CAGH

Companion Animal Genetic Health

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About the cover image: Local legend has it that when policeman John Gray passed away, his faithful companion Bobby, a Skye terrier, ceaselessly watched over his grave. Bobby died in 1872. Both master and companion are buried in the churchyard of Greyfriars Kirkyard. Today a statue of Greyfriars Bobby located on Candelmakers Row marks the entrance to the Greyfriars Kirkyard. Photo credit (Andrew Thompson).

Meeting Program

Sunday, May 13th

Arrival

19:00 Meet and greet at Summerhall

Monday, May 14th

8:30-9:15 Registration / tea and coffee

9:15-9:30 Welcome

9:30-10:50 Session 1 Talks
Led by **Catherine André** (CRNS Université de Rennes 1, France)

9:30-9:50 **Tom Marchant**, University of Edinburgh, UK
Detection and Characterisation of a Genetic Association with Norwich Terrier Upper Airway Syndrome

9:50-10:10 **Christopher Jenkins**, Animal Health Trust, UK
Identification and characterisation of a mutation associated with cerebellar ataxia in the Norwegian Buhund dog breed

10:10-10:30 **Suvi Mäkeläinen**, Swedish University of Agricultural Sciences
A novel form of retinal degeneration in Labrador retrievers

10:30-10:50 **Greg Marby**, University of Edinburgh, UK
Ontogenic Transcriptomic Profiling Identifies Signalling Pathways Driving Pathogenesis in Canine Myxomatous Mitral Valve Disease

10:50-11:10 Coffee/Tea Break

11:10-12:10 Session 2 Talks
Led by **Tomas Bergström** (Swedish University of Agricultural Sciences)

11:10:11:30 **Naomi Harvey**, The University of Nottingham, UK
Genetic and environmental risk factors for canine atopic dermatitis evaluated in a population of owned Labrador and Golden retrievers

11:30:11:50 **Maud Rimbault**, CRNS Université de Rennes 1, France
Glioma in dogs: interest of spontaneous models for the genetics of human gliomas

11:50-12:10 **Rebekkah Hitti**, Animal Health Trust, UK
Genetic analyses of Lhasa Apso dogs with retinal atrophy identifies a LINE-1 insertion in the promoter region of a retinal candidate gene

12:10-12:20 Housekeeping

12:20-13:30 Lunch

Monday, May 14th (Continued)

13:30-15:00	Session 3 Talks Led by **Eleanor Raffan,** University of Cambridge, UK
13:30-13:50	**Juliane Friedrich**, University of Edinburgh, UK "*The association of environmental and genetic factors with behavioural traits in two popular purebred dog breeds*"
13:50-14:10	**Soleanne Correard**, CRNS Université de Rennes 1, France "*A dog spontaneous model for human sensory neuropathies: Identification of a mutation in a regulatory region of GDNF and DNA screening in human patients*"
14:10-14:30	**Rebekkah Hitti**, Animal Health Trust, UK "*Extensive whole genome sequencing comparisons in dogs elucidates a putative novel candidate gene for retinal degeneration*"
14:30-15:00	Poster Introductions (odd numbered)
15:00-16:00	Poster session (coffee/tea served)
16:00-17:00	Keynote **Robert Ogden**, University of Edinburgh, UK "*A Talk on the Wild Side: Applications of Animal Genetics in Wildlife Conservation*"
17:00-19:00	Freetime
19:00	Dinner at The Rowantree (advanced meal purchase required)

Tuesday, May 15th

9:00-9:10	Announcements / tea and coffee
9:10-11:00	Session 4 Talks Led by **Lucy Davison** (Royal Veterinary College)
9:10-9:30	**Joanna Ilska**, University of Edinburgh, UK "*Use of cross-country data for estimation of heritability of longevity and heart-related deaths in Doberman Pinscher*"
9:30-9:50	**Catherine André**, CRNS Université de Rennes 1, France "*Canine breed specific cancers as natural models for rare and/or aggressive human cancer types: examples of sarcomas, melanomas, lymphomas and gliomas*"
9:50-10:10	**Louise Burmeister**, Animal Health Trust, UK "*Genetic investigation of oculoskeletal dysplasia in the Northern Inuit dog*"

Tuesday, May 15th (Continued)

10:10-10:30	**Eleanor Raffan**, University of Cambridge, UK *Deep phenotyping of an obesity-associated Labrador retriever POMC mutation*
10:30-11:00	Poster Introductions (even numbered)
11:00-12:00	Poster session (coffee/tea served)
12:00-13:00	Session 5 talks Led by **Pam Wiener** (University of Edinburgh)
12:00-12:20	**Carys Pugh**, University of Edinburgh, UK *A mutation in Olfactomedin-like 3 (OLFML3) is a candidate for severe goniodysgenesis and glaucoma in Border Collies*
12:20-12:40	**Julia Niskanen**, University of Helsinki, Finland *A novel locus in chromosome 5 is associated with dilated cardiomyopathy in Doberman Pinschers*
12:40-13:00	**Sally Ricketts**, Animal Health Trust, UK *Association of primary open angle glaucoma ADAMTS17 mutations with height in two domestic dog breeds*
13:00-13:15	Wrap-up

Abstracts (Session 1, Talk 1)

Detection and Characterisation of a Genetic Association with Norwich Terrier Upper Airway Syndrome

Thomas Marchant (1), Elisabeth Dietschi (2), Ronan Harrington (1), Michaela Drögemüller (2), Ulrich Rytz (3), Elaine A. Ostrander (4), Tosso Leeb (2) & Jeffrey J. Schoenebeck (1)

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In domestic dogs, the “flat-faced” brachycephalic head shape is a risk factor for developing the respiratory defect, Brachycephalic Obstructive Airway Syndrome (BOAS). As the popularity of breeds such as the French bulldog continues to increase in the UK, so too are the expected incidences of BOAS. For this reason, we became interested in the Norwich terrier, a non-brachycephalic breed which presents with Upper Airway Syndrome (UAS), a condition highly reminiscent of BOAS. Here, we have studied this single breed to identify genetic association(s) with UAS. Pathological assessments and grading from laryngoscopic examinations held at the Vetsuisse Faculty of the University of Bern, were used as phenotypes in conjunction with microarray genotypes to perform GWAS. In total, 233 Norwich terriers were examined. We identified the same QTL on canine chromosome (CFA) 13 to be associated with the abnormal positioning of laryngeal cartilage and everted saccules in the dogs most severely affected by UAS. We phased genotypes at the CFA13 QTL to conduct haplotype mapping, which led us to define a 413 kb critical interval which encompasses a single positional candidate gene. The derived haplotype within this interval is overrepresented: it is found to be homozygous in 61 of 81 (74%) severely affected cases. In contrast, this homozygous haplotype was identified among 7 of 86 (8.1%) mild/unaffected controls. We have resequenced four dogs representing phenotypic extremes to sixteen-fold depth to identify putatively causal variants. We will provide an update to this ongoing project, which is expected to guide Norwich terrier breeding and inspire additional exploration of the CFA13 locus to improve animal welfare.

