Project and project number

REVEALING THE PHENOTYPIC AND GENOTYPIC VARIATION IN 4 BRACHYCEPHALIC BREEDS **N2017-0017**

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Summary:

Lack of success in reduction of illness related to a brachycephalic constitution have been proposed to be due to lack of genetic variation (Pedersen et al 2016) Crossbreeding with non-brachycephalic breeds is repeatedly proposed to handle that but is neither practiced to any greater extent nor favoured by many breeders

A multicentre Nordic study with data and samples from each Scandinavian country on a wide variety of individual dogs of the brachycephalic breeds – English bulldog, French bulldog, Pug and Boston terrier aimed to reveal if there is enough phenotypic and genotypic variation to make it possible to select for a change in anatomy and predisposition for Brachycephalic Obstructive Airway Syndrome (BOAS).

Initially “Breed” -gatherings of as many individuals as possible in these breeds at various locations to attract registered as well as non-registered individuals nationally and internationally aimed to be obtained reflecting the within breed variations. Later results from BOAS testing have served the same purpose

Results: Several dogs already operated on *mainly nostrils.* Almost all considered healthy by their owners *but affected by heat and variable but* high Body Condition Scores in many dogs.

Variations within and between breeds in clinical signs, in shape of nostrils, in length of nose, in width of neck, in Cranio - facial ratio and a verification of earlier noted correlations between conformation and clinical signs.

AIM By A multicentre Nordic study with data and samples from each Scandinavian country on a wide variety of individual dogs of the brachycephalic breeds – English bulldog, French bulldog, Pug and Boston terrier aimed to reveal if there is enough phenotypic and genotypic variation to make it possible to select for a change in anatomy and predisposition for Brachycephalic Obstructive Airway Syndrome (BOAS).

to indicate animals that by fitness and morphological features would enhance the health in these breeds by being used for breeding.

to relate phenotypic measures to molecular genetics

BACKGROUND Lack of success in reduction of illness related to a brachycephalic constitution have been proposed to be due to lack of genetic variation (Pedersen et al 2016) Crossbreeding with non-brachycephalic breeds is repeatedly proposed to handle that but is neither practiced to any greater extent nor favoured by many breeders.

A working group was appointed by the Nordic Kennel Clubs (NKU/AU) to address issues related to BOAS and suggested that an inventory of existing phenotypic variation in brachycephalic breeds should be encouraged to be performed in the Scandinavian countries. By ongoing molecular genetic studies on various diseases and other phenotypes GWAS as well as Whole genome sequencing data for brachy breeds did already exist to some extent. By obtaining DNA-samples from phenotypically well-defined individuals at gatherings of these breeds at several locations we have been able to have them analysed for variations in phenotype and genotype as well as having it related to variations in clinical status as well as morphometric measures.

PEOPLE INVOLVED

The Inventories were supervised in each country by staff and members at the Scandinavian Kennel Clubs Denmark: Helle Friis Proschowsky Finland: Katariina Mäki and Liisa Lilja-Maula Norway: Kristin Wear Prestrud Sweden: Helena Skarp, Lotta Olsson.

The molecular genetic studies were conducted at the Department of Animal Breeding and Genetics in Sweden a. Prof. Göran Andersson, Docent Tomas Bergström in collaboration with i.e. Prof. Merete Fredholm (Denmark) and Prof. Frode Lingaas (Norway)

Sweden

The Inventory in Sweden was performed and analysed by **Veterinarians**: Maria Dimopoulou Elina Åsbjer Monica Stavenborn Anne-Sofie Lagerstedt Eva Skiöldebrand, Ingrid Ljungvall and Åke Hedhammar **Geneticist**: Katja Nilsson, Göran Andersson **Students:**Sara Sahlin, Ida Bertilsson, Elin Johansson and **Employed and members of the Swedish Kennel Club** (SKK):Anna Törnlöv, Maria Lönnhammar, Lotta Olsson , Helena Skarp

MATERIAL AND METHODS Initially "Breed" -gatherings of as many individuals as possible in these breeds at various locations in Sweden, Denmark, Finland and Norway to attract registered as well as non-registered individuals nationally and internationally aimed to be obtained reflecting the within breed variations. Later results from BOAS testing have served the same purpose

1. by surveying the owners, clinical examination by two veterinarians and evaluations by two show judges data by breed on
   a. *Demography:* sex, age, country of origin, registration number or not was registered.
   b. Measures of weight, BodyConditionScore (BCS)
   c. General and specific health conditions
2. by sampling and extracting DNA from each individual molecular genetic analyses were performed primarily to evaluate the genomic diversity

AVAILABLE DATA

For Phenotypic data French bulldog n-109, Pug n- 99, Boston terrier n- 77, English bulldog n- 64

For molecular genetic studies gDNA: >400 samples, Blood: 278 Pugs

Swabs: A total of 359 swabs from 4 different breeds: Pug, Boston terrier, French bulldog and English bulldog.

