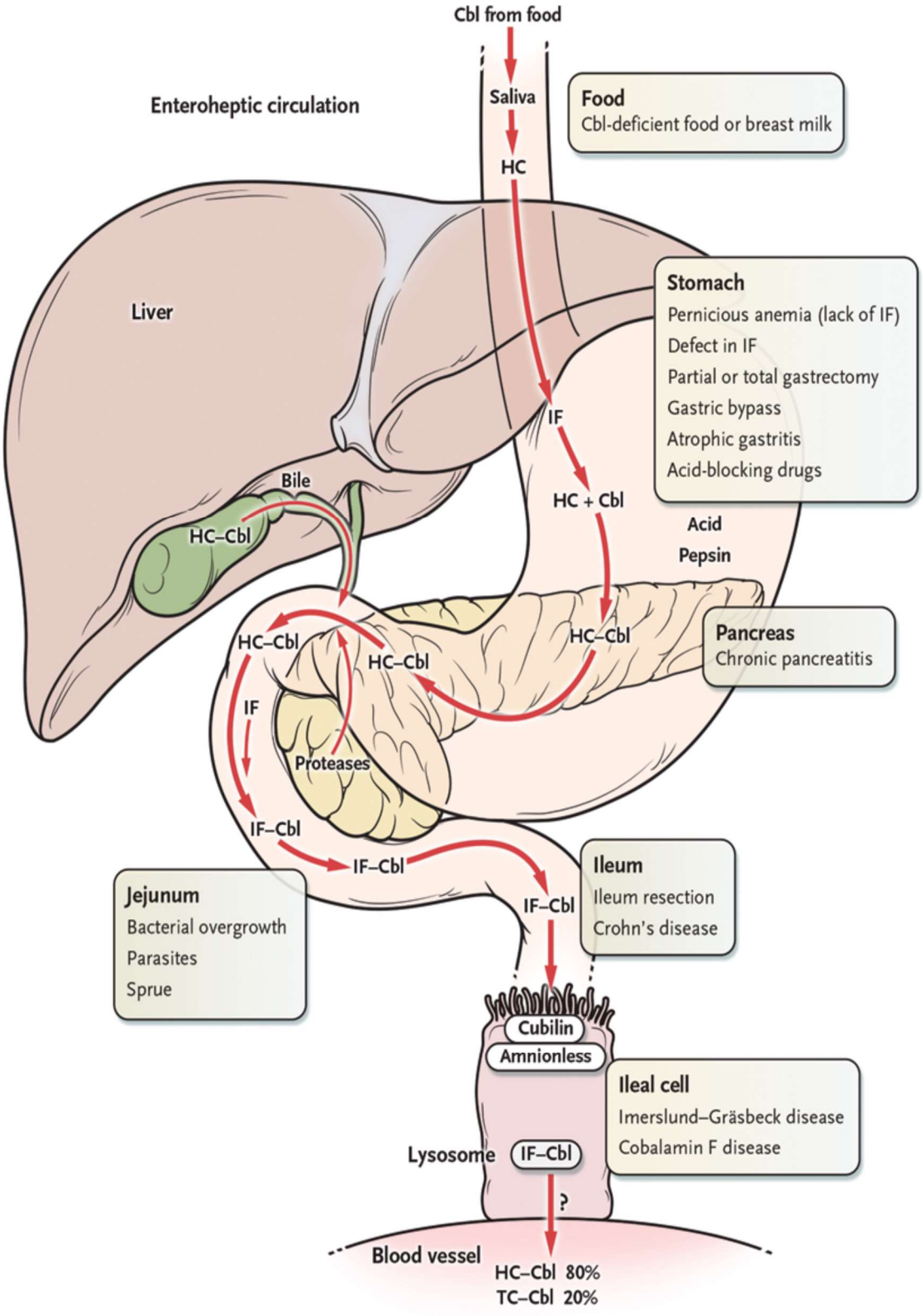


Figure 1 The Normal Mechanisms and defects of Absorption of Cobalmin in Humans. (Stabler, 2013).

Clinical manifestations present as failure to thrive, failure to gain weight despite normal linear growth, inappetence and lethargy. It is a potentially life threating metabolic disruption in the juvenile period if not diagnosed in time, all clinical manifestations can be reversed with immediate parenteral treatment of Cyanocobalamin and subsequent life-long parenteral supplementation.

Known canine breeds genetically predisposed to the disease include Giant Schnauzers; Beagles; Border Collies; Australian Shepherds and Hungarian Komondors. An associated study of Cobalamin deficiency in the Chinese Shar Pei (Grützner, et al., 2010) gives evidence of a different genetic condition resulting in cobalamin deficiency. Cobalamin deficiency in Shar Peis appear to differ from other cobalamin deficiency syndromes reported on I-GS dogs (Fyfe, et al., 1990) where gastro intestinal symptoms have not been described. The authors of the study suggest that due to lack of classical deficiency signs there are other mechanisms at work that are responsible for this condition in Shar Peis, such as malabsorption interference or defective transport of cobalamin and a slow onset of the deficiency may lead to a clinical syndrome causing gastrointestinal disease (Grützner, et al., 2010). The authors present evidence for an association of canine microsatellite markers DTR13.6 and REN13N11, located on chromosome 13, with the deficiency in Chinese Shar Peis.

The selectivity of intestinal cobalamin malabsorption coupled with failed renal tubular protein reabsorption is conferred by the mutations in cubulin (CUBN) (Aminoff, et al., 1999) or amnionless (AMN) (Tanner, et al., 2003), the subunits of the heteromeric receptor designated cubam (Fyfe, et al., 2004).

Cubam, is a multi-ligand, endocytic receptor expressed in epithelial brush borders of distal small intestine and renal proximal tubules. CUBN is a large glycosylated protein, it provides binding sites for various ligands including the intrinsic factor-cobalamin (IF-Cbl), vitamin D binding protein, transferrin, albumin, apolipoprotein A1, haptoglobin, and others (Nielsen, et al., 2012). CUBN domains 5-8 comprise the essential IF-Cbl binding site, and the N-terminal alpha helical region mediates the association with AMN. AMN is the smaller of the two proteins, it is a glycosylated transmembrane protein. There is evidence from human, mouse and canine tissues harbouring mutations in either gene that both CUBN and AMN must both be present with near-normal structure for epithelial brush border expression of the functional receptor complex. (Fyfe, et al., 2013).

The cubulin receptor is heavily expressed in the kidney proximal tubule brush border and intracellular endocytic compartments (Christensen, et al., 1998). Cubulin has been identified as an albumin binding protein and is subsequently deemed important in normal albumin reabsorption. (Fyfe, et al., 1991). Dogs with an abnormal processing and defective insertion of cubulin into the luminal membranes causing a defect in intestinal absorption of cobalamin an proteinuria display significant albuminuria and decreased tubular uptake of albumin. When selective proteinuria is recognised, it is a useful diagnostic feature because it excludes other causes of selective cobalamin malabsorption such as gastric intrinsic factor (GIF) deficiency. At present, the most efficient means to discriminate between known types of inherited selective cobalamin malabsorption without proteinuria is by molecular diagnosis, undertaking screens of GIF, CUBN and AMN for causative mutations (Tanner, et al., 2012).

