The Miniature Schnauzer and Pancreatitis

Critical review from a genetic perspective

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Alternate Title

Dr. Furrow versus Miniature Schnauzer diseases: Round 2
Outline

- Selection of diseases amenable to genetic studies
- Is canine pancreatitis a good choice?
- Critical review of candidate gene study (SPINK1) & pancreatitis in Miniature Schnauzers
- Redesigning the study
- Conclusions
- Questions, comments, suggestions?
Selection of diseases amenable to genetic studies

(using the example of calcium oxalate urolithiasis)
Question 1: Is there evidence of heritability?

- Familial clustering, breed predispositions
  - Miniature Schnauzers have 10-20 times the risk of mixed breeds for calcium oxalate stones
- Evidence for genetic component in other species
  - Numerous genetic disorders have been identified that increase calcium oxalate stone risk in people
Question 2: Does nature rule over nurture?

- Genetic factors must be the major risk
  - Stone forming Mini Schnauzers have higher urinary calcium levels than healthy controls regardless of environment and diet
  - In humans, the primary determinant of urinary calcium concentration and risk of stone formation is believed to be genetic
Question 3: Is it the primary problem?

- Keeshonden
  - Hyperparathyroidism $\rightarrow$ hypercalemia $\rightarrow$ hypercalciuria $\rightarrow$ calcium oxalate stones
  - Hyperparathyroidism would be the trait of focus for genetic studies, NOT STONES
- Miniature Schnauzers
  - Normal PTH, vitamin D, and serum calcium levels in stone formers
Question 4:
Are cases easy to phenotype?

- Must be able to clearly identify diseased animals
  - Calcium oxalate stones are easy to diagnose and analyze

Look, stones!
Question 5: Are controls easy to phenotype?

- Clearly identifiable disease-free animals
  - CaOx stones are easy to rule out with radiographs
- Need controls that are beyond the age where the disease develops to ensure they won’t become cases later in life
  - CaOx are dx around 7-8 yo, thus need control dogs at least > 8 yo

“Pick me, pick me!”
Is canine pancreatitis a good choice for genetic studies?
Question 1: Is there evidence of heritability?

- Breed predispositions have been noted
  - Miniature Schnauzers, OR = 4.1
  - Cavalier King Charles Spaniel, OR = 3.2
  - Yorkshire Terriers, OR = 4.3 (41.8 for acute fatal panc)
Question 1:
Is there evidence of heritability?

- Genetic component in other species
  - Hereditary pancreatitis (early onset – 10 yo)
  - Idiopathic chronic pancreatitis

Question 2: Does nature rule over nurture?

- Diet is a risk factor for pancreatitis in dogs
  - “Inappropriate” dietary factor such as unusual foods, table scraps, or trash; obesity (Lem et al. 2008)
- Alcohol consumption in people
Question 2: Does nature rule over nurture?

• Diet is a risk factor for pancreatitis in dogs
  • “Inappropriate” dietary factor such as unusual foods, table scraps, or trash; obesity (Lem et al. 2008)
• Alcohol consumption in people
Question 3: Is it the primary problem?

- Hyperlipidemia and gall bladder disease in people
- Hyperlipidemia in Miniature Schnauzers
  - Triglyceride levels and cPLI (Xenoulis et al. 2010)
  - Higher triglyceride levels in dogs with hx of pancreatitis than age-matched controls (Xenoulis et al. 2011)
- Endocrine diseases (Hess et al. 1998; Cook et al. 1993)
  - Diabetes mellitus
  - Hyperadrenocorticism
  - Hypothyroidism
Question 4: Are cases easy to phenotype?

- Clinical signs – non-specific
- Routine hematologic findings – non-specific
- Amylase and lipase – low sensitivity & specificity
- Radiographic findings – 24% sensitivity; no info on specificity
- cTLI – 36% sensitivity
- cPL and cPLI – 64% sensitivity; only info on specificity in completely healthy controls - may increase with IBD and hyperlipidemia
- Ultrasonographic findings – 60-70% sensitivity; no info on specificity?
- Cytology – not published
- Histopathology – gold standard but difficult to obtain antemortem
Question 5: Are controls easy to phenotype?