Keywords: Canine, Inherited Disease, Morphology

Abstracts (Session 1, Talk 2)

Identification and characterisation of a mutation associated with cerebellar ataxia in the Norwegian Buhund dog breed

Jenkins CA (1), Kalmar L (3), Mari L (2), Schofield EC (1), Mellersh CS (1), De Risio L (2, 4), Ricketts SL (1, 4)

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Inherited ataxias are typically incurable and lack disease-modifying treatments. Four Norwegian Buhunds were diagnosed with cerebellar ataxia at the Animal Health Trust. Pedigree analysis was suggestive of an autosomal recessive mode of inheritance, which is typical of inherited canine ataxias. The causal variant for ataxia in these dogs was hypothesised to be private to the breed. Whole genome sequence (WGS) was obtained for two sibling cases, which were compared to WGS from 405 dogs of other breeds. The WGS used included 44 which were generated for the study of other diseases in our laboratory, and 361 additional WGS which are part of the Dog Biomedical Variant Database Consortium. Filtering out benign variants left nine that were present only in the cases and predicted to directly affect a protein coding sequence or alter a transcript. These were assessed in 14 related and unrelated Buhunds, leaving one variant that fully segregated with the disease. Its association with ataxia was confirmed by typing in an extended set of 148 Buhunds containing two additional cases, and its absence in 359 dogs of 122 other breeds. This research has resulted in the development of a DNA test enabling breeders to avoid producing affected dogs. Importantly, the causal mutation is within a gene not previously reported to be associated with ataxia in any species. A combination of approaches was used to characterise this gene in the dog, as the current CanFam 3.1 annotation is incorrect. The gene is highly conserved and, in humans and mice, encodes multiple transcripts with alternative first exons. Expression of multiple transcripts in the canine cerebellum was confirmed through RNA sequencing, and through RT-PCR of samples from two Norwegian Buhund cases and five unaffected dogs of other breeds. RT-qPCR analysis and in-silico protein modelling have been used to further investigate the mutation’s effect on RNA expression and protein stability.

Keywords: Canine, Inherited Disease

Abstracts (Session 1, Talk 3)

A novel form of retinal degeneration in Labrador retrievers

Suvi Mäkeläinen (1), Marta Gòdia (1,†), Minas Hellsand (2), Agnese Viluma (1), Daniela Hahn (1), Karim Makdoumi (3), Caroline J. Zeiss (4), Cathryn Mellersh (5), Sally L. Ricketts (5), Kristina Narfström (6), Finn Hallböök (2), Björn Ekesten (7), Göran Andersson (1), Tomas F. Bergström (1)

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Inherited retinal degenerations are a clinically and genetically heterogeneous group of diseases affecting both humans and dogs. Approximately 30 genes have so far been identified in various dog breeds. One of the most widespread mutations is the p.C2Y mutation in the PRCD gene causing progressive retinal atrophy (PRA) which primarily affects rod photoreceptor cells. The PRCD mutation is currently the only known genetic cause of retinal degeneration in Labrador retrievers. There are, however, unexplained retinal degenerations in Labrador retrievers.

We diagnosed a sib-pair of Labrador retrievers, free from the PRCD mutation, with a novel form of retinal degeneration mainly affecting cone photoreceptor cells. The affected dogs were visually impaired under both daylight and dim light conditions. Ophthalmoscopic examination revealed signs of retinal vascular attenuation as well as abnormal mottling in the central and peripheral retina. Using optical coherence tomography (OCT) along the visual streak of the retina, we observed a general neuroretinal thinning, particularly in the outer nuclear layer.

The objective of this study was to find the genetic cause for this novel disease. We performed whole-genome sequencing of the affected siblings and their unaffected parents. We used conditional filtering of exonic variants assuming an autosomal recessive mode of inheritance. To further reduce the number of candidate variants, we filtered against 23 additional dog genome sequences. This resulted in 18 nonsynonymous SNVs and four INDELs which were then further evaluated using their predicted effect on the protein function and associations with human retinal diseases. The strongest candidate, a frameshift insertion leading to a premature stop codon, was validated using Sanger sequencing of additional Labrador retrievers with and without the disease. All the cases were homozygous for the insertion, whereas the unaffected dogs were either heterozygous or homozygous for the wild-type allele.
Next, we functionally evaluated the effect of the identified genetic variant, performing real-time quantitative PCR, Western blotting and fluorescence histochemistry. For this we used retinal tissue from dogs with the three different genotypes. The affected individual showed a lower relative gene expression in comparison to unaffected individuals. Using Western blotting, a full-length protein was detected in the retinas of the unaffected dogs with both genotypes. In contrast, no protein was detected in a dog affected by this disease. These results were confirmed with fluorescence histochemistry. We conclude that we have identified a loss-of-function mutation causing a novel form of retinal degeneration in Labrador retrievers. The details of these findings will be presented.

Keywords: Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 1, Talk 4)

Ontogenic Transcriptomic Profiling Identifies Signalling Pathways Driving Pathogenesis in Canine Myxomatous Mitral Valve Disease

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Affiliations: 1The Roslin Institute, Edinburgh, Scotland. 2Mater Research Institute, Brisbane, Australia. 3The Royal (Dick) School of Veterinary Studies, Edinburgh, Scotland.

Chronic degenerative diseases (CDGs) are a major welfare concern in canine medicine with myxomatous mitral valve disease (MMVD) being an important example. For some breeds CDGs can have an inherited basis, but often this is a polygenic trait and so understanding the mechanisms that drive disease pathogenesis requires examining molecular events in tissue. Specifically for CDGs this requires examination in both temporal and spatial terms changes in gene and protein expression. In this study we have examined the valvular gene expression at different stages of disease (temporal), different locations (spatial) and in different cell culture models of MMVD.

Methods
Transcriptomic profiling (Affymetrix canine 1.1ST microarray), with validation using RT-qPCR for selected genes, was performed on, whole valves from normal and the 4 grades of MMVD (n=6),normal and diseased regions of grade 2 valves (n=7), and cultured (all experiments n=3) normal and diseased valve interstitial cells (VICs), normal cells treated with 5ng/μL TGFβ1 and diseased cells treated with 10μM of the TGFβ pathway inhibitor SB431542. Microarray data were analysed using a range of bioinformatics platforms (Affymetrix Console, IPA, Miru (Biolayout Express)).

Results
Significantly differentially expressed genes (DEG) were identified comparing: 1) normal and the 4 grades of MMVD (1002 genes); 2) diseased and normal tissue within the same valve (315 genes); 3) normal and diseased VICs (1027 genes); 4) normal VICs and normal VICs treated with TGF-β1 (302 genes); 5) diseased VICs and diseased treated with VICs SB431542 (269 genes). Grade-dependent up and down regulated gene clusters were identified, and microarray data were validated by RT-PCR for ACTA2, TAGLN and 5HTR2B. Important GO-terms were found to be associated with myofibroblast differentiation and extracellular matrix homeostasis. In all data sets altered DEGs implicated TGF-β1 as the important up-stream regulator of disease pathogenesis, with minor contributions from TNF and IFNG. 75 DEGs were shared in common between grade 4 whole valve and the diseased sections of the dissected valves. Cultured cell data, in addition to TGFβ1, predicted genes involved in cell cycle and apoptosis as important up-stream regulators.
Conclusions
This study shows how transcriptomic profiling of chronic degenerative disease over an entire lifetime, in tandem with cell culture models, can identify the signalling pathways important in disease pathogenesis. TGFβ1 signalling has been identified as the fundamentally important pathway in MMVD initiation and development, and progression to eventual end-stage valve pathology.