Specific breeds and their specific Mutations

Separate studies on the predisposed breeds have confirmed that the inherited selective malabsorption is due to a defect in the synthesis of the cubam receptor. In addition, they have identified the precise mutation in both Cubulin (CUBN) and Amnionless (AMN) protein subunits. These subunit mutations are specific to each breed, a frameshift mutation in CUBN occurs in Border Collies (Owczarek-Lipska, et al., 2013), Beagles (Fyfe, et al., 2014) and (Murtagh, et al., n.d.) and Komondors (Fyfe, et al., 2018). Another frameshift mutation of the AMN subunit has been identified in Australian shepherds (Gold, et al., n.d.) and Giant Schnauzers (Fyfe, et al., 1990).

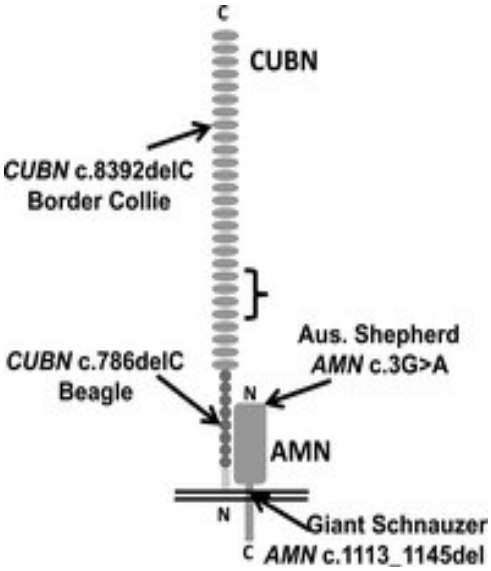


Figure 2 (Fyfe et al, 2014)

Cubam structure showing sites of I-GS causing mutations in dogs. The cartoon illustrates the protein domain structure of cubam composed of CUBN and AMN subunits. The N and C termini of each protein are indicated. The bracket around CUB domains 5–8 indicates the intrinsic factor–cobalamin binding site. The epithelial cell apical plasma membrane is indicated by double horizontal lines, and the extracellular side is to the top. Each site of breed-specific mutation causing canine I-GS is indicated by an arrow.

Diagnostic Methods

The diagnosis of IG-S in dogs can be achieved with the usual processes in diagnostic medicine.

- A full medical history
- Collection of clinical signs
- Complete blood counts
- Serum Chemistry panels, including Cbl and folate concentrations
- Blood Smears
- Bone Marrow aspirates
- Routine and Special urinalysis
- Differential diagnosis

Clinical Manifestions

On initial presentation common symptoms were recorded in all reported cases, including failure to thrive, lethargy, inappetence, low BCS. (Table 1 &2) Variable symptoms include diarrhoea(intermittent/chronic), vomiting, in severe cases seizures and cardiac abnormalities. Further investigation presented findings of nonregenerative anaemia, proteinuria and methylmalonic aciduria. In each case the levels of serum cobalamin were below the detectable limits of the assay. Haematology reports concluded the anaemia as being normochromic, normocytic non-regenerative anaemia.

	Failure to thrive, lethargy, inappetence etc.	Anaemia and type	leukogram	Proteinuria	MMAciduria	Other symptoms
Border Collies	Yes	Normochromic/-cytic non-regenerative anaemia	Results within reference ranges	Yes	Yes	Heart murmur, erosions on tongue,
Beagles	Yes	Normochromic/-cytic non-regenerative anaemia	Leukopenia; Neutropenia	Yes	Yes	Seizure, vomiting, alopecia.
Komondor	Yes	Normochromic/-cytic non-regenerative anaemia	Neutropenia, mild thrombocytopenia	Yes	Yes	Seizures, fine head tremor

Table 1

AMN breeds and clinical signs

Table 2

	Failure to thrive, lethargy, inappetence etc.	Anaemia and type	leukogram	Proteinuria	MMAciduria	Other symptoms
Border Collies	Yes	Normochromic/-cytic non-regenerative anaemia	Results within reference ranges	Yes	Yes	Heart murmur, erosions on tongue,
Beagles	Yes	Normochromic/-cytic non-regenerative anaemia	Leukopenia; Neutropenia	Yes	Yes	Seizure, vomiting, alopecia.
Komondor	Yes	Normochromic/-cytic non-regenerative anaemia	Neutropenia, mild thrombocytopenia	Yes	Yes	Seizures, fine head tremor

The leukograms of the defected AMN cases reported neutropenia with hypersegmentation.

	Day 1	Day 3	Day 5	Day 7	Day 15
Segmented neutrophils ($3x10_3$/mL) [4.0–8.2] ref range	0.4	0.1	2.3	8.8	5.3

Table 3 (Gold, et al., n.d.)

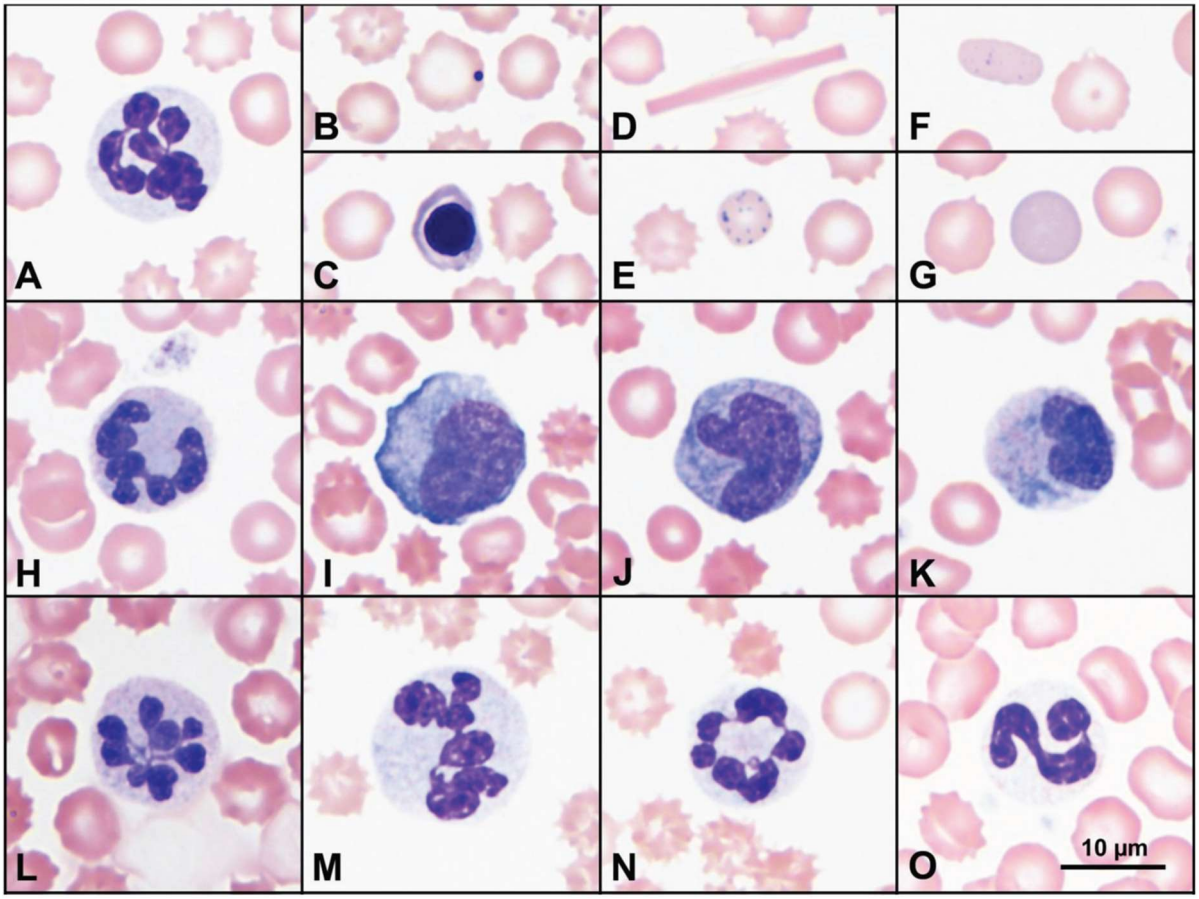


Figure 3 Neutrophil hypersegmentation. [(Gold, et al., n.d.)]

