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Sequence analysis of the coding regions of the apolipoprotein C2 (*APOC2*) gene in Miniature Schnauzers with idiopathic hypertriglyceridemia

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Abstract

It has been hypothesized that idiopathic hypertriglyceridemia in Miniature Schnauzers is hereditary, but the gene responsible has yet to be identified. The objective of this study was to determine if there were coding variants in the apolipoprotein C2 (*APOC2*) gene in Miniature Schnauzers with idiopathic hypertriglyceridemia. Blood samples from 12 Miniature Schnauzers with idiopathic hypertriglyceridemia were analyzed. Genomic DNA was extracted from whole blood, and the three coding exons of *APOC2* were amplified by PCR. The PCR amplicons were sequenced and analyzed for variants relative to the canine reference genome (CanFam3.1 assembly). A second objective was to determine the extent of variation in coding exons of *APOC2* in a large and diverse canine population using the Dog Biomedical Variant Database Consortium variant catalogue, comprised of whole genome sequencing variant calls from 582 dogs of 126 breeds and eight wolves. There were no variants detected in the coding exons of *APOC2* for any of the 12 Miniature Schnauzers with idiopathic hypertriglyceridemia. Variants in the coding exons of *APOC2* were also rare in the Dog Biomedical Variant Database Consortium variant catalogue; a single synonymous variant was identified in a heterozygous state in a Tibetan Mastiff. Thus, we concluded that coding variants in *APOC2* are unlikely to be a major cause of idiopathic hypertriglyceridemia in North American Miniature Schnauzers and furthermore, that such coding variants are rare in the canine population.

Keywords

Candidate gene; Dog; Genetics; Hyperlipidemia; Mutation

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Conflict of interest statement

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Idiopathic or primary hyperlipidemia is common in Miniature Schnauzers (MS) and is characterized by abnormal accumulation of very low-density lipoproteins (VLDL) or a combination of VLDL and chylomicrons (Xenoulis et al., 2007). A hereditary cause is hypothesized to exist but has yet to be identified (Whitney, 1993). Lipoprotein lipase (LPL) is the major enzyme involved in triglyceride clearance, and LPL deficiency is the most common cause of familial hyperchylomicronemia in humans (Lewis et al., 2015). Two studies that included both MS and dogs of other breeds with idiopathic hyperlipidemia, reported that LPL activity was significantly reduced in hyperlipidemic dogs compared to healthy control dogs (Furrow et al., 2016).

Variants in apolipoprotein C2 are the second most common cause of familial chylomicronemia in humans, after LPL (Lewis et al., 2015). Apolipoprotein C2 serves as a co-factor for LPL, and it is necessary for the activation of LPL and subsequent clearance of triglycerides (Rifai et al., 1999; Ginsberg, 1998; Bauer, 2004). APOC2 deficiency is considered a recessive disorder, often presenting in childhood, but heterozygous variants have also been associated with hypertriglyceridemia in adult patients (Wang et al., 2007).

The primary aim of our study was to determine if variants were present in the coding exons of *APOC2* in MS with idiopathic hypertriglyceridemia. A secondary aim was to ascertain the extent of *APOC2* variation in a dataset of whole genome sequencing variant calls from a large, diverse population of dogs.

Purebred MS were used in this study. The requirements for inclusion were as follows: (a) the presence of moderate to severe fasting idiopathic hypertriglyceridemia (>4.52 mmol/L; reference interval, 0.29–1.22 mmol/L) with or without hypercholesterolemia (>8.66 mmol/L; reference interval, 3.21–8.66 mmol/L); (b) no clinical signs of any disease for at least 3 months before blood collection; (c) no disease that might affect lipid metabolism (e.g., endocrine disorders); and (d) no current use of drugs that might affect lipid metabolism (e.g., glucocorticoids). Medical history was obtained through a standardized questionnaire. All owners provided written informed consent, and sample collection was reviewed and approved by the Clinical Research Review Committee at Texas A and M University (Approval Number, CRRC No. 06–35; Approval date, 08 November 2008) and the University of Minnesota Institutional Animal Care and Use Committee (Approval Number, 150933019A; Approval date, 30 September 2015).

Dogs were fasted for a minimum of 12 h before blood collection. Six to ten milliliters of blood were collected from each dog and were placed into an additive-free tubes (for serum separation) and in EDTA tubes for complete blood count and genomic DNA extraction. Measurement of triglyceride concentration, serum biochemistry profiles, and thyroid testing (total T4 concentration +/− free T4 concentration and cTSH concentration) were performed as described previously (Texas A and M University, Xenoulis et al., 2007; University of Minnesota, Furrow et al., 2016). These results were evaluated to determine study eligibility. DNA was extracted from whole blood using commercial kits, following the manufacturer's instructions (Texas A and M University, PUREGENE DNA Purification Kit; University of Minnesota, Gentra PUREGENE Blood Kit, Gentra Systems).