Keywords: Canine

Abstracts (Session 2, Talk 5)

Genetic and environmental risk factors for canine atopic dermatitis evaluated in a population of owned Labrador and Golden retrievers

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Canine atopic dermatitis (cAD) is a common clinical syndrome with no definitive diagnostic tests, which causes marked morbidity and has a high economic impact. Past efforts at evaluating the epidemiology and genetics of cAD have relied upon extensive clinical examination to identify subjects, which has limited sample sizes. In this study, we created a novel questionnaire for completion by Labrador (LR)and Golden retriever (GR) owners to evaluate canine skin health with respect to the clinical signs of cAD. A total of 4,111 dogs were registered to take part in the study, for which the owners fully completed the questionnaire (2,803 LR and 1,308 GR). Cases were identified as dogs whose owners reported a veterinary diagnosis of cAD and controls were identified as having no current or past clinical signs of cAD and aged over 3 years. Epidemiological analyses using multivariate logistic regression revealed the most salient environmental risk factors for cAD in a dog of these breeds was being reared in an urban environment, being male, being neutered, receiving flea control, and being allowed on upholstered furniture. Protective factors included living with other dogs and walking in woodlands, fields or beaches. To investigate genetic risk factors associated with the disease 784 owners of cases and controls were invited to participate in a genetic study. A total of 581 (287 cases and 294 controls) pet dogs balanced for breed between purebred Labradors and Golden retrievers were genotyped using the Illumina CanineHD BeadChip covering more than 230,000 SNPs. We will present the results from a genome-wide association analysis conducted to estimate the proportion of genetic variance attributable to the SNPs on each chromosome using restricted maximum likelihood (REML) and will report upon genome regions and SNPs significantly associated with cAD.

Keywords: Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 2, Talk 6)

Glioma in dogs: interest of spontaneous models for the genetics of human gliomas

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Human glioma are brain cancers with a dramatic 5 year survival time of 5% even applying the unique reference treatment based on radio- and chemotherapy. Interestingly, among the many dog breeds prone to spontaneously develop cancers, brachycephalic breeds (Boxers, Bulldogs, Boston terriers…) are particularly affected by glial tumors. Dogs share the same environment as humans and have also anatomical and physiological similarities, thus constituting a relevant model for the genetics and therapies of brain tumors.

Thanks to the national Cani-DNA biobank and its veterinary network (the 4 Veterinary Schools, Antagene, private practices and cancer centers) managed at CNRS Rennes (France), samples for 50 glioma affected and >100 control dogs, as well as 1400 brachycephalic dogs have been collected and DNA extracted and stored.

With the goal to compare dog and human gliomas in mind, we performed a retrospective study of 100 canine glioma cases, allowing a clinical, epidemiological and histological characterization of these canine tumors. The predominant localization of glioma to the frontal lobe, predisposed breeds (mainly brachycephalic dogs from the European Mastiff line) and mean age of onset were revealed by the analysis of 20 cases with imaging and 15 cases with histology. We showed that dog gliomas present surprising anatomic and clinical homologies, with comparable histopathological subtypes as in human gliomas.

These results led us to analyze 2 cases for which brain tissue had been collected. We identified a BRAF-MBP gene fusion in one case using RNAseq and we are currently checking for recurrence in the collected samples, as well as for the presence of this translocation in human glioma cases. Using affected cases and controls of the same breeds, we plan to pursue the identification of somatic alterations by transcriptome analyzes (RNAseq) and exome sequencing (WES) and to carry out genetic linkage and/or genetic association studies (GWAS) to identify genomic regions involved in predisposition. We will also search if and how the artificial selection that led to specific morphological characteristics, such as the shape of the dog's skull (brachycephaly), would have also led to glioma predisposition.

Keywords: Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 2, Talk 7)

Genetic analyses of Lhasa Apso dogs with progressive retinal atrophy identifies a LINE-1 insertion in the promoter region of a retinal candidate gene

Rebekkah J Hitti (1,2), Louise M Burmeister (1), Sally L Ricketts (1), Louise Pettitt (1), Mike Boursnell (1), Ellen C Schofield (1), David Sargan (2), Cathryn Mellersh (1)
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Canine progressive retinal atrophy (PRA) is a degenerative retinal disease characterised by photoreceptor degeneration over time, increasing in severity and ultimately leading to vision loss. PRA affects multiple breeds and significantly impacts welfare. In the Lhasa Apso (LA) dog, PRA manifests typically as a mid-late onset form. Utilisation of whole-exome sequencing (WES) data previously generated in our laboratory from three PRA-affected LA (cases) and three PRA-unaffected LA (controls) did not reveal any obvious exonic or splice site polymorphisms segregating with the disease, indicating a non–coding mutation. This presented the opportunity for further investigations using a genome-wide association study (GWAS) and whole-genome sequencing (WGS) approach to identify the genetic cause of PRA and develop a DNA test.

A GWAS was conducted by genotyping 44 LA dogs (17 cases, 27 controls) on the Illumina Canine HD 170K chip. Allelic association statistics were adjusted for multiple testing using the PLINK Max(T) permutation procedure, and for population stratification and relatedness using Efficient Mixed-Model Association eXpedited (EMMAX). After stringent filtering and quality control, we tested 108,263 SNPs on 42 dogs, comprising 15 cases and 27 controls (call rate ≥97%; minor allele frequency ≥95%; genotype calls ≥90%). Analysis revealed a genome-wide significant association on canine chromosome 33 (-log praw = 2.2 x 10-16) which remained significant after correcting for multiple testing (pgenome = 0.9 x 10-5) and population substructure (p=raw1.6 x 10-17). A 1.3 megabase homozygous disease-associated region was defined, harbouring two candidate genes previously associated with human retinal degeneration.

WGS was undertaken on a single PRA affected LA, and manual interrogation of the critical region in identified a long interspersed nuclear element-1 (LINE-1) insertion, situated within the predicted promotor region of a retinal candidate gene. Due to the position of the LINE-1 insertion, it was not detected in the original WES data of the same case. The LINE-1 insertion was genotyped in 447 dogs across 122 breeds, including 63 LA dogs, and is private to the LA. Seventeen LA dogs (all clinically affected with PRA) were homozygous for the LINE-1 insertions, eight were heterozygous and thirty-eight were homozygous for the wildtype allele.

As a result of this study a DNA test for this form of PRA, termed PRA4, has been developed at the Animal Health Trust. To date, 457 LA from 15 countries have been tested for PRA4 (354 UK dogs; carrier frequency 17%; allele frequency 0.9054). This study highlights the power of utilising several genetic approaches to identify a PRA mutation and develop a diagnostic test to help dog breeders make informed breeding choices, minimising the risk of producing PRA-affected LA dogs.

Keywords: Canine, Inherited Disease

Abstracts (Session 3, Talk 8)

The association of environmental and genetic factors with behavioural traits in two popular purebred dog breeds

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As a companion animal, a dog's lifestyle is mainly determined by its owner. A major component of a dog's well-being relates to its integration into the physical and social environment provided by the owner. Discrepancies between the dog's genetically-influenced temperament and the owner's lifestyle might lead to the occurrence of unwanted behaviours that affect both the owner-dog relationship and the dog's well-being. A better understanding of the relationship between genetic predisposition, environmental factors and the expression of behavioural traits may help to reduce behaviour problems in pet dogs in general and to address breed-specific needs.