Figure 3. Selected peripheral blood film findings on days 1 (A-G), 3 (H-L), 5 (M-N), and 40 (O). At presentation, neutrophils were hypersegmented (A), most with mild diffuse cytoplasmic basophilia, and red blood cells variously exhibited echinocytosis (A-F), increased Howell-Jolly bodies (B), metarubricytes (C), short to long rectangular hemoglobin crystals (D), basophilic stippling (E), mildly polychromatophilic elliptocytes (F), and polychromatophilic erythrocytes, most of which were similar in diameter to mature erythrocytes (G). On day 3, diffusely basophilic hypersegmented neutrophils without Döhle bodies persisted (H and L); there were also low numbers of blasts (I) and mostly monocytoid cells with increased diffuse cytoplasmic basophilia (J). Some cells had broad but rarely band-shaped nuclei and diffusely basophilic cytoplasm containing additional irregular patches of more intense basophilia (K). On day 5, hypersegmented neutrophils were more numerous (M and N). Throughout, some hypersegmented neutrophils were large (M). With recovery, circulating neutrophils appeared as in health, lacking cytoplasmic basophilia and hypersegmentation (O). The scale bar in frame O applies to all frames. Routine Romanowsky stains: Wright-Giemsa (A-G, M-O) and Modified Wright (H-L).

Laboratory Findings

Table 4

	Giant Schauzners	Border Collies	Beagles	Komondors	Australian Shepher
HCT	Low	Low	Low	Low	Low
Reticulocytes			Low		Low
Neutrophils	Low	Low-Normal	Normal	Low	Low
Protein	IgG	Low-normal	Low		
Albumin		Low-Normal	Low		Low
Serum Cobalamin	Below detectable limits	Below detectable limits	Below detectable limits	Below detectable limits	Below detectable limits
Folate	Norm				Low- Very Low
Urine MMA	Extremely High	Extremely High	Extremely High	Extremely High	Extremely High
Proteinuria	High	High	High	High	

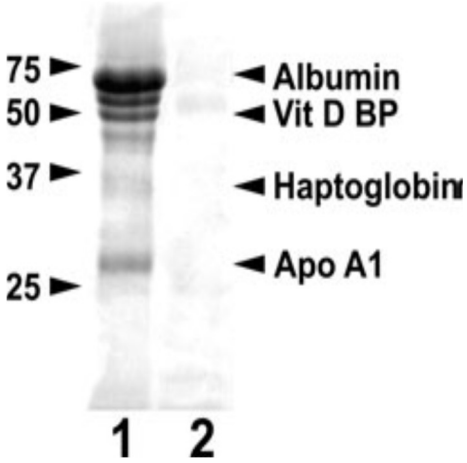
Urinalysis

As previously discussed, the cubulin receptor is also expressed in the proximal renal tubules, any defect in the receptor will result in defective protein reabsorption ultimately presenting as proteinuria. Proteinuria was present in all CUBN breeds. Of the two known AMN defective breeds, Australian Shepherds and Giant Schnauzers, there has been no reported case of significant proteinuria.

Importance of MMAciduria as a diagnostic biomarker

Vitamin B_{12} serves as a co-enzyme for methylmalonyl Co-enzyme-A Mutase and 5-methyltetrahydrofolate-homocysteine methyltransferase, deficiency of cobalamin leads to reduced activity of both of these enzymes resulting in an increase of Methylmalonic acid (MMA) and total homocysteine (tHCY). These metabolites are excreted in the urine. Measurements of these metabolites allow assessment of cellular cobalamin availability. Normal dogs excrete less than 10mg MMA/g creatinine.

Detailed evaluation of MMA in dog urine in all reported cases is available, values reported came into the range of high to extremely high. MMA levels in urine prove to be a specific biomarker for cobalamin absorption and metabolism.



Selective proteinuria in a Beagle with I-GS. Shown are lanes from a silver-stained 15% SDS-PAGE gel loaded with urine proteins of an affected (case 2; lane 1) and a clinically normal Beagle (lane 2). Proteins in each lane were concentrated from urine samples containing 50 lg of creatinine. The affected dog was in clinical and metabolic remission at the time of urine collection because of previous parenteral cyanocobalamin administration. Migration of molecular weight (kDa) markers is indicated to the left. (Fyfe, et al., 2014).

Figure 4

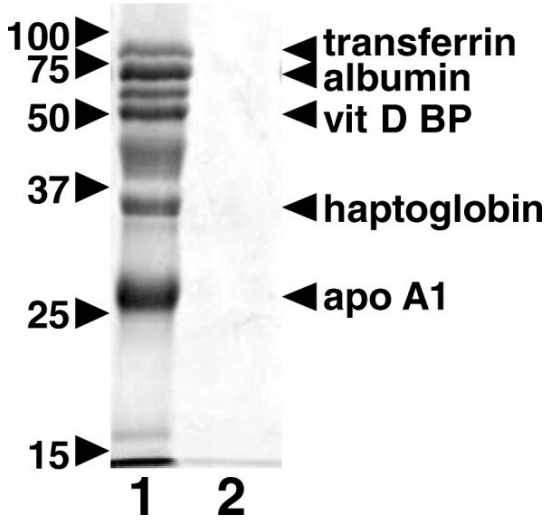


Figure 5

1 Urine protein analysis of Komondor littermates. Urine samples containing 200 µg creatinine were desalted and concentrated by centrifugation through a 10 kDa molecular weight cutoff membrane. Proteins were separated by 15% SDS-PAGE and visualized by silver staining. Lane 1 shows urine proteins obtained from an affected dog (case 5, Additional file 1) after cobalamin replenishment and during metabolic remission. Lane 2 shows urine proteins from a heterozygous but clinically healthy littermate of case 5 (Fyfe, et al., 2018)