Within ongoing studies on generalized demodicosis and ataxia in pugs (Brander et al 2023) there were 313 DNA samples in the Swedish Canine Biobank out of which 64 already have GWAS data. Also Whole genome sequencing data for many breeds including these breeds were available for comparison. Whole genome sequencing data for the four breeds are being generated as part of the "Nordic Canine Genome Project"

In addition, as an important resource, the research group in Uppsala (SLU and UU) have generated a platinum canine reference genome based on PacBio Single Molecule, Real-Time (SMRT) sequencing technology. The platinum canine reference genome, will significantly benefit research on complex traits and disorders that results from structural genomic variations not captured in CanFam.

ADDITIONAL SAMPLES

a) at RFG scooring in the BOAS clinic in Uppsala 30 well defined individuals with RFG scooring and status of Nostrils have been obtained

Denmark

A total of 79 French bulldogs were recruited via the social media platform Facebook during March 2018. A total of 90 dogs unrelated at the parental level were included on first-come, first-served basis. Both FCI-registered and non-registered dogs were accepted for the study. The dogs were 1–5 years of age and had no history of upper airway surgery or any detectable orthopaedic, cardiovascular, neurological or pulmonary disease. Intake of medication that could potentially affect the physical capacity and clinical evaluation (e.g. steroids and nonsteroidal anti-inflammatory drugs) within the past 6 weeks was also a reason for exclusion. Informed consent forms were signed by all dog owners before the clinical study was performed.

The clinical part of the study aimed to register morphometric characteristics that could be correlated to BS, as well as other phenotypic characteristics that could be confounders for the correlations discovered (13 parameters in total), and to use the BSF score as a proxy for degree of BS.

The morphometric and phenotypic characteristics included height (H), neck length (NL), neck girth (NG), the neck-length-to-neck-girth ratio (NLGR), muzzle length (ML), cranial length (CL), craniofacial ratio (CFR), nostril stenosis (NS), BCS and body weight (W). Measurements of the skull, neck and height were performed]. In addition to the morphometric measurements, the sex, neuter status and age were noted for each participating dog, based on owner information.

Finland

Within research at University of Helsinki development of a submaximal exercise test phenotypic data and genomic material was made available

Results

Swedish

The outline and result of the inventory of four Brachy breeds in Sweden is reported in a video and ppt presentation to Agria SKK. ***Is there enough variation of the appearance in four brachycephalic breeds for a healthy life in the next generation*** P*henotypic variation in pugs, French and English Bulldog and Boston terrier* (Hedhammar 2021)

Results: Several dogs already operated on *mainly nostrils* Almost all considered healthy by their owners *but affected by heat and variable* but high Body Condition Scores in many dogs.

Variations within and between breeds in clinical signs, shape of nostrils, length of nose, width of neck, in cranio - facial ratio and a vverification of earlier noted correlations between conformation and clinical signs

For Pugs: Low variation in all parameters of relevance for BOAS, Length of nose for almost all dogs 15-20mm. The lowest craniofacial ratio and *affection of Cornea in 50% and in skin in 25%*

For French bulldogs: Almost all - varying degree of stenotic nares, low variation in length of nose with most just 20-40mm with somewhat more variation in width of neck and chest *and Almost no* ***affection of eyes but many with skin affection***

For English bulldog: Almost one third with affected breathing, Highest fraction of unaffected nostrils, Some variation in width of neck and chest and *many of the dogs expressing affected eyes*

For Boston terrier: *Few dogs with unaffected nostrils but most of them slight to moderate, Some variation in length of nose but most of them around 25mm and Few with affected eyes but many with skin disorders*

- **General conclusions:**

- High frequency of already operated dogs and clinical signs
- A correlation - but breed dependent - between anatomical parameters and clinical signs
- Limited variation to make a selection. High Body Condition score in many dogs
- **In a master thesis by Sara Sahlin** the phenotypic variation is reported and discussed. The results of the study show that the majority of the measurements do not vary much. Some of the measurements have been associated as risk factors for BOAS show some variation and therefore show potential for a selection towards healthier breeding (Sahlin 2021)

