

STUDY SUMMARY

PLOS ONE: Identification of modifiable pre- and postnatal dietary and environmental exposures associated with owner-reported canine atopic dermatitis in Finland using a web-based questionnaire

The diet eaten by a puppy or its mother may determine their risk for developing atopic dermatitis

The goal of this study was to identify causes of allergy that can be controlled by owners to help reduce the incidence of atopic dermatitis (or environmental allergies).

Method of study: The DogRisk questionnaire was used to gather data. A total of 2236 dogs were divided into allergy prone vs non-allergy prone groups. The dogs had to be eating one of two diets – either a raw meat-based diet or a dry kibble.

Results:

- If a puppy's mother ate raw, or the puppy's first solid food (from 1-2 months of age) was raw, there was a **decreased** chance that it would have allergies as an adult.
- If a puppy's mother ate kibble, or the puppy's first solid food (from 1-2 months of age) was kibble, there was an **increased** chance that it would have allergies as an adult
- The diet consumed by puppies from 2-6 months of age tended to be associated with the risk of developing atopy in adulthood similar to above, but the results failed to reach statistical significance.

Discussion: These findings may represent the "Old friends hypothesis" at work. Raw food contains microbes that educate the immune system in such a way as to prevent hypersensitivity reactions later in life. Another theory involves the biochemical differences in raw food vs processed kibble – perhaps processing changes food in some way that increases the chances of becoming allergic. Finally, the nutrient profiles of the raw diet (high protein, broader range of functional compound and no AGEs) may protect the immune system from developing allergy later in life.

This is an observational study which has inherent weaknesses, although for this type of study it is well controlled. One weakness is that owners were asked to remember aspects of care and feeding years previously and may not have reported them accurately. Second, some of the diagnoses and descriptions of medical conditions were based on the owners' judgements and were not verified by professional veterinary diagnosis.

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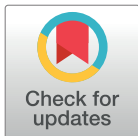
RESEARCH ARTICLE

Identification of modifiable pre- and postnatal dietary and environmental exposures associated with owner-reported canine atopic dermatitis in Finland using a web-based questionnaire

Manal Hemida^{1,2}, Kristiina A. Vuori¹, Siru Salin¹, Robin Moore¹, Johanna Anturaniemi¹, Anna Hielm-Björkman^{1*}

1 Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland, **2** Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt

* anna.hielm-bjorkman@helsinki.fi



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Abstract

A cross-sectional hypothesis generating study was performed to investigate modifiable exposures such as whether feeding pattern (a non-processed meat based diet, NPMD, or an ultra-processed carbohydrate based diet, UPCD), certain environmental factors and their timing of exposure might be associated with the development of canine atopic dermatitis (CAD). Also, genetic and demographic factors were tested for associations with CAD. The data was collected from the validated internet-based DogRisk food frequency questionnaire in Finland. A total of 2236 dogs were eligible for the study (the owners reported 406 cases and 1830 controls). Our main interest was to analyze modifiable early risk factors of CAD, focusing on nutritional and environmental factors. We tested four early life periods; prenatal, neonatal, early postnatal and late postnatal periods. Twenty-two variables were tested for associations with CAD using logistic regression analysis. From the final models we identified novel dietary associations with CAD: the NPMD during the prenatal and early postnatal periods had a significant negative association with the incidence of CAD in adult dogs (age above 1 year). Oppositely, UPCD was associated with a significantly higher risk for CAD incidence. Other variables that were associated with a significantly lower risk for CAD were maternal deworming during pregnancy, sunlight exposure during early postnatal period, normal body condition score during the early postnatal period, the puppy being born within the same family that it would stay in, and spending time on a dirt or grass surface from 2 to 6 months. Also, the genetic factors regarding maternal history of CAD, allergy-prone breeds and more than 50% white-colored coat all showed a significant positive association with CAD incidence in agreement with previous findings. Although no causality can be established, feeding NPMD early in life seemed to be protective against CAD, while UPCD could be considered a risk factor. Prospective intervention studies are needed to establish the causal effects of the protective role of NPMD on prevalence of CAD during the fetal and early postnatal life.

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Abbreviations: AD, atopic dermatitis (in humans); CAD, Canine atopic dermatitis; NPMD, non-processed meat based diet; UPCD, ultra-processed carbohydrate based diet; DOHaD, developmental origins of health and disease; FFQ, Food Frequency Questionnaire.

Introduction

Canine atopic dermatitis (CAD) is considered an incurable inflammatory and pruritic allergic skin disease in dogs, mostly diagnosed based on clinical skin symptoms and to see if it is food-induced, the clinician uses the results from an elimination diet [1, 2]. The disease prevalence is up to 10% in the dog population [3] with usual eruption within the first three years of age [1, 4]. Atopic dermatitis (AD) in humans and CAD in dogs are complex multifactorial diseases resulting from an interaction between genetics, epigenetics, immune system and environmental exposures including diet [5–8].

The genetic component and heritability of CAD has been confirmed by several genomic studies which have suggested several genes to be important in the pathogenesis [9–11]. Other studies support the heritability of CAD, for example, 50% of dogs with a paternal history of atopy develop CAD themselves [12], and the maternal history of CAD greatly increase the risk of CAD incidence in offspring [13]. A strong breed predilection to develop CAD has also been confirmed in several studies [13–16]. The most frequently affected breeds are West Highland White Terrier, Boxer, English bulldog, Dalmatian, Golden Retriever, French Bulldog, Bull Terrier, German Shepherd Dog, and English springer spaniel [7, 13, 14]. Gender plays a part in human AD predisposition, [17] and this is the same with CAD in dogs [18]. Some studies have also suggested an association between the expression of genes responsible for coat color and CAD in dogs [19–22]. Studying the genetic and background factors along with the modifiable early life factors will help us understand the etiopathogenesis of CAD and to provide ways of preventing the disease.

There is growing evidence from human epidemiological studies that early exposures during pregnancy and postnatal life are crucial for the programming of the immune system, and therefore predisposition to allergy later in life [23–29]. Early life is a critical time window when one can influence allergy risk and/or prevention. Potential risk factors for human AD and/or allergy that might have an impact on the early immune development include early life diet [30–32] and environmental exposures [33, 34], which are regarded as modifiable, whenever they affect the risk of the disease. In human studies, early nutrition and lifestyle factors have been shown to be key factors in programming for health during the critical periods, from antenatal life to early postnatal life, resulting in long-term changes related to later health conditions [29, 35, 36]. The mechanisms by which this health related programming occur is unclear, some proposed mechanisms include genetics [37], epigenetics [38] and intergenerational effects [39].