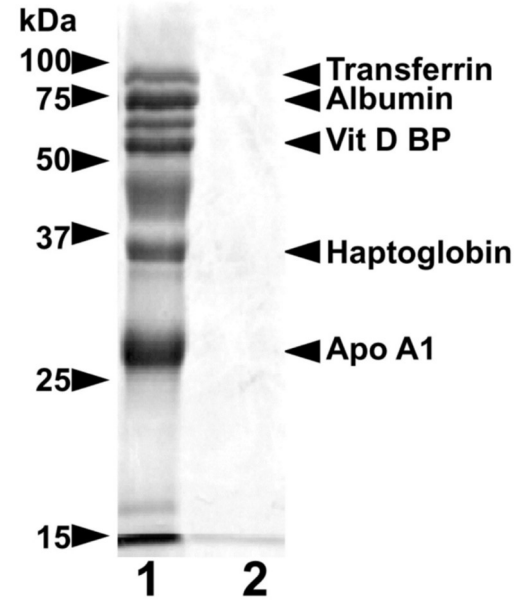


Figure 6

Selective proteinuria in BC with I-GS

Shown are lanes from a silver-stained 15% SDS-PAGE gel loaded with urine proteins of an affected (lane 1) and a clinically normal BC (lane 2). Proteins in each lane were concentrated from urine samples containing 20µg of creatinine. The affected dog was in metabolic remission at the time of urine collection due to previous parenteral cyanocobalamin administration. Identities of the labelled protein bands were confirmed by immunoblotting as previously reported. (Fyfe, et al., 2013)

Haematological methods

Complete blood counts were performed in all cases along with biochemistry panels. A summary of clinically relevant findings in predisposed breeds can be found in table **1** and **2.** In humans bone marrow analysis produced a diagnosis of macrocytic and megaloblastic anaemias which reveals low haemoglobin, pancytopenia, increased red cell size objectively indicated by high mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) indexes, and typical morphological changes in both red and white cells and platelets and their precursors, e.g. megaloblasts, granulocytes with poly-lobulated nuclei, and so on. The myelogram helps to rule out malignant and other conditions that may resemble megaloblastic anemia. (Scheibel, et al., 1981). However, the association between cobalamin and folate deficiencies and macrocytic, non-regenerative anaemia established in humans is not routinely present in dogs. (Stanley, et al., 2019).

Bone Marrow Aspirates

On bone marrow examinations, megaloblastic changes were particularly evident in the myeloid series. Giant metamyelocytes and band forms were present. Erythroid precursors were reduced in number, and cellularity of the marrow was normal or decreased. Bone marrow iron stores appeared normal, and serum iron concentrations and total iron-binding capacities were normal. (Fyfe, et al., 1990)

Bone marrow investigations can be informative in some cases, however can be a quite invasive, painful and a costly procedure in most cases.

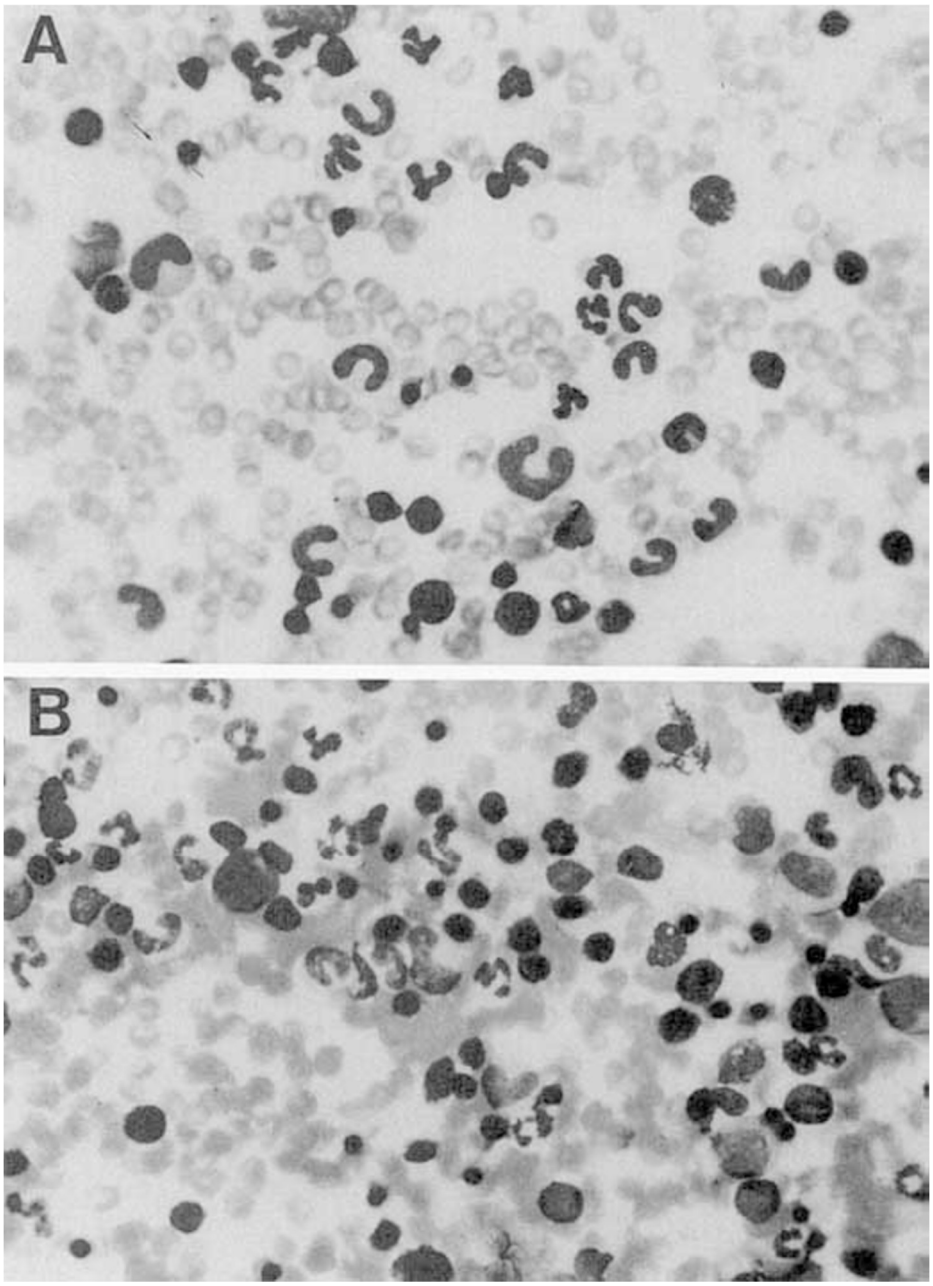


Figure 7 (Fyfe, et al., 1990)

Bone marrow aspirate from a Cbl-deficient and an agematched normal puppy.

A, a typical aspirate from a 20-wk-old, untreated, affected puppy exhibiting clinical and laboratory signs of Cbl deficiency.
Megaloblastic changes are visible in some of the myeloid cells shown.
Giant metamyelocytes and band neutrophils are present. Erythroid precursors are lacking and cellularity of the marrow is reduced from normal.

B, a normal bone marrow aspirate is presented for comparison.

Differential Diagnoses

The predominating clinical signs/client complaints

- Inappetence
- Failure to gain weight
- Failure to thrive

With none of these signs being specific, a high index of suspicion based on breed disposition and age of onset is needed for correct and efficient diagnosis of hereditary cobalamin absorption. The differential diagnosis is of equal importance to the normal diagnostic.