In a master thesis by Ida Bertillson on part of the surveyed dogs the aim was to investigate the phenotypic variance correlated to BOAS in the Swedish population of four brachycephalic breeds; English Bulldog, French Bulldog, Pug and Boston Terrier and discuss their welfare implications. The project consisted of two parts; one inventory and one survey. The inventory consists of conformational description of the four brachycephalic breeds and the survey was to investigate the BOAS related problems in the same breeds and their owner's perspective of health and welfare. The conformational risk factors correlated to BOAS found in this study were snout length and craniofacial ratio for French Bulldogs, snout length, chest girth, neck length, sternum length and craniofacial ratio for English Bulldogs and none for either Pugs or Boston Terriers. According to the survey noisy breathing and heat intolerance were the most common BOAS-related problems for all the four breeds in this study. The conclusion of the study was that there was enough phenotypic variance to improve the BOAS problem for both French Bulldogs and English Bulldogs. For Boston Terriers the BOAS problem was not severe enough in this study to get a result and for the Pugs the phenotypic variance is too small. For the welfare aspect this study showed that BOAS is a welfare problem and that there was a normalization for some of the problems connected to BOAS for English Bulldogs, French Bulldogs and Pugs. (Bertilsson 2019)

In a master thesis by Elin Johansson on part of the dogs surveyed the aim was to investigate the genetic variation in the genes SMOC2, BMP3, and DVL2 in the Swedish population of the four brachycephalic breeds; Boston Terrier, English Bulldog, French Bulldog and Pug.

One hundred and two privately owned brachycephalic dogs were genotyped for the mutation in the SMOC2 gene. Additionally, 45 of these dogs were also genotyped for the mutations in the BMP3 and DVL2 gene.

Results: The mutant variant of the SMOC2 gene was fixated in all four breeds. The mutant variant of the BMP3 gene had high allele frequency in all breeds. In Bull type breeds (Boston terrier, English bulldog and French bulldog) the mutant version of the DVL2 gene was fixated, whilst all Pugs were tested wild type for the DVL2 mutation.

Conclusions: Low genetic variation will make it more challenging to improve health through genetic selection within the breeds (Johansson 2019)

Danish

In a published paper on French bulldogs in Denmark - ***Breeding French bulldogs so that they breathe well-A long way to go*** (Ravn-Mölby et al 2019) the correlations between morphometric and other phenotypic characteristics and BS was evaluated in a population of 69 French bulldogs from Denmark to identify parameters that could serve as a basis for breeding against BS.

Furthermore, the genetic variation was monitored to determine whether it would be possible to breed based on these characteristics without simultaneously causing a critical reduction in genetic variation.

Six phenotypic characteristics were correlated with the Brachycephalic Syndrome Functional (BSF) score. Among the morphometric risk factors, nostril stenosis (NS) and neck girth (NG) had the highest impact on the BSF score, accounting for 32% and 4% of the variation, respectively.

Finnish

A paper about based on an evaluation of the submaximal exercise test (Lilja-Maula 2016) result of Finnish Kennel Club Boas breeding test data (2017-2022) have been submitted to a peer reviewed journal In which phenotype information (BOAS grade, nostril stenosis grade and CFR and exercise result as the main “outcome” saved to FKC database). Preliminary BOAS grade heritability’s were calculated.

Ongoing and further studies

The phenotypic data presented (Bertilsson 2019, Hedhammar 2021, Sahlin 2021,) together with further analyses of correlations will be published within the frame of a two PhD projects (Åsbjer 2024, Dimopoulou 2024)

The genomic data already available together with additional Whole genome sequencing and GWAS data for Pugs are analysed and the result will be published in a per-review journal during 2024

Primarily, the level of genetic variation in the Swedish Pug population and the U.K. Pug population will be defined. The genomic data already available together with additional novel Whole genome sequencing and genotype data of Pugs from Sweden and U.K. will be analysed in this project. Data from U.K. has been made available through a collaboration with Prof. David Sargan, Cambridge University. Genotyping the Swedish Pugs will be performed using high density Axiom array of a total of 359 gDNA samples to be prepared from swabs of the four dog breeds and previously genotyped data from two GWAS on juvenile demodicosis (manuscript in preparation) and thoracolumbar myelopathy (Brander et al 2023) will be analysed to detect the level of genetic variation, the extent of homozygosity and to identify selective sweeps will be analysed and published 2024/25.

Further planned publications:

Johnsson M et al. Genetic variation and genomic diversity in Swedish and British pugs. Manuscript to be published

Andersson G et al Epigenomic studies on pugs affected with BOAS. Manuscript to be published

In a follow up study of dogs participating in the Swedish Inventory study the owners will in a questionnaire be surveyed about the outcome (dead /date or alive, BOAS or other Brachy related clinical signs/ diagnoses since their participation.