As in humans, recent studies have shown that maternal diet during lactation has an effect on the development of allergies in canines [40, 41]. The impact of the diet on human and canine health is not only tied to their macro- or micronutrient content but also to the processing; whether the food items are consumed as raw / non-processed or as ultra-processed diets, or mixes of them, as described in the NOVA guidelines [42–45]. Wild canids naturally consume diets high in raw animal protein and fat and low in carbohydrates [46–49]. In contrast, pets are usually served ultra-processed foods with a high carbohydrate content. The so-called “Developmental Origins of Health and Disease” (DOHaD) hypothesis is the current term of the ‘Fetal Origins of Adult Disease’ concept that was established for humans in the 1990s [26], and it presumes that exposure to certain environmental stimuli during critical developmental periods may have significant impacts on an individual’s future health [27]. The evidence come from animal models and human studies [29]. As far as we know, the DOHaD hypothesis has not been tested on dogs a priori, before.

Two hypotheses were tested in our study, the developmental DOHaD hypothesis, which emphasizes the role of early life nutrition on allergy susceptibility in adult life [50], and the

hygiene hypothesis which speculates that early exposure to dietary and environmental microbiota stimulates the early immune system development [51]. The influence of the maternal diet during pregnancy on prevalence of CAD has so far been poorly investigated. Moreover, the possible effects of a non-processed meat based diet (NPMD) or an ultra-processed carbohydrate based diet (UPCD) on CAD prevalence has not been tested previously. The aim of this study was to investigate whether a NPMD or an UPCD feeding pattern as well as environmental factors and their timing of exposure (in the prenatal, neonatal and postnatal life) may be associated with the development of CAD in adult dogs. We additionally aimed to test the already known genetic and demographic risk factors for CAD, in part to support them and in part to thereby validate our new results.

Materials and methods

Study design and data collection

This is a cross-sectional epidemiological study with longitudinal data. The data was a subset from the data of the large validated internet-based DogRisk food frequency questionnaire (FFQ) [52]. The DogRisk FFQ as described in a previous study [13] was launched in 2009 at the Faculty of Veterinary Medicine, University of Helsinki, Finland. All in all, it generates 1332 data points per dog. The questions are answered by the owners and include different aspects of nutritional and environmental exposures during the whole life of the dog, starting from prenatal life and depending on the dog's age at time of answering, often until adult age. Besides that, there are some questions on the essential demographic information for the dogs and their owners. Moreover, the FFQ contains two question sets about the diseases of the dogs in question and those of their mothers; each of them covers 117 different canine diseases. All questions and answers given have been written using more layman terms than professional veterinary or epidemiological terms, as the FFQ was answered by laymen, e.g. "very chubby" instead of "obese". The DogRisk FFQ is still open online <http://www.ruokintakysely.fi/> and is so far available only in Finnish. The ethical approval for the questionnaire (29.4.2016) was applied from the University of Helsinki, Viikki campus ethical board.

This binary outcome (dependent) variable was tested for any potential associations with 22 different categorical and ordinal variables (listed in Table 1) at different time points in the dog's early life. The early life periods studied here (Fig 1) are as follows: 1) The prenatal period is the period of fetal life or intrauterine life during pregnancy. 2) The neonatal period is the period directly after birth and extends until the first 3 to 4 weeks of the puppy's life. 3) The early postnatal period for the young puppy is the period from one to two months of age. 4) The late postnatal period for older puppies, is the period from 2–6 months of age.

Study population

The total population consisted of 12,011 dogs (Fig 2). After excluding the duplicates and the robot answers (12.92%) our population consisted of 10,460 individuals. All breeds (allergy-prone and non-allergy prone) and both sexes (males and females) were eligible for this study. Then, according to study inclusion criteria, we handled the data as follows: We excluded all puppies under one year of age in order to avoid reverse causality [53], and further, excluded all dogs under 3 years of age from the control group to avoid cases that had not yet erupted, as the "age of disease onset" is considered to be 0–3 years of age [4]. Furthermore, the participants who did not respond to the questions about having CAD or not, and those who had not given any data about the early life diets, were excluded from this study (Fig 2). Finally, to avoid wrongly diagnosed dogs (as the diagnosis of CAD was based only on owner reported diagnosis) we only included CAD dogs that whose owners had also responded that their dogs had

Table 1. Variable characteristics and their distribution within study cases, controls and the total study population.

N.	Covariates	Categories	Canine atopic dermatitis %(n)		
			Cases, % (n = 406)	Controls, % (n = 1830)	Total, % (n = 2236)
Prenatal period					
1	Maternal history of CAD	Non-atopic mothers	80.1 (113)	97.8 (881)	95.4 (994)
		Atopic mothers	19.9 (28)	2.2 (20)	4.6 (48)
2	Dog breed	Non-allergy prone breeds	40.2 (137)	65.4 (952)	60.6 (1089)
		Allergy prone breeds	59.8 (204)	34.6 (503)	39.4 (707)
3	Dog gender	Male	49.4 (195)	42.9 (765)	44.1(960)
		Female	50.6 (200)	57.1 (1017)	55.9 (1217)
4	Dog color	White >90%	12.9 (49)	7.9 (136)	8.8 (185)
		White >50%	14.5 (55)	10.7 (184)	11.4 (239)
		Less white	21.1 (80)	26.8 (461)	25.8 (541)
		Little or no white	51.5 (195)	54.5 (936)	54.0 (1131)
5	Mother's diet during pregnancy	NPMB	3.9 (10)	8.3 (94)	7.5 (104)
		UPCD	96.1 (244)	91.7 (1041)	92.5 (1285)
6	Was the mother dewormed during pregnancy?	Yes	94.8 (221)	97.7 (1135)	97.2 (1356)
		No	5.2 (12)	2.3 (27)	2.8 (39)
7	Was mother vaccinated during pregnancy?	Yes	50.4 (63)	48.5 (329)	48.8 (392)
		No	49.6 (62)	51.5 (349)	51.2 (411)
Neonatal period					
8	Mother's diet during lactation	NPMB	5.4 (13)	7.9 (86)	7.4 (99)
		UPCD	94.6 (227)	92.1 (1004)	92.6 (1231)
9	Season of birth	Winter (Dec-Feb)	26.5 (106)	25.5 (460)	25.7 (566)
		Spring (March-May)	33.3 (133)	32.0 (576)	32.2 (709)
		Summer (June-Aug)	21.3 (85)	23.4 (422)	23.0 (507)
		Autumn (Sept-Nov)	19.0 (76)	19.1 (344)	19.1 (420)
Early postnatal period					
10	Puppy's first solid diet	NPMD	3.3 (8)	8.8 (99)	7.9 (107)
		UPCD	96.7 (233)	91.2 (1020)	92.1 (1253)
11	Frequency of outdoor activity	Many times / day	51.1 (157)	60.0 (843)	58.4 (1000)
		Once / day	16.6 (51)	15.3 (215)	15.5 (266)
		A few times / week	12.1 (37)	12.2 (172)	12.2 (209)
		A few times / month	9.1 (28)	4.2 (59)	5.1 (87)
		Not at all	11.1 (34)	8.3 (117)	8.8 (151)
12	Sunlight exposure, hours / day	Not at all	20.9 (44)	14.2 (143)	15.4 (187)
		≥ 1 hour	79.1 (167)	85.8 (861)	84.6 (1028)
13	Type of flooring	Dirt / lawn floor	5.3 (17)	7.0 (102)	6.7 (119)
		Non-dirt / lawn floor	94.7 (301)	93.0 (1355)	93.3 (1656)
14	Body condition score	Overweight puppies	17.1 (60)	13.8 (213)	14.5 (273)
		Normal weight puppies	72.0 (252)	77.2 (1188)	76.3 (1440)
		Underweight puppies	10.9 (38)	8.9 (137)	9.3 (175)
Late postnatal period					
15	Puppy diet	NPMD	16.7 (41)	23.7 (247)	22.4 (288)
		UPCD	83.3 (205)	76.3 (794)	77.6 (999)
16	Was the dog born into the same human family as where it stayed as adult?	No	97.3 (395)	90.4 (1654)	91.6 (2049)
		Yes	2.7 (11)	9.6 (176)	8.4 (187)