The most common cause of cobalamin deficiency is malabsorption, dietary insufficiency is becoming increasingly uncommon. Acquired cobalamin malabsorption causes include:

- Gastrointestinal dysbiosis
- Surgical resection
- Exocrine pancreatic insufficiency
- Diffuse mucosal disease
- Atrophic gastritis leading to lack of intestinal factor
- Transport derangement (aberrant TC-II)
- Errors in Cbl-dependant enzymes
    - Resulting in hematological or metabolic derangement causing MMAciduria and Homocysteinuria
- Infiltrative or inflammatory intestinal disease
- Porto-systemic shunts
- Infectious diseases
    - Toxoplasmosis
    - Neosporosis
    - Distemper
    - Parvoviral Infection
    - Intestinal Parasites
- Congenital hypothyroidism
- Toxic ingestion
- Thiamine deficiency

A cobalamin deficiency leads to a deceleration of nuclear maturation in rapidly proliferating hematopoietic and intestinal epithelial cells creating the recognised alterations in the tissues. An increase in the concentration of mitochondrial pools of acyl-CoA esters secondarily inhibit additional metabolic pathways including the Urea cycle, gluconeogenesis and glycine cleavage leading to hyperammonemia, hypoglycaemia and hypoglycinemia. Therefore, signs of chronic cobalamin deficiency are global because of the disturbance of several metabolic pathways and could be confused with a number of disorders including hepatoencephalopathy.

A complicating factor is that enterocyte function is affected by cobalamin deficiency leading to secondary malabsorption. Until deficiency state has been corrected, absorption tests should then be carried out. (Scheibel, et al., 1981).

Cobalamin Absorption tests.

Cobalamin absorption tests have been used in the past to determine the precise location of cobalamin absorption in the intestinal tract. Direct evidence was provided in 1959 by Booth and Mollin (Booth & Mollin, 1959) who studied the distribution of radioactivity in the intestine after oral administration of labelled B_{12}. Using a Geiger Muller counter during laparotomy, radioactive B_{12} was found concentrated in the ileum. Vitamin B_{12} is unique in several respects; it is absorbed only in the ileum in contrast with most other substances; it requires a gastric intrinsic factor to be efficiently absorbed; and because the number of specific receptor sites in the ileum is restricted, only tiny amounts can be absorbed (Schj0nsby, 1989).

Cobalamin absorption tests have been used regularly in determining inherited selective intestinal cobalamin malabsorption in dogs. (Fyfe, et al., 1990) have used radioactively labelled Cobalamin to assess the intestinal absorption of orally administered Cbl (0.66µg [^{57}Co]CN-Cbl) alone or with oral administration of IF or normal dog gastric juice on affected (IGS) and non-affected dogs. Final measurements of Cbl levels in blood and faeces. Malabsorption of Cbl in affected dogs was documented by [^{57}Co]CN-Cbl absorption tests in which plasma in the control dogs was similar to that observed in dogs previously and was comparable to levels in humans studied in a similar way. The complete and rapid response

of affected dogs to parenteral CN-Cbl administration as the sole treatment suggested that the defect was selective Cbl malabsorption. (Fyfe, et al., 1990).

Cobalamin absorption tests using radioactively labelled cobalamin has proved to be a useful diagnostic test, however, the performance of these tests has been increasingly difficult because of the limited availability of radioactively labelled cobalamin and the decreasing acceptance of a radioactively labelled vitamin in diagnostic tests. More over the human IF used in these tests (Schilling test II) has been removed from the market in most countries. This leads to a need for Nonradioactive Vitamin B_{12} Absorption Tests. *Bor M et al 2005* devised a test that removes the disadvantage of complex ingredients and also the inconvenience of the collection of urine/faecal samples by focusing on the absorption of free Vitamin B_{12}.

In the study, they evaluated the use of this new approach as a vitamin B_{12} absorption test in patients with inherited malabsorption of vitamin B_{12} attributable to IGS or lack of intrinsic factor, their obligate heterozygous parents, and healthy controls. The results of the study indicate that measurement of holo-TC after an oral dose of vitamin B_{12} can identify patients with hereditary disorders of absorption of vitamin B_{12}. The patient group was not able to actively absorb vitamin B_{12}; therefore, any absorption of the vitamin during the proposed test would suggests the occurrence of passive absorption.

The results suggested that there was no sign of passive absorption, the result is an important prerequisite for the use of the proposed test. Passive absorption of vitamin B_{12} is believed to account for ~1% of the administered dose of the vitamin; we were therefore concerned whether the high physiologic dose used in our new test would lead to passive absorption, mimicking active uptake. Results strongly support the conclusion that significant passive absorption does not occur when a challenge involving three 9-µg doses of vitamin B_{12} is used, at least not in patients with the 2 hereditary disorders of absorption of vitamin B_{12} studied.

However, in one of the most recent studies (Kook & Hersberger, 2019) on treatment of selective malabsorption, the use of 1mg cyano-Cbl (11kg Beagle) daily indicates for the first time that passive intestinal Cbl absorption alone leads to a normal clinical and cellular Cbl status in dogs. More on this study will be discussed later in regards to I-GS treatment. The measurement of holo-TC after the dose of oral B_{12} can be very useful in diagnosing hereditary B_{12} malabsorption errors and subsequently I-GS. Recombinant human IF is now

available commercially, this test is designed to examine whether IF supplementation can correct negative absorption of free vitamin B_{12}. In suspected patients, this would help distinguish those with vitamin B_{12} malabsorption attributable to a defective receptor from those with inherited lack of IF. Only in the latter group would one expect to be able to correct vitamin B_{12} absorption by addition of IF. (Bor, et al., 2005)

These absorption tests are necessary because no single laboratory marker is suitable for the assessment of B_{12} status in all patients (Harrington, 2017). Serum Cbl concentrations are not measured routinely in absorption studies because long-term experience has shown that low or even undetectable serum concentrations often are encountered in clinically healthy affected (I-GS) dogs that have received parenteral treatment. Speculation as to why, is that the half-life of Cbl bound intracellularly as a coenzyme is longer than the residence time of Cbl in the circulation, which would mean that Cbl continues to be active in tissues and Cbl dependent metabolism remains normal even when serum concentrations are low. Interestingly, the few available serum Cbl concentrations in this study were all within reference Interval, similar to what has been published recently. (Kook & Hersberger, 2019). For evaluating response to treatment, Cbl absorption tests such as *Bor M. et al* methods or the measurement of Urine MMA could be clinically more conclusive than the measurement of serum Cbl levels. In the diagnosis of I-GS, measuring serum Cbl levels are vital in determining a Cbl deficiency when the case is first presented.

Suspect cases

Yorkshire Terrier

In 2015 a case of methylmalonic Aciduria secondary to cobalamin malabsorption in a Yorkshire terrier was reported in the UK (McLauchlan, et al., 2015). An 8-week old male Yorkshire Terrier presented with a 2-week history of hypoglycaemia, lethargy and seizures. Investigations revealed undetectable serum cobalamin levels, hyperammonemia and methylmalonic aciduria. Yorkshire terriers are known to be predisposed to congenital portosystemic shunts and portal venous hypoplasia which can result in hyperammonemia subsequently and identical signs to those seen in dogs with hypocobalaminemia and MMAciduria. The treatment of the two conditions is very different therefore documenting

the existence of MMAciduria in Yorkshire terriers is crucial. The report concluded with the elimination of causes of hepatic origin with the complete remission of clinical signs from parenteral cobalamin, the MMAciduria was due to hypocobalaminemia. However, the patient was also treated for hepatic encephalopathy, it is therefore impossible to determine how much the hyperammonemia contributed to clinical signs and how much they were due to MMA. The report also highlights that it found it impossible to exclude that the malabsorption of cobalamin was secondary to small intestinal bacterial overgrowth because of the antibiotic treatment. But the sustained and complete response to cobalamin supplementation without any other long-term therapy would make other differentials unlikely. The difficulty of differentials in this case suggests a molecular analysis of the CUBN and AMN genes could be the diagnostic method of choice in determining I-GS.