We characterised owner-assessed behavioural traits in Labrador Retrievers (LRs) and German Shepherd dogs (GSDs), which are among the most popular dog breeds worldwide and are used as both pets and working dogs, to analyse the association of behavioural traits with environmental factors and genetic markers.

First, principal component analysis (PCA) was used to define distinct behavioural traits based on the established Canine Behavioral Assessment and Research Questionnaire (C-BARQ) in both dog breeds separately. The resulting behavioural traits were generally consistent with the behavioural traits typically found by analysing C-BARQ questions in other dog breeds, but in addition, breed-specific behavioural traits were identified, e.g. "Fetching" and "Barking tendency" in LRs and "Resource guarding" and "Aversion being stepped over" in GSDs.

General linear models were applied to analyse the interaction between these behavioural traits and demographic factors of the dog (e.g. sex, neuter status, age, diseases experienced), its living situation (number of children, adults and other animals living with the dog, living place) and its management (e.g. puppy socialisation, amount of exercise and stimulation, training, activities). The analysis showed that various demographic and management factors were associated with behavioural traits in LRs and GSDs. In particular, levels of exercise and the "role" of the dog (i.e. pet, show or working dog) were strongly associated with behaviour in LRs and age and the number of commands for which the dog was trained in GSDs.

Although a large number of environmental factors were associated with behavioural traits, they still only explained a small proportion of the variance observed in the traits and thus, the genetic influence on behaviour in the dogs was also investigated. For LRs, several behavioural traits exhibited moderate pedigree- and genomic-based heritabilities and showed suggestive association with specific genomic regions in a genome-wide association study (GWAS). Estimation of heritabilities and GWAS in GSDs are being carried out to allow across-breed comparison.

Keywords: Behaviour, Canine, Genomics and Variation

Abstracts (Session 3, Talk 9)

A dog spontaneous model for human sensory neuropathies: Identification of a mutation in a regulatory region of GDNF and DNA screening in human patients.

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In humans, there are many forms of sensory neuropathies, associated or not with a loss of sensitivity to pain and sometimes accompanied by self-mutilation. Although to date 13 genes have already been implicated in this disease, they do not explain the genetic causes of all patients. Similar neuropathies are diagnosed in dogs and several breeds are at risk to develop certain forms. Neuropathy has been described in hunting dogs, where the condition results in progressive mutilation of the distal extremities of the paws (Paradis et al., 2005). Pedigree analysis led to conclude to a monogenic autosomal recessive mode of inheritance. Blood samples from affected and unaffected hunting dogs from France and from Canada were collected through the French CaniDNA biobank (dog-genetics.genouest.org). Genetic studies (GWAS and sequencing) led to the identification of a locus on canine chromosome 4, and to a mutation located 90kb upstream GDNF, a gene encoding a neurotrophic factor involved in the survival of dopaminergic neurons. This mutation segregates as expected in 300 hunting dogs of known clinical status and is not found in 900 dogs of 90 other non-predisposed breeds. Functional experiments have shown that the mutation causes a decrease of GDNF expression in the dorsal root ganglia and also a decrease in the affinity of a regulatory complex for the DNA sequence to which it binds (Plassais et al., 2016). This gene had not previously been involved in human forms of sensory neuropathy and appears a good candidate. Through French and Belgium reference centers, we collected 111 DNAs of patients affected with different forms of sensory neuropathies and we sequenced GDNF exons as well as two regions predicted as regulatory, orthologous to the mutated regulatory region in the dog.

Abstracts (continued)

23 variants were identified and classified:

i. New variants (not listed in human databases).

ii. Rare variants (listed in databases with a minor allele frequency inferior to 1%).

No new variants have been found in the coding parts of the gene, however, 6 new variants have been identified in the UTRs and regulatory regions upstream GDNF and 17 rare variants were also identified.

In conclusion, the dog model has allowed to identify a new gene for canine and potentially human sensory neuropathies. New and rare variants in this gene are being analyzed to tentatively identify their potential role in human neuropathies.

Plassais et al. 2016. A point mutation in a lincRNA upstream of GDNF is associated with a canine insensitivity to pain: a spontaneous model for human sensory neuropathies. Plos Genetics 2016

Keywords Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 3, Talk 10)

Extensive whole genome sequencing comparisons in dogs elucidates a putative novel candidate gene for retinal degeneration

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Retinitis pigmentosas (RP) are genetically heterogeneous, progressive diseases characterised by retinal degeneration and causing loss of vision before middle age, and affecting in 1 in 2000 humans. The canine equivalent, progressive retinal atrophy (PRA) is untreatable and affects multiple dog breeds, significantly impacting dog welfare.
A novel form of PRA was diagnosed in a family of Giant Schnauzer dogs, where three out of seven littermates presented with clinical signs of PRA around four years of age. The sire and dam were clinically unaffected and therefore considered likely to be obligate carriers of an autosomal recessive mutation causing PRA in this family. We sought to identify the causal mutation of PRA in this Giant Schnauzer family with the ultimate aim of developing a DNA test, a tool which breeders could utilise to prevent this form of PRA becoming widespread in the breed.
Whole genome sequencing (WGS) of two PRA affected full-siblings and their unaffected parents was performed. Variants were filtered based on those segregating appropriately for an autosomal recessive disorder and those with a predicted pathogenic effect on the coding sequence and protein. Successive filtering against a total of 568 genomes (including genomes from the Dog Biomedical Variant Database Consortium and the Animal Health Trust Give a Dog a Genome bank) reduced an initial set of > 20 million variants down to a single candidate variant in a novel gene not previously associated with retinal degeneration in any species.
The candidate variant was genotyped in a total of 1,444 dogs of 175 breeds, 10 cross breed dogs and 3 wolves, with our three PRA-affected Giant Schnauzers being the only homozygotes identified to date. Nine Giant Schnauzer heterozygotes were identified in addition to heterozygotes in three additional breeds of German origin, including the German Giant (Gross) and Medium (Mittel) Spitz and Miniature Longhaired Dachshund (MLHD). The genotyping of German Spitz varieties, including 110 Giant Spitz, 21 Medium Spitz, 24 Miniature (Klein) Spitz, 17 Pomeranian (Zwerg) Spitz, and an additional 27 Giant Schnauzers was carried out by collaborators at the University of Bern. We screened a total of 163 MLHD for the candidate variant. Seven German Giant Spitz, one German Medium Spitz and six MLHD heterozygotes were identified, suggesting this may be an ancestral, but rare, mutation.
This study highlights the power of using WGS to identify novel genes associated with disease using a very small number of cases. This novel candidate gene, harbouring a variant that is predicted to be the causal mutation of PRA in the Giant Schnauzer, could provide insights into gene discoveries in human retinal degenerations. Further functional study options are being explored to confirm the candidate gene's role in retinal function and maintenance.