(Continued)

Table 1. (Continued)

N.	Covariates	Categories	Canine atopic dermatitis %(n)		
			Cases, % (n = 406)	Controls, % (n = 1830)	Total, % (n = 2236)
17	Outdoor activity, hours / day	< 0.5	2.4 (8)	2.3 (35)	2.3 (43)
		0.5–1.0	32.3 (108)	26.1 (397)	27.3 (505)
		1.0–2.0	50.6 (169)	51 (775)	50.9 (944)
		> 2.0	14.7 (49)	20.5 (312)	19.5 (361)
18	Sunlight exposure, hours / day	≤ 1	30.8 (95)	25.5 (348)	26.5 (443)
		> 1	69.2 (213)	74.5 (1018)	73.5 (1231)
19	Type of flooring	Dirt / lawn	7.1 (29)	11.8 (216)	11.0 (245)
		Non-dirt / lawn	92.9 (377)	88.2 (1614)	89.0 (1991)
20	Body condition score	Overweight puppies	6.3 (22)	6.0 (95)	6.1 (117)
		Normal weight puppies	67.6 (236)	68.5 (1083)	68.3 (1319)
		Underweight puppies	26.1 (91)	25.5 (403)	25.6 (494)
21	Was the puppy vaccinated 2–4 times under 1 year of age?	Yes	99.3 (400)	99.1 (1786)	99.1 (2186)
		No	0.7 (3)	0.9 (16)	0.9 (19)
22	Was the puppy dewormed 2–10 times under 1 year of age?	Yes	98.7 (387)	99.3 (1760)	99.2 (2147)
		No	1.3 (5)	0.7 (12)	0.8 (17)

(n): number of dogs, CAD: canine atopic dermatitis, NPMD: non-processed meat based diet, UPCD: ultra-processed carbohydrate based diet.

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skin symptoms (n = 406) while the allowed controls had to report that they neither had CAD nor skin symptoms (n = 1830) giving us a final adult dog population for analysis of n = 2236. This study was carried out to test the association between CAD and different nutritional, environmental, genetic and demographic variables in the pre- and postnatal periods (Fig 1).

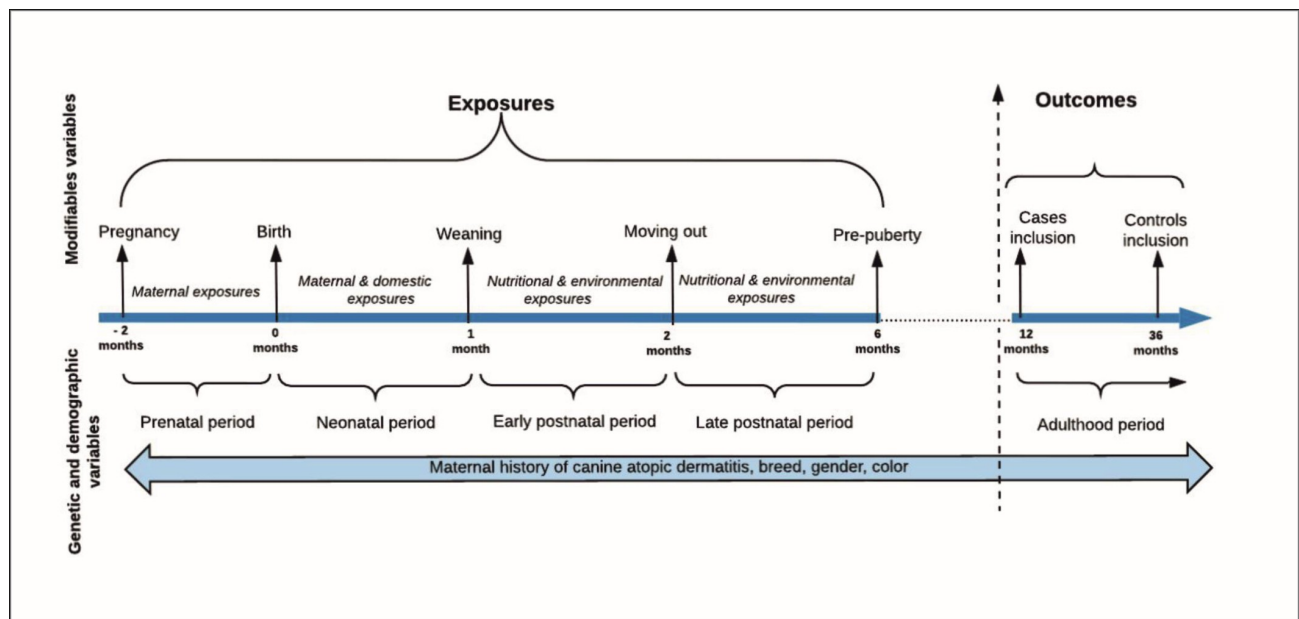


Fig 1. Timeline of the study design.