Chinese Shar Peis

Suspicion already exists within the Chinese shar pei breed that they have a hereditary disorder affecting cobalamin deficiency (Dandrieux, et al., 2013). An association study (Grützner, et al., 2010), characterised that the case of Shar Pei cobalamin deficiency as different to the cobalamin deficiency in I-GS dogs.

Shar Peis commonly present with clinical signs similar to chronic gastrointestinal disease (usually small bowel diarrhoea and weight loss). The lack of classical clinical signs of Cobalamin deficiency suggest that other mechanisms may be responsible for this condition in Shar Peis, such as malabsorption interference or defective transport of cobalamin and a slow onset of cobalamin deficiency may lead to a clinical syndrome causing gastrointestinal disease. (Dandrieux, et al., 2013).

The study could not conclusively narrow down the region on chromosome 13 as the major locus or primary gene responsible for cobalamin deficiency in the Shar Pei. Further studies to fine map the region as well as the whole genome using a single nucleotide polymorphism is warranted.

The study presents the first evidence of an association in a region located on canine chromosome 13. In this region, no previously identified genes reported to be associated with cobalamin deficiency in dogs or any other species. The authors concluded an association of canine microsatellite markers DTR13.6 and REN13N11, located on chromosome 13, with

cobalamin deficiency in Chinese Shar Peis. With a future aim to utilise a finer mapping tool, such as SNPs, to both verify and fine map this particular region to locate candidate genes for further investigation (Grützner, et al., 2010).

Conclusion on suspected cases.

In the case of the Yorkshire Terrier, one can conclude with the extensive differential diagnoses and the elimination of other suspected causes, the suggestion of I-GS is acceptable. However, because a molecular analysis/genetic screen was not performed it is impossible to determine if this is an I-GS case.

In relation to the Chinese Shar Pei and the molecular genetic studies performed one can conclude that the Shar Pei is not a breed predisposed to I-GS but rather to a different and possibly unique genetic disorder affecting the absorption of cobalamin.

Treatment and management of lifelong cobalamin supplementation

A vitamin B_{12} deficiency can be a life-threatening syndrome. The severity of the deficiency associated with I-GS requires both immediate treatment and lifelong supplementation of cobalamin.

Immediate treatment involves Parenteral SC or IM injections of either cyanocobalamin or hydroxycobalamin. Doses range from 50-75 µg/kg BW once daily for 1-3 days, depending on protocols that individual clinicians or private veterinary practices follow. Puppies can receive supplementation from 2 weeks old at 2.5µg/kg BW. With immediate treatment one can expect appetite and weight gain to return to normal within 12-48 hours, bone marrow regeneration within 10-14days, MMAcid levels to return to normal in 7 days and reticulocytosis in 12 days.

Lifelong treatment protocols can vary. In regards to dosage and frequency. The various case reports and studies researched in this thesis the dosage range is a subcutaneous 50µg/kg BW or 1mg injection every 3-4 weeks or bimonthly depending on follow up re-evaluations of the patients health/cobalamin status.

A comparison of the efficacy of oral and parenteral Cobalamin supplementation was performed by (Toresson, et al., 2018). The study was carried out on dogs with chronic enteropathies and hypocobalaminemia using a randomised protocol with dogs either receiving oral or parenteral supplementation. The oral group received 0.25-1mg CN-Cbl daily and the parental group received 0.4-1.2mg OH-Cbl once weekly for the first 6 weeks then once every 4 weeks. Analysis of Serum Cbl levels happened at 28 days and 90 days. As expected, an increase of serum Cbl was significantly greater in the parenteral group at the 28-day measurement interval. However, at the 90-day measurement interval the serum Cbl level was significantly greater in the oral group. The conclusion of the study was that both oral and parenteral cobalamin supplementation are effective ways to treat hypocobalaminemia due to chronic enteropathies. In comparison of the two methods of supplementation the authors agree that oral supplementation is as effective, if not better than parenteral supplementation, in regards to cost effective treatment oral cobalamin supplementation could be deemed the preferred way of treatment.

The cobalamin malabsorption associated with chronic enteropathies mimics the cobalamin malabsorption associated with I-GS. The treatment is somewhat identical, however, with

time, an enteropathy may heal however, in the case of I-GS life-long supplementation is needed. In a 2018 study on Daily oral cyanocobalamin supplementation in Beagles with hereditary cobalamin malabsorption (Imerslund‐Gräsbeck syndrome) the authors concluded that daily oral supplementation can maintain normal clinical and cellular cobalamin status. The study is particularly interesting because the results using IGS‐affected dogs with a known functional defect of cubam but otherwise healthy intestinal tract, indicates for the first time that passive intestinal Cbl absorption alone leads to a normal clinical and cellular Cbl status in dogs. In the present study, all dogs entered the PO supplementation phase after having been supplemented parenterally for an extended period of time (15, 19, and 21 months). Therefore, it can be confidently ruled out that possible negative effects of pre-existing Cbl deficiency on intestinal absorptive capacity. In conclusion, a maintenance dose of 1 mg cyano‐Cbl administered PO appears efficacious for maintaining normal clinical status and cellular markers of Cbl metabolism in dogs with IGS. The findings further corroborate the hypothesis that an alternative absorptive pathway of Cbl absorption exists in dogs. Assuming that comparable results can be achieved when treating other breeds of dogs with IGS caused by different mutations, but this warrants further study. (Kook & Hersberger, 2019).

Finally, it is important to note that since cobalamin is a water-soluble vitamin, excess cobalamin is excreted through the kidneys and clinical disease due to over-supplementation should not occur and has never been described in any species.

I-GS an autosomal recessive disorder

Genetic investigations

I-GS is a rare autosomal recessive disorder. Since the discovery of the disorder, studies have been carried out proving the hereditary nature if the disease. Genetic studies have determined the exact mutations causing defective cobalamin absorption in the aforementioned dog breeds. Mutations in *CUBN* cause I-GS in border collies and beagles, and mutations in *AMN* cause I-GS in giant schnauzers and Australian shepherds (Fyfe, et al., 2014), (Fyfe, et al., 2013). Genetic tests are available for each breed. While I-GS is uncommon among all dogs, in the affected breeds there can be a substantial carrier

frequency and more common occurrence of disease regionally or worldwide. Affected dogs of the various breeds have been reported from Canada, USA, UK, continental Europe, and Australia. Examples from the genetic studies of the various breeds are included below.

Segregation analysis of I-GS in the BC kindreds was consistent with simple autosomal recessive inheritance. The number of affected pups born in such matings was not different from what is expected under that hypothesis (χ 2=0.35, p>0.55, df=1), and both male and female affected dogs were observed, always born in matings of clinically normal parents. Due to incomplete breeding records, a common ancestor was not determined to all obligate carriers of all kindreds. However, affected dogs of this study appeared to be distantly related despite that ascertainment of the 3 kindreds occurred over 17 years and 2 continents.