Keywords: Canine, Inherited Disease

Abstracts (Session 4, Talk 11)

Use of cross-country data for estimation of heritability of longevity and heart-related deaths in Doberman Pinscher

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Genetic improvement with the use of Estimated Breeding Values (EBV) is a method which, after decades of successful and validated use in livestock species, slowly gains recognition in the world of dogs breeding. However, accurate EBV prediction for complex traits requires large datasets of phenotyped and related animals. While generation of such datasets is possible in the most popular dog breeds, for many other breeds reaching sufficient numbers within any national database is not likely. Further, collection of the data pertaining to diseases through national Kennel Clubs is usually limited to very few already established grading systems for specific diseases, such as British Veterinary Association's scheme for Hip and Elbow Dysplasia. Thus, databases created by independent breed societies combining records across countries and on breed-specific issues, could become sources of data for the genetic analyses and EBV predictions in numerically small breeds. We present a preliminary analysis of the heritability of longevity and heart-related deaths (HEART) in Doberman Pinscher, based on data collated by The Doberman Welfare Community (DWC) – an independent group of breed enthusiasts. The data included over 350,000 dogs over 37 generations, born between 1890 and 2017, and from 18 regions. Phenotypic records on longevity and cause of death were recorded for 10,549 and 5,844 dogs respectively. Neither longevity nor causes of death are currently recorded by national kennel clubs, thus highlighting the role of the DWC in collecting this type of data. Among the causes of death, HEART were most common (48%), and more frequent in males than females (55% males, 45% females). The average longevity (LONG – number of months between birth and death) was 89 months (7 years) for males and 100 months (8 years) for females.

A number of mixed linear models were fitted to identify significant environmental factors affecting LONG and HEART, and to estimate heritability of the traits. LONG was Box-Cox transformed to improve normality of the data, and binomial models were fitted for the heritability estimation of the underlying liability for the HEART. Factors identified as significant for HEART were sex, region, season of birth, and year of death. LONG was affected by year and season of birth, as well as year of death. Heritability of the HEART and LONG was 0.29 (0.02) and 0.11 (0.02) respectively. To the best of our knowledge, these are the first published estimates of heritability of longevity and heart-related deaths in Dobermans using owner-collated data. A significant genetic variance detected for both traits indicates that selection could bring improvement in these traits, which is particularly important for HEART – heart conditions are believed to affect as many as 20% of Dobermans, and the symptoms of the disease often appear after a dog has already been used for breeding. Further, significant estimates obtained in the presented analyses indicate validity of the data, thus opening a new window of opportunity for genetic analyses of complex traits in numerically small breeds through the recruitment and collation of data by breed enthusiasts.

Keywords: Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 4, Talk 12)

Canine breed specific cancers as natural models for rare and/or aggressive human cancer types: examples of sarcomas, melanomas, lymphomas and gliomas.

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Through the French Cani-DNA biobank, developed in the team since 2005, we have collected over 3000 samples (blood and paired tumour/normal tissues) form many dogs affected by breed specific cancers, as well as controls of the same breeds, for which there are specific issues in the human corresponding cancers. Indeed, naturally occurring canine cancers are recently receiving attention in comparative oncology because of their high similarity to human cancers both in their clinical and histological presentations as well as in their response to treatments.

We have constituted large collections of cases and controls as well as large family pedigrees and through genome wide association studies (GWAS) and genetic linkage approaches, we have identified predisposition loci for Histiocytic Sarcoma (HS) and oral melanomas. In parallel, through the search of somatic genetic alterations in the tumour (whole exome sequence -WES-; capture/sequencing and RNAseq techniques), we have identified relevant genetic alterations in canine lymphomas, sarcomas, melanomas and gliomas. Either we found new genes implicated in dogs and we could identify the same genes in the corresponding human cancers, or we found already known genes, especially oncogenes with the same hotspots than in humans, as well as gene fusions with the same partners and the same over-expression mechanism than in humans, for lymphoma, sarcoma and glioma (Ulvé, Rault et al., 2017). We have identified such genes and their pathogenic somatic alterations for HS (TP53 and a MAPK oncogene), melanomas (over 50 genes, including PTEN, NRAS), lymphomas (26 genes, including cyclins) and gliomas (a BRAF-MBP fusion). For oral melanomas, we also have identified specific Copy Number alterations (CNA) that we showed to be significantly linked to survival.

Finally, we developed cell lines for these canine cancers (8 for HS, 10 for oral and uveal melanomas, 2 for gliomas and 1 for lymphoma), and were able to demonstrate the effect on proliferation, of drugs targeting genes coding for MAPK pathway oncogenes and cyclin genes, for HS and lymphomas respectively. We thus showed that canine cancers might be highly useful for clinical trials, as in vitro and in vivo models to screen drugs in dog/human homologous cancers, prior to test them in humans, in the frame of the treatment of the dogs and with the owner partnership and consent. Finally, to also benefit breeders, we also developed a genetic risk test for Histiocytic Sarcoma, made of 9 markers predictive of a "protective" or "at risk" haplotype and status, available for breeders to help their selection against HS in the Bernese Mountain Dog breed.

To conclude, these genetic findings bring a better understanding of the genetics and potential treatments for dog but also for human medicine. More widely, these results show the interest of the dog model to decipher the genetic bases and plan clinical trials in dogs for rare and/or aggressive-refractory human cancers.

Abstracts (Session 4, Talk 13)

Genetic investigation of oculoskeletal dysplasia in the Northern Inuit dog

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Oculoskeletal dysplasia (OSD) is a hereditary condition in dogs characterised by skeletal and ocular defects. Mutations in collagen genes, COL9A2 and COL9A3, have previously been implicated in the disease in the Samoyed and Labrador retriever breeds respectively. OSD was first observed in the Northern Inuit dog (NID) breed in 2012, with six dogs from two related litters diagnosed. Orthopaedic examinations revealed shortened long bones, hip and elbow dysplasia and osteoarthrosis. Ophthalmic examination revealed multiple ocular defects including macroglobus, cataracts, lens coloboma, retinal detachment and retinal degeneration, resulting in impaired vision. Whole genome sequencing (WGS) was carried out using DNA from two OSD-affected dogs, one of which has been and the other will be shared with the Dog Biomedical Variant Database Consortium (DBVDC). The sequences of nine candidate genes were interrogated for single nucleotide polymorphisms (SNP) and insertions/deletions (indels) by comparing WGS from OSD-affected NID with those from 26 dogs of different breeds without OSD. Twenty-five variants homozygous in both cases but absent from the controls were identified within the candidate genes, and in silico pathogenicity prediction tools excluded 24/25 variants. The remaining variant was predicted to be a nonsense SNP resulting in a premature termination codon and therefore a truncated protein product. This variant was genotyped in a total of 1,233 dogs comprising 123 NID (7 OSD affected, 116 unaffected) and 1,110 dogs of other breeds. All seven OSD-affected NID were homozygous for the mutant allele (T/T), confirming an autosomal recessive mode of inheritance. All 116 OSD-unaffected NIDs were either heterozygous (n=31) or homozygous for the wildtype allele (n=85), confirming that the variant is statistically associated with OSD (Fisher's exact P-value=1.4×10-11). All 1,110 non-NID dogs were homozygous wildtype. Analysis of the genotypes of NIDs unrelated at the grandparent level resulted in an allele frequency of approximately 8%, suggesting carrier and affection rates of 15% and 0.6% respectively. Analysis of messenger RNA from retinal tissue of an OSD-affected NID and an unaffected golden retriever revealed comparable expression levels, suggesting that the aberrant mRNA does not undergo nonsense-mediated decay. It is likely that a truncated protein product is therefore produced. A DNA test was launched by the AHT in September 2017 and 87 dogs have been tested so far, resulting in the identification of 13 carriers in addition to the 31 carriers identified through the research study.