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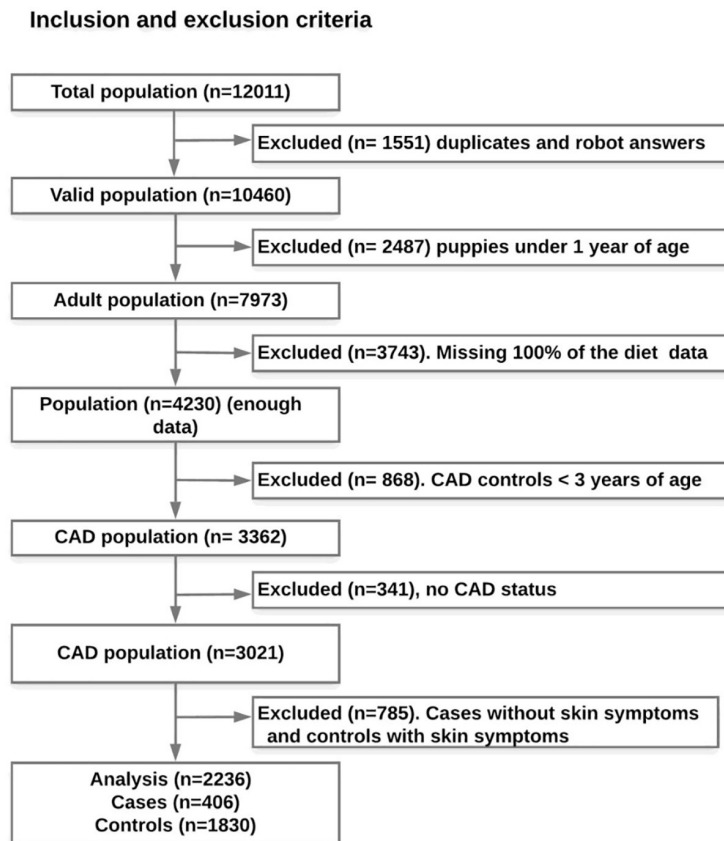


Fig 2. Flow chart of the study population. The study population was extracted from the DogRisk food frequency questionnaire population in the period between 2009 and 2018.

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Additionally, to pinpoint both potential risk and protective effect of a variable on CAD incidence we tested the association between each category in all variables with CAD.

Selection of variables

Our main interest was on the modifiable dietary and environmental exposures within pre and postnatal life. The nutritional variables during the prenatal, neonatal and early postnatal life as well as the maternal deworming and vaccination in the prenatal life were testing the DOHaD hypothesis. The hygiene hypothesis was tested by using the nutritional variables in the early and postnatal life and the environmental exposures in the postnatal life. Also, the already known genetic and demographic factors were tested, including dog breed, gender, color and maternal history of CAD.

Newly created independent variables. A dichotomous variable for dog breeds was created including allergy-prone breeds and non- allergy-prone breeds. These breed lists were modified from a previous article [14] with data from CAD research [15, 16, 54–58] and are included as a supplementary S1 Table. Two questions (Q26-27) were about the maternal diet during the pregnancy and the lactation period and a third about the young puppy's first solid diet (Q32), before two months of age. For this study we only used an answer to the question "Do you remember what (type of food had been used)?" where the owner had typed in the type of food used, themselves. From the owners' answers, we selected only dogs that either had

answered that they were using one of two extreme diets; either they used a raw or non-processed meat based diet (NPMD) or a dry or ultra-processed carbohydrate based diet (UPCD). NPMD and UPCD in our study also represent the two extreme groups of the most recently applicable NOVA classification system of human foods, the non-processed foods versus the ultra-processed foods [42, 43, 59]. However, the NPMD tested diets in our study are not totally identical to group 1 of the NOVA classification [59], as they might have been deep-frozen and might have contained supplements like salt, artificial vitamins, mineral premixes etc. The similarity is that they are not heat-treated (not warmed over 45° C).

The answers of the body condition score (Q31 and 36) at the age of 2 months and 6 months respectively, were as follows: very chubby, chubby, normal, slim and very slim. The first two categories were merged into one category of overweight dogs. The last two categories (slim and very slim dogs) were merged into a category of underweight dogs. As such, the variable contained three categories of overweight, normal, and underweight puppies. Later the underweight and overweight were combined, resulting in a dichotomous variable of normal or not normal.

The answer to how many hours the dog spent in sunlight (Q29 and 34) at the age of 2 months and 6 months, respectively, was in form of a continuous numeric variable. Based on preliminary cut point analyses the answer at two months was changed into the dichotomous variable of zero hours versus one hour or more (up to 24 h) of sunlight exposure per day. In the case of the older puppies (6 months of age), it was changed into the dichotomous variable of zero or one-hour sunlight exposure versus more than one-hour sunlight exposure per day.

In questions 30 and 35 we were asking for the kind of “floor” the puppy was living on at 2 and 6 months of age, respectively. The multiple choices were; mainly a slippery floor, mainly a non-slippery floor, outside slippery ice, dirt/lawn, newspaper, soft carpet / rugs, or I do not know. Due to our previous research on urban and rural environments [14] we divided these into a dichotomous variable of dirt/lawn floor and non-dirt/lawn floor.

All other questions and answers were used as such.

Statistical analysis

The data was analyzed using IBM SPSS statistics for windows, version 25.0. Armonk, NY: IBM Corp. Package ‘forest plot’ [60] in R software version 3.5.1 [61] was used for visualizing odds ratios. The distribution of the variables within the cases and the controls was calculated using the crosstabs descriptive analyses. The prevalence of CAD cases was calculated using the participants who answered the question regarding CAD, including all breeds, all ages, and both sexes.

The variables were initially screened individually for a potential association with having CAD or not, using univariate logistic regression analysis. The variables with a $p \leq 0.2$ were then included in the multiple logistic regression analysis. The multiple regression analyses were run in five models; one for the non-modifiable genetic and demographic variables and four models for the tested four early life periods modifiable variables, using the method enter. The first model included dog gender as a tested variable and the last four models were adjusted for the dog gender. Further, variable interactions were run and the interacted variables were assessed for associations with CAD using univariate logistic regression analysis. In the logistic regression analysis, univariate and multivariate, the model was run twice for the dichotomous variables or more than two times for the variables including more than two categories, using a different category as a reference each time.

The correlation between the variables in each model was tested using bivariate Pearson correlation to avoid multicollinearity. The correlations within the variables in each model were

weak (≤ 0.1), except the correlation between the frequency of outdoor activity and hours of sunlight exposure in the early postnatal period that was ≥ 0.5 . As such, the variable of outdoor activity was excluded from model 4. The missing values in our data have not been imputed and are handled by the listwise deletion in the program. Significance was considered when the p -value ≤ 0.05 . The fitness of the logistic regression analysis was checked using the Omnibus test (a p -value < 0.05 being good), the Hosmer and Lemeshow test (> 0.05 being good) and the Nagelkerke's R (where a larger value is better) [62, 63].

Results

Variable characteristics

The prevalence of atopic cases within the participants in the food frequency questionnaire by answering the question regarding CAD was 18.8%. The mean age \pm SD of the total study population was 5.40 ± 2.84 , while for the cases and controls the mean ages were 4.88 ± 2.71 and 5.70 ± 2.75 , respectively. Variable characteristics and their distribution within the canine atopic dermatitis (CAD) cases and the non-atopic control dogs are shown in [Table 1](#).