The carrier frequency of dogs being heterozygous for *CUBN:c.8392delC* variant in a cohort of 200 selected Border Collies is relatively low at 6.2%. (Owczarek-Lipska, et al., 2013).

Figure 8

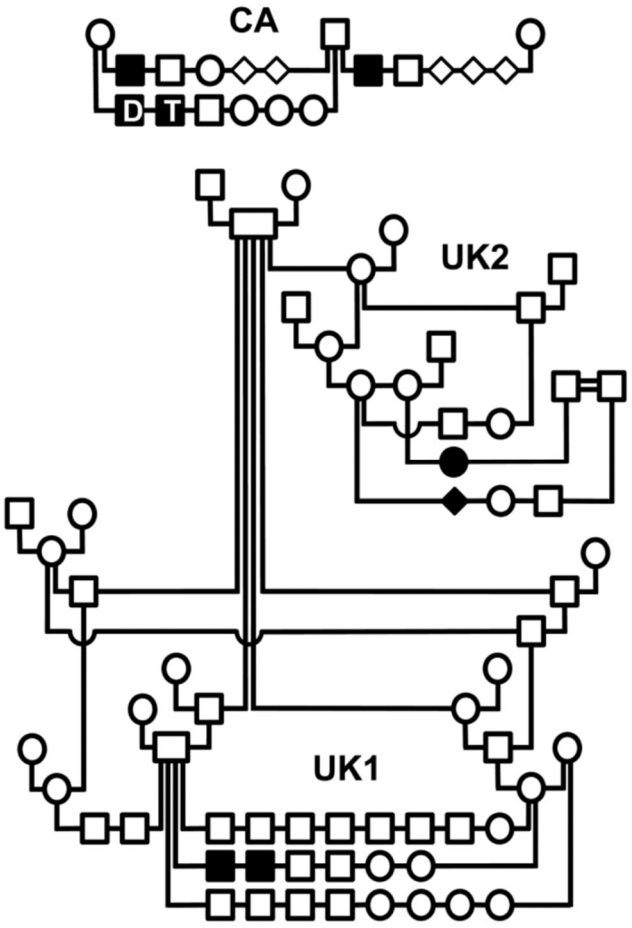


Figure 1. Pedigrees of 3 border collie families segregating canine I-GS
Squares indicate males, circles indicate females, and filled symbols indicate affected dogs. Offspring of a mating are arranged on a horizontal line extending between vertical lines that descend from the parents. Clinical findings of the dogs labeled **D** and **T** in the CA kindred are described in detail in Results. The filled diamond indicates an affected dog of unreported sex. The double line connecting two males in the UK2 kindred indicates that they share common great-grandparents but of unknown relationship to the other BC
(Fyfe, et al., 2013)

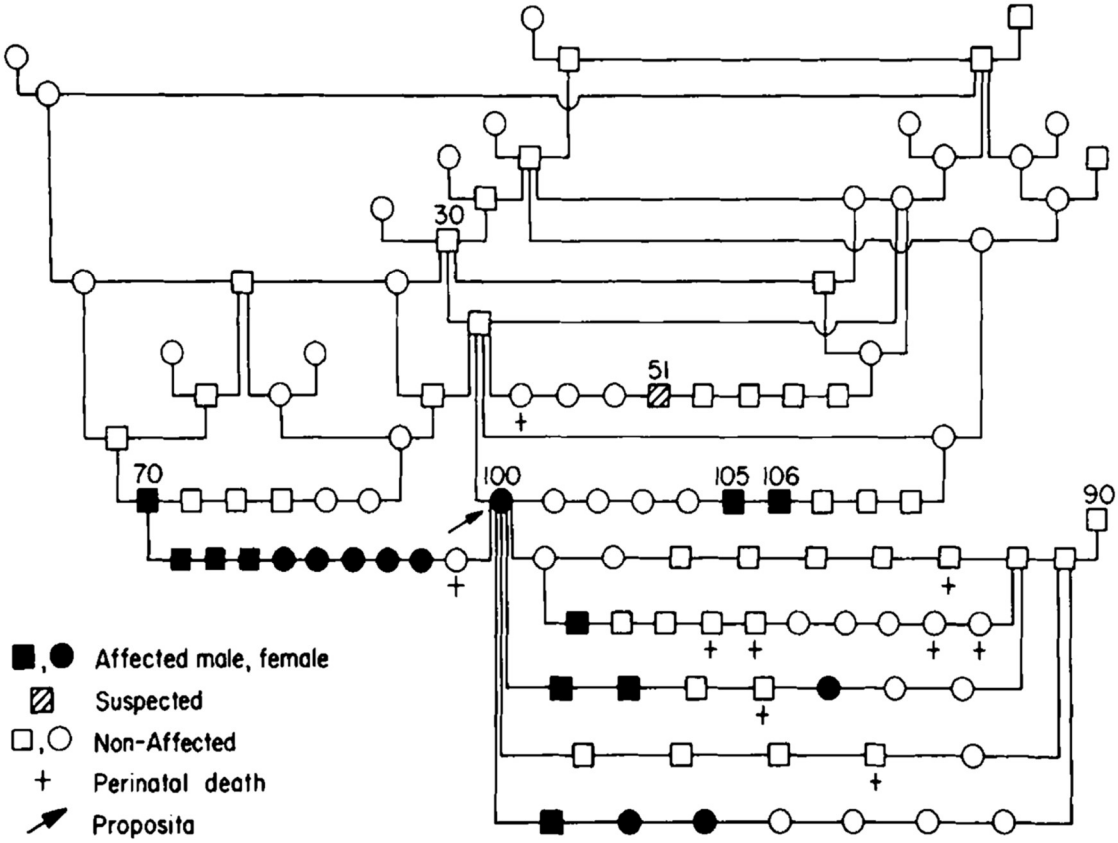


Figure 9

Fig. 3. Pedigree of a family of dogs with inherited selective intestinal Cbl malabsorption. **A** mating of two dogs is indicated by the symbols for offspring arranged on a horizontal line joining a vertical line from the bottom of the symbol for each parent. The proposita (arrow) and dog no. 70 were purebred giant schnauzers. Dog no. 90 was an unrelated, normal mongrel. Note that affected dogs were born to normal parents, that both sexes were affected, and that dog no. 30 is a common ancestor of every parent of an affected dog. These features and the numerical results of experimental outcross and FI-backcross matings are consistent with simple autosomal recessive inheritance.
(Fyfe J. , et al., 1991).