Keywords: Canine, Inherited Disease

Abstracts (Session 4, Talk 14)

Deep phenotyping of an obesity-associated Labrador retriever POMC mutation

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Following our report of a 14 base pair deletion in the gene Proopiomelanocortin (POMC) associated with body weight and adiposity in Labrador and flatcoat retriever dogs, we have tested the effect of the mutation on canine eating behaviour. The mutation prevents production of β-MSH, a neuroactive peptide derived from the POMC protein and is predicted to disrupt signalling through the leptin-melanocortin signalling pathway, which has a well-studied role in the control of food intake.

The mutation was previously reported to affect canine behaviour in the home environment, as assessed using a validated owner-reported measure of food-related behaviour. We developed tests to measure eating behaviour in pet dogs and applied them to Labrador dogs wild type or heterozygous for the POMC mutation. Affected dogs displayed significantly greater wanting behaviour (measured using an inaccessible food task). Using a modified ad libitum meal protocol, food intake was not significantly different but affected dogs were less likely to vomit/regurgitate after eating large amounts.

The mutation appears to increase wanting which translates into greater persistence in pursuing opportunities to eat in the home. In contrast, it appears not to affect food intake when offered a large meal, suggesting β-MSH is primarily involved in governance of hunger and less important to satiety signalling.

Keywords: Canine

Abstracts (Session 5, Talk 15)

A mutation in Olfactomedin-like 3 (OLFML3) is a candidate for severe goniodysgenesis and glaucoma in Border Collies

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Goniodysgenesis is a developmental abnormality of the anterior chamber of the eye. It is generally considered to be congenital in dogs and has been associated with glaucoma and blindness. Goniodysgenesis and early-onset glaucoma emerged in the Border Collie breed in Australia in the late 1990s and has since been found in Europe and the USA. Increasing concern regarding the condition led to its inclusion in the Schedule B list of 'Conditions Under Investigation' for Border Collies in the British Veterinary Association Eye Scheme and the objective of this study was to determine its genetic basis in the breed. Clinical diagnosis was based on results of examinations by veterinary ophthalmologists of affected and unaffected dogs from eleven different countries. Reports were gleaned from a publicly available database, managed by breeders, and from test results submitted directly by owners. Genotyping using the Illumina high density canine SNP chip and whole genome sequencing were used to identify candidate genetic regions. Expression profiles and evolutionary conservation of candidate genes were assessed using public databases. Analysis of pedigree information was consistent with an autosomal recessive mode of inheritance for severe goniodysgenesis (potentially leading to glaucoma) in this breed. There was a highly significant peak of association over chromosome 17, with a p-value of 5 x 10-13. Whole genome sequences of three dogs with glaucoma, three dogs with severe goniodysgenesis and three unaffected dogs identified a missense variant in the olfactomedin-like 3 (OLFML3) gene in all six affected animals and this was homozygous in all nine cases with glaucoma and nine of 11 other animals with severe goniodysgenesis. None of 56 unaffected animals was homozygous for this variant. The identification of a candidate genetic region and putative causative mutation will inform breeding programs to reduce the frequency of goniodysgenesis and the risk of glaucoma in the Border Collie population.

Keywords: Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 5, Talk 16)

A novel locus in chromosome 5 is associated with dilated cardiomyopathy in Doberman Pinschers

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Dilated cardiomyopathy (DCM) is a breed-specific, high prevalence disorder in Doberman Pinschers. However, the genetic background of DCM and its relationship to cardiac arrhythmias remains largely unknown. To uncover the genetic risk loci and variants that are associated with DCM, we have established a cohort of over 550 Dobermans with cardiological examination, which included echography, electrocardiogram and 24-hour Holter monitoring. The cohort was genotyped using Affymetrix Axiom 2.0 arrays with ~1.2 M SNPs, and 180 cases and 180 controls that met inclusion criteria were selected for analysis. About 290k informative SNPs remained for analysis after quality control. Genome wide association analysis was performed with single-locus and mixed model approaches and multiple testing was corrected by permutation or Bonferroni correction. A novel locus in chromosome 5 was revealed with genome wide significance (praw=1.376×10-11, pBonf=4.099×10-6). This new locus is different from the previously published locus in chromosome 5. The ~1.4 Mb critical region covered by the 15 SNPs passing genome wide significance contains several genes potentially relevant for cardiac function and for DCM specifically. Ongoing whole genome sequencing analyses aim to discover the causative variant in the associated region. This study demonstrates the power of the new high-density SNP array and is expected to provide a novel DCM candidate gene, new insights to disease mechanism and tools for breeding purposes.

Keywords: Canine, Genomics and Variation, Inherited Disease

Abstracts (Session 5, Talk 17)

Association of primary open angle glaucoma ADAMTS17 mutations with height in two domestic dog breeds

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There are currently five known ADAMTS17 mutations in the dog that are associated with the development of either primary open angle glaucoma or primary lens luxation. Interestingly, these mutations have been identified in breeds of generally short stature including terriers and Basset breeds. In humans, mutations in the ADAMTS17 gene are associated with Weill-Marchesani syndrome – a disorder whose clinical characteristics include ocular manifestations such as microspherophakia, myopia, glaucoma, and cataract, in addition to brachydactyly and short stature. This led us to hypothesise that these mutations may also be associated with height in these breeds. To test this, we conducted an association analysis between breed-specific ADAMTS17 mutations and height in two of these breeds – the Petit Basset Griffon Vendeen and Shar Pei. Two hundred and twenty-seven Petit Basset Griffon Vendeen and 65 Shar Pei were genotyped for their breed-specific ADAMTS17 mutations. The height of each dog was measured at the withers. We used linear per allele regression to assess the association between ADAMTS17 mutations and height as a continuous variable, and linear regression and log-likelihood ratio tests to assess the shape of the association by comparing a general model with a linear per allele model. The mean heights of affected (n=21), carrier (n=84) and clear (n=122) Petit Basset Griffon Vendeen were 33.41 cm, 34.78 cm and 34.93 cm, respectively. The mean heights of affected (n=9), carrier (n=30) and clear (n=26) Shar Pei were 43.32 cm, 47.93 cm and 48.38 cm, respectively. Each breed-specific ADAMTS17 mutation showed a strong association with height in both breeds: Petit Basset Griffon Vendeen (P=7.9 x 10-3); Shar Pei (P=6.9 x 10-5). The shape of the associations appeared similar between the two breeds. In humans, ADAMTS17 affects skeletal development by modulating the extracellular matrix. A similar mechanism may be present in the dog. We speculate that selection for short stature might have inadvertently increased ADAMTS17 mutant allele frequencies and thus increased prevalence of primary open angle glaucoma in these breeds.

Keywords: Canine, Morphology

Poster 1

Swedish actions to "handle" Brachycephalic Obstructive Airway Syndrome (BOAS) related health issues

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The poster is reviewing Swedish actions to improve health issues related to Brachycephalic Obstructive Airway Syndrome (BOAS) and handling the increased attention and awareness of the syndrome.