Associations between the early life exposures and the incidence of CAD

From the univariate logistic regression analysis, we found that there were 12 variables from a total of 22 variables in the different periods of life, that were significantly associated ($p \leq 0.05$) with CAD in dogs over one year of age. In [Fig 3](#) and in the supplemental [S2 Table](#) we present odds ratios with confidence intervals OR (CI) for each category in all the variables. Blue and red lines represent decreased and increased risk when OR is below or above one, respectively ([Fig 3](#)). Contributing to open access, this also makes it easier for laymen potentially interested, to understand our results.

Univariate logistic regression analysis was done to assess the associations between the interacted variables and CAD incidence. No new significant associations were found between the interacted variables and CAD in comparison to the single variables and CAD.

The final models were created using multivariable logistic regression analysis. There were three significant variables in model 1 for the genetic and demographic non-modifiable variables, two significant variables in model 2 for the prenatal modifiable variables and three significant variables in model 4 for the early postnatal modifiable variables. In model 5 for the late postnatal modifiable variables there were two significant variables. Model 3 gave no significant factors. OR (CI) are given for each category in the dichotomous variables used in the five models as shown in [Fig 4](#) and in the supplemental [S3 Table](#). Blue and red colors in [Fig 4](#) represent the decreased and increased risk when OR is below or above one, respectively.

Discussion

In the current hypothesis generating study some modifiable early risk factors, nutritional and environmental, for CAD have been identified. Also, to verify the validity of our data, some genetic and background-related variables were also analyzed. We will discuss our findings against previous research and the data we have at hand.

Genetic and background-related non-modifiable variables

The dogs with a maternal history of CAD were at high risk to develop CAD when becoming adult while those without a maternal history of CAD had a low chance of developing CAD during adulthood. However, as the offspring live in the same physical environment during their first 2 months and consume the mother's milk, they are also usually first fed the same

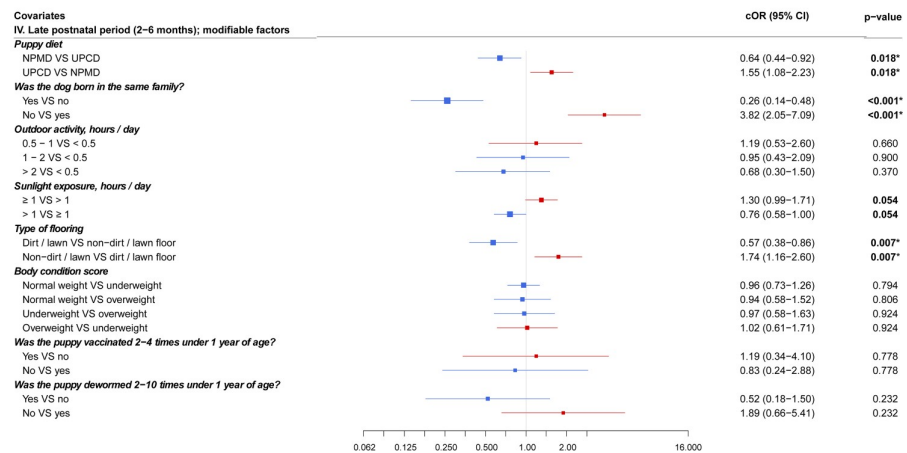
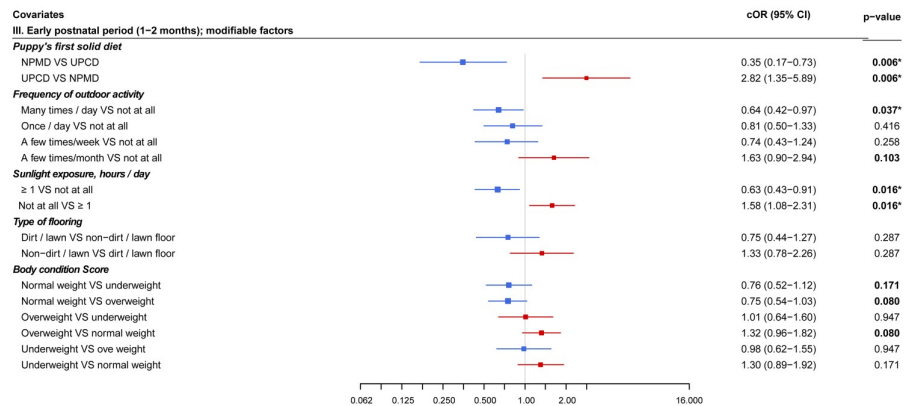
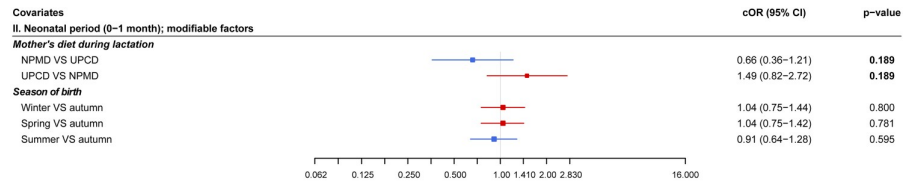
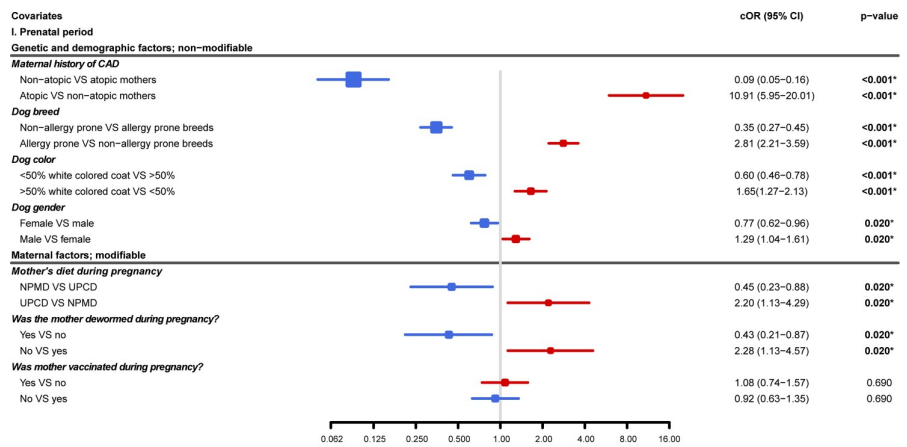


Fig 3. Forest plot of odds ratios for associations between pre- (A), neo- (B), early post- (C) and late postnatal (D) period variables and canine atopic dermatitis based on univariate logistic regression analyses (n = 2236). cOR: crude odds ratio, CI: confidence interval, CAD: canine atopic dermatitis, **bolded**: $P \leq 0.2$, *: $P \leq 0.05$, NPMD: non-processed meat based diet, UPCD: ultra-processed carbohydrate based diet, VS: versus.