- Can be subclinical
  - 26% prevalence of chronic pancreatitis and 2% acute pancreatitis on post-mortem in dogs with unknown history (Watson et al. 2007)

- Average age 8-9 yo
  - A dog without current evidence of pancreatitis could still go on to develop pancreatitis later on – it’s never too late!
SPINK1 and pancreatitis in the Miniature Schnauzer

“I’ve had the panc!”
SPINK1 and pancreatitis in Schnauzers

- Bishop et al. 2010
- SPINK1 as candidate gene
  - Encodes for a pancreatic secretory trypsin inhibitor

SPINK1 and pancreatitis in Schnauzers

- Bishop et al. 2010
- SPINK1 as candidate gene
  - Variants are disease modifiers/risk factors in humans
SPINK1 and pancreatitis: **Results**

- Sequenced SPINK1 in 39 cases and 25 controls
- Three variants were identified and found to be associated with pancreatitis
  - Two missense mutations in an exon & a duplication mutation in an intron
- Having at least one copy of the variant alleles \( \rightarrow \) increased risk of pancreatitis
  - OR = 9.5, cases versus all controls (n = 25)
  - OR = 21.7, cases versus controls >5 yo (n = 11)
- **Authors’ conclusion:** “...defects in the SPINK1 gene likely play a role in the development of pancreatitis in Miniature Schnauzers”
SPINK1 and pancreatitis in the Miniature Schnauzer: Critical review

“I don’t buy it.”
SPINK1 and pancreatitis: Cases

- Clinical signs & elevated cPLI or cPL
  - Without knowing specificity of cPLI and cPL, may have included false positives in case group
  - No other clinical information provided on cases
  - No histopathology
SPINK1 and pancreatitis: Controls

- No clinical signs for 3 months, no prior “history of pancreatitis” & normal cPLI or cPL
  - Is 3 months sufficient?
  - How is “no prior history of pancreatitis” determine?
  - Only measured cPLI/cPL at one time point!

“The panc? That’s old news. I haven’t had it for a long time.”
SPINK1 and pancreatitis: Controls

- Any age (mean 5 yo)
  - Inappropriate!
  - Given that the average age for pancreatitis in studies is 8-9 yo, confidence that a 5 yo will not go on to develop disease is very small

“I hope I don’t grow up to get the panc.”
SPINK1 and pancreatitis: Population stratification

- Pedigrees obtained for control dogs to ensure that no closely related dogs were enrolled
- No information on relatedness was provided for case dogs
- Thus, the higher proportion of variant alleles in the case group could be due to increased relatedness between these dogs rather than a true association with pancreatitis
SPINK1 and pancreatitis: Population stratification

- **Definition** – Differences in allele frequencies between groups due to systematic differences in ancestry
SPINK1 and pancreatitis: Summary of design flaws

- How would the problems with case and control selection affect study results?
  - False positive cases and false negative controls → *increased risk of type II error*
- How would population stratification affect study results?
  - Associations between cases due to increased relatedness rather than true disease association → *increased risk of type I error*
SPINK1 and pancreatitis: Conclusion

- **Authors’ conclusion**: “...defects in the SPINK1 gene likely play a role in the development of pancreatitis in Miniature Schnauzers”

- **My conclusion**: It is not possible to interpret the results without knowing whether population stratification was present and given the limitations with phenotyping of cases and controls
Dr. Furrow, against all her instincts, takes on a genetic study of canine pancreatitis.
Clinical relevance

- Role of SPINK1 in pancreatitis is worthwhile to investigate further
- If associations with pancreatitis are real, dogs could be screened for this genetic risk factor early in life
  - Low fat diets could be initiated
  - Improved awareness of disease when clinical signs develop
- Better understanding of disease etiology may lead to innovative therapeutic or preventative measures
Redesigning the study – Miniature Schnauzers, pancreatitis, and SPINK1