These actions are

1. forming of working groups composed of various stakeholders, arranging conferences and production of educational material for breeders, judges and veterinarians (a,b).
2. reviewing the epidemiology of BOAS in Swedish dogs by breed club health surveys and insurance data and focusing on a published report in Swedish (Lagerstedt et. al 2018) on 300 dogs operated at 12 clinics in Sweden during 2014-15-16 (including information on breed, gender, age, procedures performed and outcome) Improved diagnostic criteria and follow ups are proposed.
3. initiating a Nordic inventory on the phenotypic and genotypic variation in four brachycephalic breeds – English bulldogs, French bulldogs, Pugs and Boston terriers

Aim
To investigate if there is sufficient phenotypic and genotypic variation in four brachycephalic breeds to allow selection for a change in anatomy and thereby reduce the predisposition for Brachycephalic Obstructive Airway Syndrome (BOAS)

The project is based on "Breed" -gatherings – for dogs of various background arranged for by the breed clubs and supported by the four Nordic Kennel Clubs

Material
Data collection by breed on demography: gender, age, country of origin, measures: of weight, BodyConditionScore (BCS) and conformational measurements (i.e. width of Nares (WN), craniofacial ratio (CFR), neck girth ratio (NGR), photo in standardised position (whole body and skull)and surveying general and specific health conditions by a survey to owners and a veterinary examination (clinical data including BOAS)

Cheek swabs will be collected from each dog for genomic analyses

Analyses of data is intended to compare variation within and between breeds regarding age, gender, origin and the indicated measures

Out of all dogs described and sampled, 100 individuals of each breed from each country will be selected for more extensive studies across-breed quantitative trait locus. By performing genotyping using the Illumina high density 170K SNP array of around 400 brachycephalic dogs we will estimate the genomic variation in each breed.

Poster 1 (continued)

4. Control measures by a reporting form where deaths and performed surgical procedures regarding BOAS are registered as well as an obligatory puppy health certificate to be issued at time of delivery to a new home.

5. The development of a screening procedure for evaluation of breathing capacity , thermoregulation and anatomical features relevant for breathing in adult dogs to initially be used and registered voluntarily but intended to serve in the future as a mandatory request for breeding animals.

6. international collaborations on these issues with Nordic Kennel Clubs and the veterinary organisations (WSAVA / FECAVA) are described as well as Swedish involvement in the BOAS activities within International partnership for dogs(IPFD) and DogWellNet (c,d,e)

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Poster 2

Candidate genes for feline hypertrophic cardiomyopathy: analysis of 18 sarcomeric and non-sarcomeric genes

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Hypertrophic cardiomyopathy (HCM) is a common heritable myocardial disease in cats. In humans, HCM is typically caused by mutations in cardiac sarcomere protein genes and occasionally in non-sarcomeric genes. In cats only 2 causative mutations for HCM have been identified so far; both in the sarcomeric gene MYBPC3. We hypothesised that HCM in cats is associated with mutations in the same genes as in human HCM. To investigate this, we performed targeted re-sequencing of 18 genes known to cause HCM in humans in a group of 48 cats (25 cases and 23 breed-matched controls) of 8 different breeds. Variant discovery was performed using the GATK tools for best practice. Allelic/genotypic frequencies at each single nucleotide polymorphism (SNP) locus with a likely high, moderate, or modifier functional impact were compared between cases and controls using the Chi-squared and T-tests. Using this re-sequencing data we also performed genome-wide association (GWAS) and selective sweep (Fst) analyses using the PLINK and GEMMA algorithms. A mixed model that accounted for age, weight and breed, and included the genomic relationship matrix among individuals as a random effect was implemented. Significance level was set at $P< 0.05$ and a Bonferroni correction for multiple testing was performed. More than 9,000 SNPs and insertions and deletions (INDELs) were detected in 15 out of the 18 genes. 10 SNPs located in the TNNT2, TPM1, ACTN, PDLIM3 and CSRP3 genes had a significantly higher frequency in cases compared to controls. The GWAS detected one SNP with a genome-wide significant association with HCM located within the TNNT2 gene and several others with a suggestive significant association. Fst results supported GWAS results with the highest Fst peak also spanning the TNNT2 gene. These results suggest an association of HCM with TNNT2 and other candidate genes across feline breeds. These results should be validated in a larger population.

Keywords: Feline, Genomics and Variation, Inherited Disease

Poster 3

Population structure of Leonberger dogs

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The Leonberger is a giant dog breed formed in the 1850s in Germany. This breed appears to have higher predisposition to neurodegenerative disorders and osteosarcoma than other breeds. Every second polyneuropathy-diagnosed Leonberger can be explained by dominantly inherited ARHGEF10 or GJA9 variants and a recently described recessive NAPEPLD variant identifies a juvenile-onset leukoencephalomyelopathy. Breeders also report shorter lifespan and lower fertility in Leonberger dogs. These problems, combined, imply inbreeding depression. We assessed the genetic diversity of the Leonberger population from extensive pedigree data (including more than 145,000 animals) as well as single nucleotide polymorphism (SNP) genotypes based on 170K array data of 1,175 dogs.

Pedigree analysis was done using open source software EVA v3.0. The completeness index over 5 generations of available pedigrees was above 99% for animals from the latest cohorts and exceeded 80% in 1935. We identified 22 founder animals in the population and a severe bottleneck during the 1940s with only 17 inbred dogs registered in 1946. Since the year 2000 approximately 4,400 dogs are born every year worldwide. The average litter size across cohorts was 6.5 puppies and a constant generation interval of 4 years was observed. The average inbreeding coefficient F was estimated to be 0.29 with a maxF of 0.60. The popular sire effect is apparent since a quarter of all sires produces two thirds of all offspring and the three top males sired more than 330 registered animals each. Additionally, SNP array data of 1,175 individuals sampled worldwide were investigated. These animals represent the current population of Leonberger well and therefore subpopulations were expected due to large geographic distances between breeders. However, multidimensional scaling (MDS) of pairwise genetic distances was carried out and revealed no significant clustering. Additionally, a high level of homozygosity was observed.

Despite increasing population size observed in last cohorts, considerable genetic diversity has been lost due to the bottleneck in the last century. The use of popular sires and high level of inbreeding may have facilitated undesirable genetic traits to spread rapidly within the gene pool of the Leonberger population. Maintaining the genetic diversity is possible through informed selection decisions (especially to include more animals in breeding practice, avoid the use of popular sires and aim to minimize inbreeding) which would contribute to reduce the incidence of health problems. Crossbreeding with several candidate breeds could help optimize long-term genetic diversity.

Keywords: Breed Composition, Canine, Inherited Disease

Poster 4

Cani-DNA: a French National biobank of canine samples for dog/human compared biomedical research.

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Dog represents a spontaneous model of many genetic diseases including cancers and other multifactorial diseases as well as monogenic diseases. The last 10 years, genetic tools dedicated to dogs allowed to identify the genetic causes or predisposition to canine and human homologous genetic conditions. More recently, the idea emerged that dogs can be of help for therapeutic trials, to screen and validate new drugs in homologous human cancer types. To this aim, we created the canine Cani-DNA biobank in 2003 to collect blood and tissue samples from dogs affected with genetic diseases and healthy dogs. Cani-DNA contains the native samples and extracted nucleic acids as well as clinical and genealogical data, to distribute samples with high quality controlled procedures. This resource is implemented by a French veterinarian network based on vet practices, specialized vet centres and histopathology laboratories. In 2012, a contract signed between Cani-DNA and the 4 French vet schools and the Antagene Company allowed a national organization and international visibility. Cani-DNA joined the French consortium of domesticated animals, CRB-Anim (funded by French government 2012-2020), aiming to combine genetic and reproductive resources.To date, Cani-DNA, with its primary site at CNRS Rennes and secondary sites at the fourth Vet Schools and Antagene contains almost 20 000 DNA extracted from blood and 3000 nucleic acids (DNA, RNA) extracted from tissue samples (tumoral and controls), representing 300 breeds and over 100 genetic diseases. Samples can be sent to implement Cani-DNA and can be distributed for research purposes, upon e-mail ordering (cani-dna@univ-rennes1.fr) or through : http://dog-genetics.genouest.org. We plan to facilitate exchanges and access to such samples for biomedical research with other European biobanks such as those in Bern with 40 000 samples from ~100 breeds (T. Leeb) and Helsinki with 60 000 samples from 330 breeds. (H. Lohi).