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type of food (NPMD or UPCD) that the mother eats. The challenge hence remains to determine whether a multitude of genes are indeed the causative factor or whether it is the environment or the diet that matters more, possibly through altering relevant gene expression. In a previous study investigating the genetic predisposition of CAD in Labrador and Golden retrievers, they found that breeding two atopic parents resulted in 65% atopic offspring, breeding one atopic parent resulted in 21–57% atopic offspring and breeding two non- atopic parents resulted in 11% atopic offspring [12]. Unfortunately, there was no information provided regarding the environment or the diet of neither dams nor offspring. A recent human study showed that a family history of allergic disease is a risk factor for AD [64]. Also, Eichenfield et al. [65] reported that 70% of people with AD have a family history of atopy. Moreover, in our study the allergy prone breeds were associated with a high risk for CAD while the non-allergy prone breeds were protected from CAD. These findings are in accordance with Hakanen et al. [14], Anturaniemi et al. [13], Gedon and Mueller [66], and Jaeger et al. [55]. The

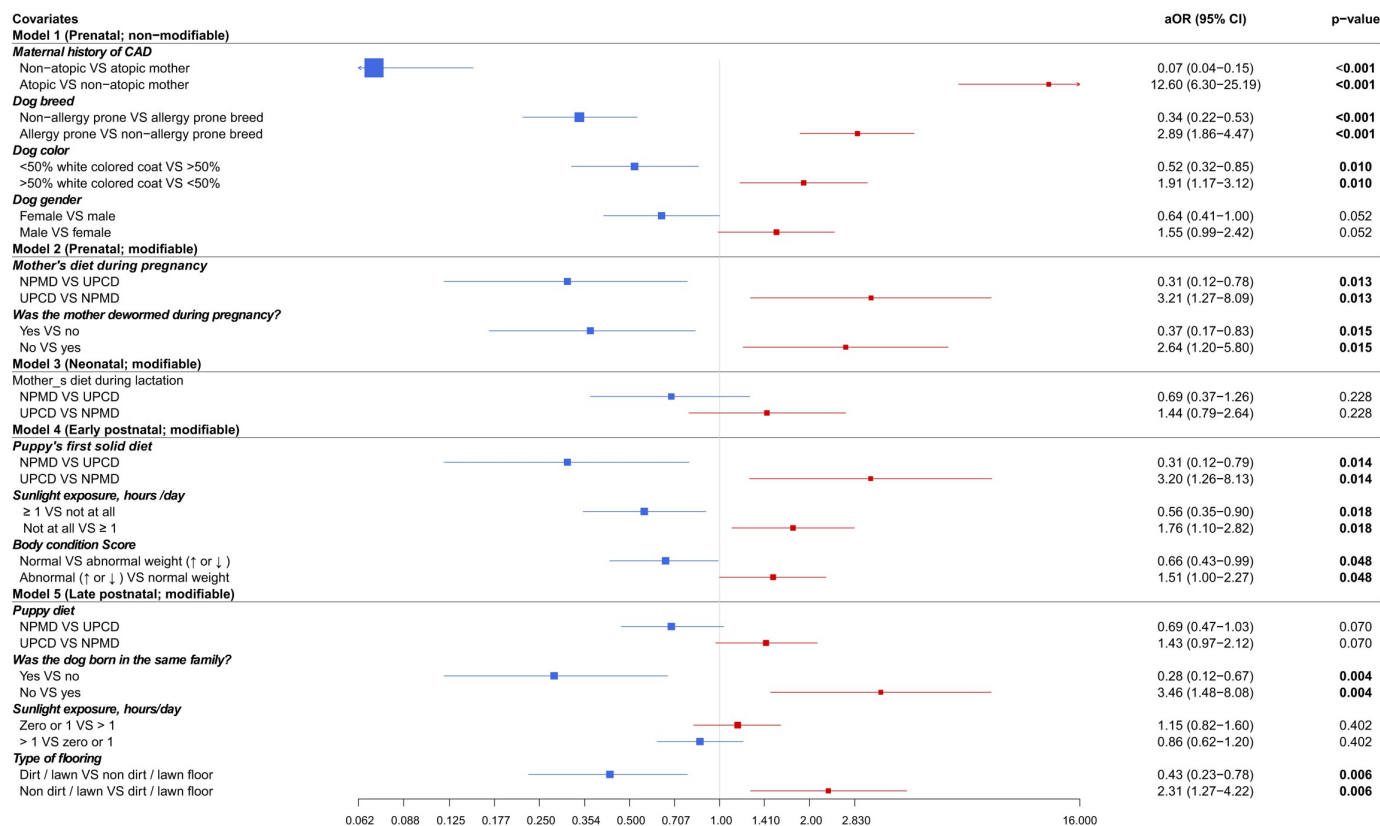


Fig 4. Forest plot of odds ratios for associations between pre-, neo-, early post- and late postnatal period variables and canine atopic dermatitis based on multivariable logistic regression analyses (n = 2236). Included/missing dogs for each model: model 1 (789/1447), model 2 (1357/879), model 3 (1297/939), model 4 (782/1454) and model 5 (1071/1165). Models 2–4 have been adjusted for dog gender. aOR: adjusted odds ratio, CI: confidence interval, CAD: canine atopic dermatitis, **bolded**: $P \leq 0.05$, NPMD: non-processed meat based diet, UPCD: ultra-processed carbohydrate based diet, VS: versus.

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dogs with a less than 50% white-colored coat were less susceptible to develop CAD while having a more than 50% white-colored coat put the dogs at high risk to develop CAD. This finding was in line with Anturaniemi et al. [13] and Nodtvedt et al. [41]. Almqvist et al. [17] found that gender is a contributing factor as boys are more susceptible to atopic sensitization than girls, although the mechanism is not clear. In our study we saw no difference in the final model which was in agreement with Anturaniemi [13].

Prenatal modifiable variables

In the present study, having been subjected to NPMD during pregnancy resulted in a lower risk of CAD, while subjection to UPCD was associated with a higher risk of CAD in the offspring at adulthood. The potentially protective effects and risks of NPMD and UPCD can be attributed to many reasons. The dietary effect could be related to the time periods when they were served, their potentially healthy microbial load, the processing methods, and/or the macro- or micronutrient content.