- **Cases** – group 1 (high confidence)
- **Cases** – group 2 (moderate confidence)
- **Controls** – group 1 (high confidence)
- **Controls** – group 2 (moderate confidence)
- **Diabetes mellitus “control” group**
Redesigning the study: Miniature Schnauzers, pancreatitis & SPINK1

- Cases – group 1 (high confidence)
  - Compatible clinical signs, no other diagnosis to explain signs AND
  - Major pancreatic AUS findings AND/OR
  - Histopathology AND/OR
  - Elevated cPLI or cPL
Redesigning the study: Miniature Schnauzers, pancreatitis & SPINK1

- Cases – group 2 (moderate confidence)
  - Compatible clinical signs, no other diagnosis to explain signs **AND**
  - Minor pancreatic AUS findings **AND/OR**
  - AXR **AND/OR**
  - Elevated lipase or amylase
Redesigning the study: Miniature Schnauzers, pancreatitis & SPINK1

- Controls – group 1 (high confidence)
  - At least 9 years of age, not diabetic AND
  - No history of vomiting, diarrhea, anorexia for >24 hours, or abdominal pain EVER AND
  - Normal ultrasound of pancreas AND/OR
  - Normal macroscopic appearance of pancreas AND/OR
  - Normal pancreatic histopathology
  - Exceptions
Redesigning the study: Miniature Schnauzers, pancreatitis & SPINK1

- Controls – group 2 (moderate confidence)
  - At least 9 years of age, not diabetic AND
  - No history of vomiting, diarrhea, anorexia >24 hours, or abdominal pain
  - No exceptions

Agility Schnauzers 11 yo and panc free!
Redesigning the study

- **Diabetes mellitus “control” group**
  - Dogs that meet the high or moderate confidence control criteria but are also diabetic
  - We will evaluate this group separately in case the risk for DM in Schnauzers is secondary to chronic pancreatitis
Redesigning the study

- **Cases and controls**
  - Pedigrees will be requested from all participants & analyzed to ensure that no dogs in the case or control groups are related within 2 generations.
Redesigning the study

- **Power calculation**
  - To confirm the difference in proportions of dogs with at least one copy of the variant alleles - 97% for cases versus 64% for controls
  - Alpha = 0.05, power = 0.8, & 1:3 ratio of cases to controls
  - **15 case dogs and 46 controls**
Redesigning the study

- Power calculation
  - 15 case dogs and 46 controls

- Currently have DNA from 120 Miniature Schnauzers with complete medical histories
  - 11 pancreatitis (6 high & 5 moderate confidence)
  - 51 controls ≥9 yo without pancreatitis (14 high & 37 moderate confidence)
  - 7 diabetic “controls” ≥9 yo without a history of gastrointestinal signs or pancreatitis

- Seeking Schnauzers
  - Only “need” 4 more case dogs, but aim to enroll 16 more
  - Don’t “need” any more controls, but aim to enroll at least 5 more
Potential pitfalls

- Histopathology on cases would be ideal
- Need for histopathology on controls is debatable
  - Evaluating for an association of SPINK1 variants with the development of CLINICAL disease
- Stricter age requirements for controls such as ≥ 12 yo
- Dogs with hyperadrenocorticism, diabetes, hypothyroidism, and hyperlipidemia should ideally be analyzed separately

“Fibrosis? Couldn’t care less doc. It’s not going to stop me from eating this ball of delicious yarn.”
Conclusions
Conclusions

- Pancreatitis is a complex disease likely influenced by genetic, metabolic, and environmental factors.
- Recent study found association between SPINK1 variants and pancreatitis in Miniature Schnauzers.
- **HOWEVER**, the problems with the study design preclude interpretation of the results.
- Goal is to redesign the study to determine validity of the association between SPINK1 and pancreatitis.
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Questions, comments, suggestions?