Discussion and practical applications

During the extended project period

a) Two BOAS related PhD projects (Dimopoulou, Åsbjer) and further BOAS studies have been initiated (Åsbjer et al 2024)
b) practical application of BOAS grading have resulted in screening and Health Programs for BOAS in all the Nordic countries

The result from this study already have had significant spin off effects by serving as a basis for the two BOAS focused PhD projects and extensive funding from Svenska Djurskyddsföreningen for BOAS studies at SLU. Further practical applications is to be expected by wider use of scoring for BOAS in clinics as well as in selection for breeding.

Funding

Funding of quite extensive manpower within the project was funded by the fact that gatherings, examinations and DNA-sampling was performed by staff /volunteers from SKK and students/academic personal within their positions.

The grant from Agria-SKK research foundation was entirely spent on the molecular analyses (preparation of DNA, whole Genome sequencing and GWAS) that was not already performed/ available by being used and funded within other projects.

Publications

Pedersen, Niels C., Ashley S. Pooch, and Hongwei Liu. "A genetic assessment of the English bulldog." *Canine genetics and epidemiology* 3 (2016): 1-16. https://scholar.google.com/scholar?q=A+genetic+assessment+of+the+English+bulldog+Pedersen+2016

Ida Bertilsson, 2019. Fenotypisk *variation for BOAS för fyra brakycefala hundraser – Kan god djurvälfärd uppnås?* Degree Project in Animal Science • 30 credits Agronomprogrammet – Husdjur Uppsala. https://stud.epsilon.slu.se/15103/

Johansson, Elin, 2019. *Genetic variation in genes associated with brachycephaly.* Avancerad nivå, A2E. Uppsala: SLU, Institutionen för husdjursgenetik. https://stud.epsilon.slu.se/14846/

Hedhammar 2021 Is there enough variation of the appearance in four brachycephalic breeds for a healthy life in the next generation? ppt and video report to Agria SKK Research foundation. Inventering av 4 brakycefala raser | Svenska Kennelklubben (skk.se)

Sahlin, Sara, 2021. *Fenotypisk karaktärisering av 375 brakycefala hundar i Sverige.* Avancerad nivå, A2E. Uppsala: SLU, Institutionen för kliniska vetenskaper (KV). https://stud.epsilon.slu.se/17133/

Ravn-Mølby EM, Sindahl L, Nielsen SS, Bruun CS, Sandøe P, Fredholm M. Breeding French bulldogs so that they breathe well-A long way to go. PLoS One.

2019 Dec 16;14(12):e0226280. doi: 10.1371/journal.pone.0226280. PMID: 31841527; PMCID: PMC6913956. https://pubmed.ncbi.nlm.nih.gov/31841527/

Lilja-Maula L, Lappalainen AK, Hyytiäinen HK, Kuusela E, Kaimio M, Schildt K, Mölsä S, Morelius M, Rajamäki MM. Comparison of submaximal exercise test results and severity of brachycephalic obstructive airway syndrome in English bulldogs. Vet J. 2017 Jan;219:22-26. doi: 10.1016/j.tvjl.2016.11.019. Epub 2016 Dec 6. PMID: 28093105. https://pubmed.ncbi.nlm.nih.gov/28093105/

Dimopoulou M, Engdahl K, Ladlow J, Andersson G, Hedhammar Å, Skiöldebrand E, Ljungvall I. The epidemiology of upper respiratory tract disorders in a population of insured Swedish dogs (2011-2014), and its association to brachycephaly. Sci Rep. 2023 May 30;13(1):8765. https://pubmed.ncbi.nlm.nih.gov/37253809/

Brander G, Rohdin C, Bianchi M, Bergvall K, Andersson G, Ljungvall I, Hultin Jäderlund K, Häggström J, Hedhammar Å, Lindblad-Toh K, Tengvall K. Multiple Genetic Loci Associated with Pug Dog Thoracolumbar Myelopathy. Genes (Basel). 2023 Feb 1;14(2):385. https://pubmed.ncbi.nlm.nih.gov/36833311/

Åsbjer E, Hedhammar Å, Engdahl K. Awareness, experiences, and opinions by owners, breeders, show judges, and veterinarians on canine Brachycephalic Obstructive Airway Syndrome (BOAS). Canine Med Genet. 2024 Mar 8;11(1):3. https://pubmed.ncbi.nlm.nih.gov/38459530/