To the extent of our knowledge, the study at hand is the first study to test the DOHaD hypothesis [27] on dogs. This “Developmental Origins of Health and Disease” concept, which is used extensively in observational human research [23, 33, 39, 67–74] analyzes the role of intrauterine and early postnatal nutrition on future health outcomes. Our results were consistent with the DOHaD hypothesis. The maternal diet during pregnancy is the only source of nutrition for the fetus as the fetus receives nutrients from its mother via the placenta. Any disturbance or imbalance of the maternal diet during gestation reflects on the newborn. This may result in an impaired immune system and high susceptibility to chronic diseases in the future [26].

As far as we know, the canine maternal gestation diet has not been analyzed before in conjunction with CAD. In a human study, Niinivirta-Joutsa [75] found interesting associations between maternal diet and the risk of allergy in children. He found a lower risk of atopic eczema in children whose mothers consumed milk and hen’s egg during pregnancy while consumption of cheese increased the risk. Moreover, antenatal exposure to maternal allergens was found to have an influence on the incidence of allergic diseases in genetically predisposed individuals [76]. But, in a conflicting study, there were no consistent associations between the maternal diet and the occurrence of atopy in their children, although some separate protective foods were reported [77].

The effect of the diet on allergy development depend on the kind of the diet, some diets are protective while others are a risk of allergy development [78]. Heat processing of UPCD has been shown to destroy nutrients, active enzymes and antioxidants, making it non-nutritionally sound [79]. Indeed, heat processing boosts the denaturation of food proteins, which interact with other food components, promoting immunogenicity and allergenicity [44]. On the other hand, the NPMD is highly palatable, the proteins and fats are highly digestible, it reduces blood triglycerides, maintains fecal quality and serum chemistry, and modifies the fecal microbiota community in adult dogs [80]. Moreover, eating a NPMD provides a variety of microbes when compared to eating UPCD, as the latter one is heat processed, which consequently kills the bacteria, making it sterile [40]. There is an indication that the transmission of microbiota from the intrauterine environment to the fetus may occur during pregnancy which seems to have a better impact on offspring future health [81].

Dogs are carnivores and have evolved on eating diets rich in animal proteins and fats (meats, fish, organs, edible bones etc.) as well as animal fiber (e.g. feather, scales, fur etc.) and low in carbohydrates [46, 47]. The UPCD contains high amounts of highly processed, and therefore highly fermentable, cereal grains (wheat, rice, oats, barley, rye, etc.) which are not a

part of the canine ancestral diet. Cereal grains often contain gluten or other possibly harmful proteins [82]. Gluten intolerance or celiac disease occurs in humans and therefore gluten-containing grains are avoided by these patients [83, 84]. There are, however, very few studies or data on these diseases in dogs [85–92]. Gluten-related disorders might mimic the skin findings in humans and gluten avoidance seems to play a role in the prevention of AD [93]. As there are no minimum requirements for carbohydrates for dogs [94], a more species-appropriate, low or non-carbohydrate diet has been suggested to be used in cases of chronic skin diseases [95]. In a human study, the intake of allergenic foods such as margarine and vegetable oils during pregnancy increased the risk of allergic diseases in children, while consumption of foods rich in fish oil, decreased the risk [96]. However, discordant findings have been found when testing the effect of avoiding maternal dietary allergens in children with AD [97–101]. Furthermore, the Mediterranean diet during pregnancy seemed to be protective against allergic diseases in children [102, 103] which are in accordance with our results: the human Mediterranean diet contains more raw protein elements such as cured meats, raw shellfish, as well as raw eggs and raw vegetables, making them much more comparable to the NPMD of our study.

Other maternal factor tested in our study, maternal deworming during pregnancy, was negatively associated with CAD incidence in the puppies. This may indicate that deworming is essential to avoid the direct effects of worms on gastrointestinal pathology [104]. Moreover, helminth infection of the mother during pregnancy have been reported to have a long-lasting impact on the fetal immune system development and both risks and opportunities for diseases later [105]. The mother's vaccination during pregnancy was non-significant in our study. In a previous study on dogs [13] neither of the variables were significant. In a human study [106], maternal inflammatory cytokines during pregnancy were associated with corresponding cytokines in children at one year of age, but did not associate with increased IgE or atopic dermatitis in children.

Neonatal modifiable variables

The NPMD and UPCD failed to show any associations with CAD incidence in the offspring during the neonatal, i.e. lactation period. Thereby our negative results of an association between the mother's lactation diet and adult CAD disagree with Nodtvedt et al. [41]. In another conflicting study, feeding the mother a non-commercial animal products during lactation was protecting its puppies from CAD, regardless of whether the puppy was eating commercial or non-commercial meat before 6 months of age [40]. The findings of maternal lactation diets and their association with allergic diseases are also conflicting in human epidemiological studies [107, 108]. In a meta-analysis of 27 prospective cohort human studies, there was no strong AD protective effect of exclusive breastfeeding for a minimum of 3 months, even among children with a positive AD family history [109]. Moreover, in another study there was no significant association between duration of exclusive breast-feeding and development of sensitization in the first 6 years of life in children [110].

The environmental variable during the neonatal period, season of birth, had no association with CAD incidence at adulthood neither in the univariate nor in the multivariate logistic regression analysis. In a previous study, season of birth was reported to have an effect: dogs born in autumn had a higher risk for CAD [111]. Furthermore, born in winter has been associated with development of AD in children [112].

Early postnatal modifiable variables

In this period of life, puppies that consumed NPMD had a lower risk of CAD, while those that consumed UPCD were associated with a higher risk of CAD at adulthood. Postnatal nutrition

is essential for optimal physiological development of vital organs [113]. Our findings for the early postnatal diet are also in line with the DOHaD hypothesis [23, 27]. In the early postnatal period, the newborn gut is exposed to food allergens for the first time. The acquired dietary microbiota and their interactions also stimulate the immune tolerance development [114, 115]. This also supports the hygiene hypothesis which states that early life exposure to dietary microbes influences the immune system development against atopic dermatitis [116]. In a human study, child adherence to the Mediterranean diet was protective against allergic diseases [117, 118] while child adherence to the western diet was a risk factor for AD [118]. Moreover, some farming environment studies have shown that unprocessed farm milk consumption by the child is associated with a lower prevalence of allergic disease, without depending on other farm-related covariates [119, 120]. The decreased and increased risk of CAD with the NPMD and UPCD have been interpreted above with the maternal diet during pregnancy.

Sunlight exposure for one or more than one hours per day during the early postnatal period (1–2 months of age) was significantly associated with lower risk of CAD in dogs at one year of age. Daily outdoor sunlight exposure in early life increases the exposure to both sun and external allergens. Some human research suggests that insufficient sunlight exposure in the first two years of life might increase the risks of development of AD [112] while exposure to ultraviolet sun light showed a positive significant association with AD symptoms in children [121]. In addition, the exposure to the outdoor environmental microbes has been found to influence the immune system against sensitization [14, 122]. The environmental exposures in the early life period are very critical. The hygiene hypothesis clarified that early exposure to microbiota is important in stimulating the immune tolerance in humans and this is probably also true for dogs. Recent studies in dogs showed an increased risk for CAD with urban environment and regular washing of healthy puppies, and a decreased risk of CAD with rural environment and forest walks [14, 122, 123].