Canine *APOC2* is located at chr1:110,504,815–110,506,961 (canFam3.1 build) and consists of three coding exons with a total cDNA sequence length of 306 bp (transcript ENSCAFT00000007426.4). Two sets of primers were designed using Primer3 (Untergasser et al., 2012;[1]). Primer details and PCR conditions are listed in Table 1. Standard PCR reactions were performed using HotStarTaq DNA polymerase (Qiagen, Hilden) and were run on a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories).

Sanger sequencing of the PCR amplicons was performed, and results were compared to the canFam3.1 reference build using commercially available software (Sequencher 5.1, Gene Codes). Exon sequences, as well as non-coding sequences captured, were analyzed for variants. To determine the prevalence of *APOC2* variants in a large and diverse dog population, the Dog Biomedical Variant Database Consortium (DBVDC) variant catalogue was evaluated for all variants in the coding exons of *APOC2*. The DBVDC is comprised of whole genome sequencing data of 582 dogs from 126 breeds and eight wolves (Jagannathan et al., 2019). Fourteen of the dogs in the DBVDC are MS. Health information is not publically available for dogs in the DBVDC.

We performed a sample size calculation to determine the number of dogs required to achieve a greater than 80% probability of detecting a variant present in at least 20% of the population. Using the formula -

$$1 - (0.8)^{n}(\text{basic probability of events})$$

we determined that eight dogs yielded a probability of 83%. Because dogs are diploid, the probability of detecting a variant was greater than this estimate, but depended on the inbreeding coefficient.

Twelve dogs were included in the study and had a median age of 10.3 years (range, 4.6 – 13.8 years). Two dogs were male (both neutered) and 10 were female (nine spayed and one intact). Eight dogs had ideal BCS (4–5/9; Laflamme 1997), three were overweight with a BCS of 6/9, and one dog was obese with a BCS of 9/9. Median fasting triglyceride concentration was 12.20 mmol/L (range, 7.86 – 23.60 mmol/L). Median cholesterol concentration was 8.69 mmol/L (range, 4.29 – 14.87 mmol/L).

No variants were identified in the coding exons of *APOC2* in any of the 12 MS with idiopathic hypertriglyceridemia or in the intronic sequence captured (83–131 bps 3' of intron 1, all 165 bp of intron 2, 16–82 bp 5' of intron 3, 53–90 bp 3' of intron 3, and all of the 3' UTR). A synonymous variant in the first coding exon of *APOC2* (chr1:110,505,825A>G, p.Leu11Leu) was identified in the DBVDC variant catalogue. This variant was present in a heterozygous state in one dog (Tibetan Mastiff). No other variants were present in coding exons.

The findings of our study suggest that *APOC2* deficiency is unlikely to be the major cause of idiopathic hypertriglyceridemia in MS, and coding variants in this gene are generally rare

[1]See: Primer 3 version 0.4.0; http://bioinfo.ut.ee/primer3-0.4.0/ (Accessed 8 September, 2020).

in dogs. We did not evaluate for regulatory or structural variants that could alter the expression of these genes without changing the coding sequence. Additionally, the presence of comorbidities that cause secondary hypertriglyceridemia cannot be fully excluded in these dogs.

It should be noted that four dogs were slightly overweight (n=3) or obese (n=1). As in humans (Hegele et al 2014; Jagannathan et al., 2019; Lewis et al., 2015;), the genetic cause of idiopathic hypertriglyceridemia in MS might be complex, meaning that multiple genes may contribute to the phenotype. Other genes that modulate LPL function, such as *APOA5*, *LMF1*, and *GPIHBP1*, could be involved in the pathogenesis of the disease (Lewis et al., 2015). Studies using a candidate gene sequencing approach, genome-wide association studies (Johansen et al., 2010; Teslovich et al., 2010; Willer et al., 2013), whole genome sequencing and/or RNA-Seq analysis (Seo et al., 2016; Tada et al., 2019) might prove effective in detecting genetic variants associated with the development of hypertriglyceridemia in MS.

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Highlights

- Causes of idiopathic hypertriglyceridemia in Miniature Schnauzer (MS) are unclear.
- No variant was found in the coding exons of *APOC2* in MS with hypertriglyceridemia.
- Coding variants in *APOC2* are unlikely to be a cause of hypertriglyceridemia in MS.
- Variants in coding exons of *APOC2* were rare in a canine variant database.
- Whole genome sequencing and/or RNA-Seq analysis might be more appropriate methods.

Table 1

Primers and PCR conditions for amplification of coding exons of canine *APOC2*.

Exon(s)	Primers	Product Size	PCR conditions
2 and 3	Forward: 5'CAACTGGATCAGCAGGGAAG3' Reverse: 5' CCCAGTGCAGGACTCAATC3'	645 bp	Initial denaturation: 94 °C × 15 min 35 cycles: 94 °C × 30 sec, 60 °C × 30 sec, 72 °C × 30 sec Elongation: 72 °C × 10 min
4	Forward: 5' TCCCCACAGGACACATGAC3' Reverse: 5' AACCTCGGTGAAGGACAATC3'	513 bp	Slowdown PCR method, 49 cycles with varying temperatures (Frey et al., 2008)