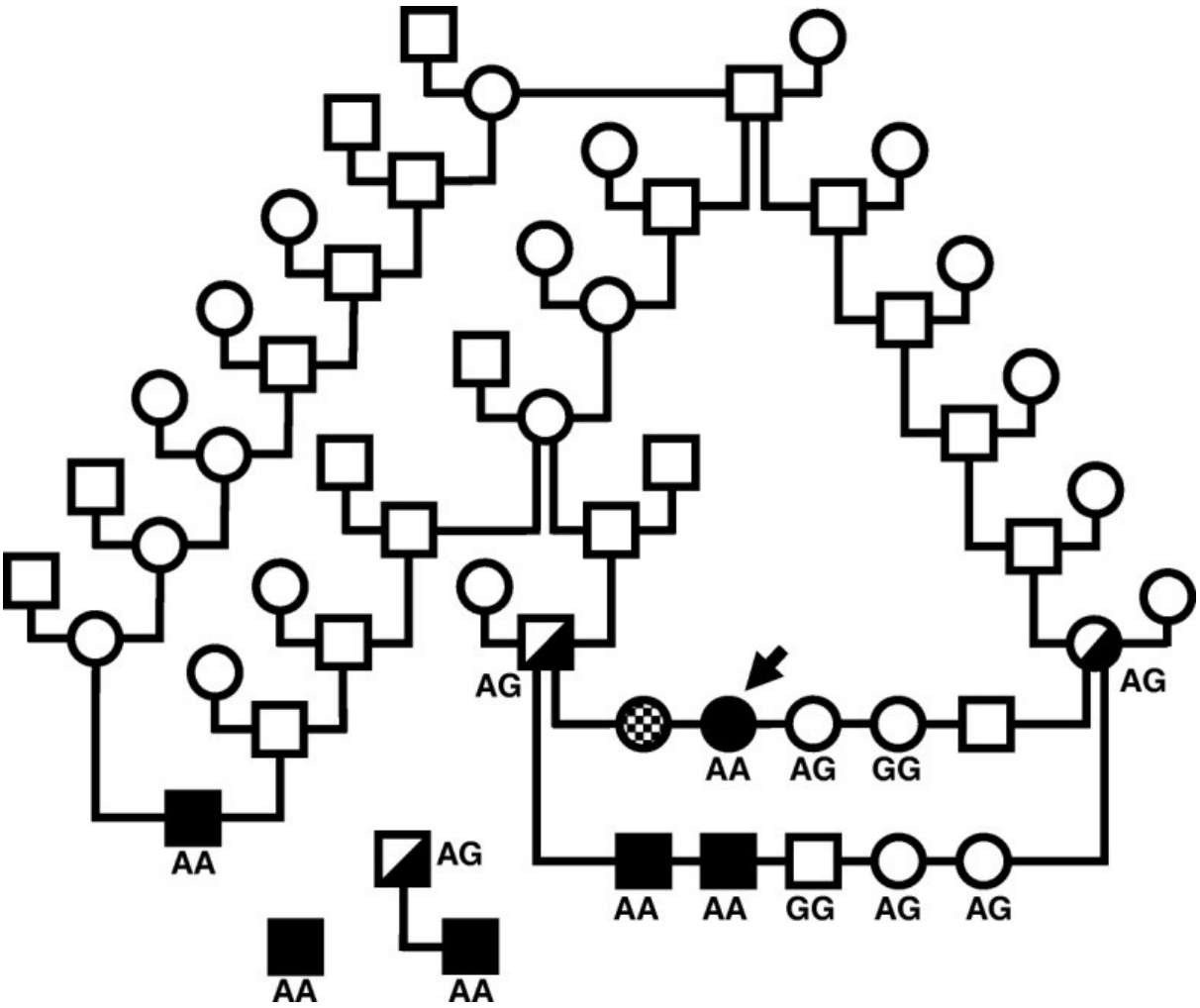


Figure 10

Pedigree of a Komondor dog kindred with hereditary selective intestinal cobalamin malabsorption. Squares and circles are males and females, respectively. Filled symbols indicate affected dogs. Offspring in a single litter are arranged on a horizontal line that connects lines descending from the parents' symbols. The arrow points to the index case (case 5, Additional file 1), and the checkered symbol indicates case 4 that died without biochemical or molecular diagnosis. Half-filled symbols indicate carriers of the disease allele determine by genotyping the *CUBN* c.NM_001003148.1; c.8746 + 1G > A variant. Genotypes of dogs determined are indicated below or immediately adjacent to their symbols. Open symbols indicate dogs which were not genotyped.

(Fyfe, et al., 2018)

Prognosis

When sufficient amounts of B_{12} is supplied the prognosis is relatively excellent. The first cases in humans have been documented for over 50 years with no reported fatalities. (Scheibel, et al., 1981). Proteinuria will persist, but no report of any loss of kidney function. The practioner must stress that the owner must never stop giving the medication even though symptoms seem not to reappear immediately following cessation of treatment. Due to the very early age of onset the prognosis is highly dependent on early diagnosis.

Genetic Counselling

I-GS is an autosomal recessive disease; therefore, it is advised to make an effort to inform relatives/kindred of the patient when possible.

Conclusion

Vitamin B_{12} is an essential micronutrient for mammals, obtained by monogastric species from animal derived foods via a complex receptor mediated mechanism in the GI tract. This complex receptor mediated mechanism is the main focus in this review. Malabsorption of vitamin B_{12} is most commonly seen in the elderly, selective pediatric, nondietary-induced B_{12} deficiency is generally due to inherited disorders including the Imerslund-Gräsbeck syndrome and the much rarer intrinsic factor deficiency. Biochemical, clinical and genetic research on these disorders considerably improved our knowledge of vitamin B_{12} absorption.

The Imerslund-Gräsbeck(I-GS) syndrome is a rare autosomal recessive disorder characterised by cobalamin deficiency due to selective malabsorption of this vitamin. The selectivity of intestinal cobalamin malabsorption coupled with failed renal tubular protein reabsorption is conferred by the mutations in cubulin (CUBN) or amnionless (AMN) the subunits of the heteromeric receptor designated cubam, both CUBN and AMN must both be present with near-normal structure for epithelial brush border expression of the functional receptor complex

The canine Imerslund-Grasbeck syndrome mimics the human disorder with similar clinical manifestations, except the characteristic megaloblastic anaemia. Clinical manifestations present as failure to thrive, inappetence and lethargy. Laboratory findings present as non-regenerative anaemia, proteinuria (CUBN breeds), neutropenia, MMAciduria and serum Cbl levels below detectable limits.

Known canine breeds genetically predisposed to the disease include Giant Schnauzers; Beagles; Border Collies; Australian Shepherds and Hungarian Komondors. A suspect case of a Yorkshire Terrier in the UK presenting with similar clinical signs and the elimination of predisposed liver vascular abnormalities could suggest a possible I-GS case. However, molecular analysis was not performed, it is therefore impossible to determine I-GS in this case. The Chinese Shar Pei is another breed predisposed to cobalmin deficiency, studies were performed to identify the exact genetic cause, the authors present evidence for an association of canine microsatellite markers DTR13.6 and REN13N11, located on chromosome 13, with the deficiency in Chinese Shar Peis.

For diagnosis following normal diagnostic pathways, attention must be paid to the age of onset, serum Cbl levels and using MMAciduria as a specific diagnostic biomarker. The

purpose of this thesis ultimately, is to increase awareness of this rare disorder as the prognosis is highly dependent on early diagnosis. The immediate treatment of parenteral supplementation leads to complete remission of clinical signs followed by life-longoral or parenteral cobalamin supplementation the disorder can be managed easily and relatively inexpensive.

In review of the literature available and the research that has already been carried out in both human and veterinary medicine, I can conclude that it provides a reliable amount of groundwork to base an understanding of the disorder and for performing further investigation in relation to identifying other predisposed breeds. The molecular analysis that is available at this present time is sufficient to screen for I-GS and provide more accurate information on prevalence of the disorder. Ideally to aim to a complete list of predisposed breeds within the canine species.

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