The normal body weight of the young puppy before two months of age seems to be associated with lower odds of suffering from CAD while an increased or decreased body weight were both associated with a higher risk of CAD incidence in adulthood. In the Anturaniemi et al. [13] study the dogs that were obese or very slim at the age of 2 months were more susceptible to develop CAD when compared to the dogs with a normal body condition score. Recently, a positive association between a high body condition score/overweight and atopic dermatitis in cats [124] and in infants [125] have been reported. Moreover, our results also agreed with Zhang and Silverberg [126] who found an association between obesity and prevalence of AD in North America and Asia. Furthermore, longtime obesity in early life is a risk factor for atopic dermatitis and weight loss is essential for the prevention of atopic dermatitis in children [127]. This can be due to the low immunological tolerance in obese puppies [128].

Late postnatal modifiable variables

The puppy diet consumed from 2 to 6 months of age associated with CAD but did not reach statistical significance. Similar to the other time frames the NPMD had a tendency to decrease the risk of CAD incidence in adulthood, while the UPCD tended to increase the risk. In a human study, high fish intake in late infancy was associated with a reduced AD risk [129, 130]. In contrast, Sallander et al.'s (2009) canine study found that feeding the puppy non-commercial meat before 6 months of age, had no effect on the risk of CAD later [40] but they only had 106 dogs compared to our over 2000 dogs. Also, the processing of the food was not clearly addressed and they just called the foods home-made diets / table diet. It is therefore possible

that feeding only a meat-based diet might not be enough, it is possible that it also needs to be raw? So, for more evidence future prospective interventions are needed.

During 2–6 months of age, being born in the same family as it would proceed to continue living in, was associated with a lower risk of CAD in adulthood. These findings were also in accordance with our previous findings [13]. When the dog was born within the same family it meant that the dog continued to live in the same family where it was born and therefore it also continued to be exposed to at least one other dog, its mother. The protective effect of living with other dogs was in agreement with Meury et al. [122] and Fall et al. [131]. The protective effect is also in line with a human hygienic hypothesis study which emphasizes that the more siblings, the less atopic dermatitis [132]. Moreover, when a dog continues to live in the same environment as it did in puppyhood, it would imply that the puppy already created immunity towards the external factors of that environment [13]. Another significant environmental factor during this period was the kind of flooring used by the puppy. We found a negative association between a dirt floor and CAD incidence in comparison with a non-dirt floor, which associated positively with CAD incidence. This was in accordance with Lehtimäki et al. [123] who found that exposure to arable land and forest in the surroundings of the birthplace associated with less allergic symptoms in dogs. The same has also been reported by Ruokolainen et al. [133] who found that early-life exposure to green environments (forest and agriculture land) is associated with lower atopic sensitization in 6 year old children. The environmental effect may be intermediated by the effect of land or floor microbiota on the commensal microbiota influencing immune tolerance.

Strengths and limitations of the study

The strengths of this study are the use of validated data [52], using a wide range of potential covariates (nutritional, environmental and genetic), investigating four early life periods, and a satisfactory sample size (2236 dogs included in the analysis). Moreover, the reverse causality effect has been avoided here by excluding the puppies under the age of one year from the cases and under 3 years from the controls. Hereby we eliminate most patients that either eat something because of already having the disease and we eliminate controls that later might have turned into cases. Furthermore, this study is the first study to investigate the feeding pattern during the gestation period and its association with CAD incidence in the offspring, hence testing the DOHaD hypothesis.

However, our study, like any observational epidemiologic study, has some limitations. The first limitation is the study design. This is a cross-sectional study where the questions have been asked regarding different time points. This enabled us to use longitudinal data, but possibly with a bit less certitude, as memory comes into play. A second limitation is that the cases and controls were based on owners' reports and not verified by professional veterinary diagnosing. This limitation, however, was amended by validating the questionnaire for CAD [52].

Conclusion and recommendation

In conclusion, there were many modifiable variables that were associated with the prevalence of CAD: The NPMD during the prenatal and early postnatal periods suggested a protective effect on CAD, while the UPCD during the same periods suggested an increase of the risk of adult CAD incidence. In addition, deworming the mother dog during pregnancy, getting one hour or more of sunlight exposure per day at 1–2 months of age, having a normal body condition score at the age of 2 months, being born in the same family that the dog still lives in, and having spent time on a dirt/lawn floor at the age 2–6 months, were all associated with a significantly lower risk of CAD incidence in the adult dog. As predicted, the non-modifiable risk

factors such as dogs with maternal history of CAD, from an allergy prone breed and with more than 50% white colored coat, were highly correlated with CAD. The modifiable risk factors, nutritional and environmental, during prenatal and postnatal periods provided by this study are critical starting points for further research. Prospective, randomized, longitudinal dietary intervention studies should now be undertaken both in dogs with a genetic predisposition, as well as in dogs without the genetic burden, to develop individualized primary prevention strategies for future prevention of CAD.

Supporting information

S1 Table. Breeds' predisposition to develop canine atopic dermatitis.

(DOCX)

S2 Table. Associations between pre-, neo-, early post- and late postnatal period variables and canine atopic dermatitis based on univariate logistic regression analyses.

(DOCX)

S3 Table. Associations between pre-, neo-, early post- and late postnatal period variables and canine atopic dermatitis based on multivariate logistic regression analyses.

(DOCX)

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Author Contributions

Conceptualization: Manal Hemida, Robin Moore, Johanna Anturaniemi, Anna Hielm-Björkman.

Data curation: Manal Hemida, Siru Salin, Anna Hielm-Björkman.

Formal analysis: Manal Hemida, Kristiina A. Vuori, Anna Hielm-Björkman.

Funding acquisition: Anna Hielm-Björkman.

Investigation: Anna Hielm-Björkman.

Methodology: Manal Hemida, Kristiina A. Vuori, Robin Moore, Anna Hielm-Björkman.

Project administration: Anna Hielm-Björkman.

Resources: Anna Hielm-Björkman.

Supervision: Anna Hielm-Björkman.

Validation: Johanna Anturaniemi, Anna Hielm-Björkman.

Writing – original draft: Manal Hemida, Kristiina A. Vuori, Siru Salin, Robin Moore, Johanna Anturaniemi, Anna Hielm-Björkman.

Writing – review & editing: Manal Hemida, Kristiina A. Vuori, Siru Salin, Robin Moore, Johanna Anturaniemi, Anna Hielm-Björkman.

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