Keywords: Canine, Genomics and Variation, Inherited Disease

Poster 5

Integrated genomic and transcriptomic analyses of long non-coding RNAs in dog as a model of human melanoma

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Recent efforts have extended the dog genome annotation with the discovery of thousands of long non coding RNAs (lncRNAs) using the machine-learning based tool FEELnc [1]. Although lncRNAs have been shown to play important roles in many biological processes, and particularly in cancers [2], it remains challenging to assign functions and classify lncRNAs in order to interpret their impact on cancers and genetic diseases. Here, we integrated genomic and transcriptomic features from the extended canFam3.1-plus annotation to perform bioinformatic functional predictions of lncRNAs. We first characterized expression patterns of 10,444 canine lncRNAs in 26 distinct tissues representing various histological and anatomical localizations. We defined tissue specificity profiles of lncRNAs and deduced potential functionality and evolutionary origins through comparative genomic and transcriptomic analysis with human data from the ENCODE project (ENCyclopedia Of DNA Elements).

As in human, we show that canine lncRNAs are lower expressed than protein coding genes (mRNAs). Among the 26 tissues, we detected 4,600 tissue-specific lncRNAs. Unsupervised hierarchical clustering based on lncRNA expression levels recapitulates tissue origins and pinpoint candidate lncRNAs likely associated with specialized functions, such as nervous and integumentary clusters. Furthermore, we identified more than 900 conserved dog-human lncRNAs for which we show their overall reproducible expression patterns in both species through comparative transcriptomics. We then constructed co-expression networks and found significant correlations (| rho | > 0.5 and adjusted p-value (BH) < 0.05) for 7,615 lincRNA:mRNA and 524 antisense:mRNA pairs. These results revealed co-expressed modules that may predict regulatory relationships and/or the evolutionary origin of subsets of lncRNAs. Using functional annotations based on GO biological processes terms, we found 23 clusters significantly enriched (adjusted p-value (BH) < 0.05) corresponding to developmental processes 'sensory organ development', 'axon development' or 'hindbrain development'.

Poster 5 (continued)

We conducted a pilot study of melanoma in dogs, we performed differential expression analysis using matched tumour/control RNA-seq samples from canine buccal melanomas. We identified 930 lncRNAs with significant differential expression between tumour and control samples (FDR < 0.01). These lncRNAs represent potential biomarkers and/or candidate to study tumorigenesis of melanomas in dogs. Moreover, more than 100 of the 930 lncRNAs are conserved in human and can be used for further evaluating their therapeutic potential in both human and veterinary medicine. Altogether, this genomic and transcriptomic integrative study of lncRNAs constitutes a major resource for biomedical research in the dog species

[1] Wucher V. et al. FEELnc: a tool for long non-coding RNA annotation and its application to the dog transcriptome. Nucleic Acids Research. 2017.
[2] Gillard M. et al. Naturally occurring melanomas in dogs as models for non-UV pathways of human melanomas. Pigment Cell & Melanoma Research. 2014.

Keywords: Canine, Genomics and Variation, Inherited Disease

Poster 6

Data cleaning; is it time to stop sweeping it under the carpet? An example from the Dogslife project.

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Even with careful study design and extensive validation, large datasets are often heterogeneous and require cleaning prior to analysis to prevent losses in research validity, quality and statistical power. Many publications report that data was 'cleaned' but few studies document the process reproducibly and values identified as 'outliers' are commonly deleted without reporting the possible causes of error. Our aim was to develop a novel, automated data cleaning algorithm for growth (height and weight) that could be applied to large datasets.
Dogslife is an internet-based, longitudinal cohort study of Kennel Club registered Labrador Retrievers living in the UK, which was launched in 2010 and has over 7500 registered dogs to date. The main objective of Dogslife is to identify risk factors for canine health and disease by collecting information from owners via regular questionnaires. In addition to questionnaire data, the study has collected DNA and faecal samples from subsets of the cohort, which has produced genomic and microbiome data.

We developed our data cleaning pipeline in R software and used rule-based approaches, non-linear mixed-effects mathematical models and text analysis to identify common errors such as duplicate entries, typing, decimal point, unit, menu/option, intentional, website-generated and measurement errors. Individuals were permitted to differ from the population by making use of repeated measurements and alternative data sources. The method avoids the modification of unusual but biologically plausible values, prioritise data repair over removal and explicitly report the decision making process behind why a particular data entry is modified or deleted.
We validated our cleaning algorithm for growth variables (weight and height) on three other independent data sources from studies with fundamentally different designs; veterinary consultation Labrador Retriever weight records from the SAVSNET (Small Animal Veterinary Surveillance Network), clinical Labrador Retriever weight records from a veterinary hospital network and a publically available (via the UK Data Service) human weight and height data from CLOSER (Cohort & Longitudinal Studies Enhancement Resources) with varying proportions of artificially simulated errors. We found that our algorithm could be reproducibly applied as an effective data cleaning method on all of the validation datasets. We also compared our method to uncleaned data and six different cleaning methods and found that our algorithm out-performed these with greater accuracy and fewer unnecessary data deletions.

There is an increasing demand for data cleaning methodologies to be thoroughly reported so that they can be reproduced, tested and adapted by the wider research community. In the future, it is vital that data cleaning is considered an integral part of study design and should be considered as early as possible in order to ensure that the quality of the data is conserved. Our methods have broad applicability to longitudinal and cross-sectional growth data and we propose that they could be adapted for use in other breeds, species and fields.

Keywords: Canine, Morphology, Technical Advances

Poster 7

Give a Dog a Genome: generating a stake-holder funded bank of whole-genome sequences with which to elucidate benign and disease-associated variation within the canine genome

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The advent of whole genome sequencing (WGS) has promised to revolutionise genetic research, and the rapid fall in per-sample costs in recent years has made the revolution an affordable reality for geneticists. The technology is especially useful for the study of simple Mendelian conditions where disease-causing mutations have the potential to be identified from the WGS of a single case. However, when comparing a typical canine genome with the reference sequence (CanFam3.1) or a control genome, at least 2-3 million variants will typically be identified. Many of these variants are likely common polymorphisms which could be excluded by comparing with multiple control genomes. We devised the Give a Dog a Genome (GDG) project to build a resource of canine genetic variants across the genome using WGS; currently projected to contain 90 genomes from 78 breeds, and investigate genetic diseases in at least 69 breeds. We used a crowd-funding approach, with the costs of the project being shared between multiple stakeholders. To date (two years after GDG was launched), 74 samples from 69 breeds have been sequenced comprising 62 dogs affected with a suspected genetic condition (27 conditions in total) and 12 apparently healthy older dogs. The GDG variant bank has been used to validate several disease-associated mutations and DNA tests have been developed to improve the health and wellbeing of dogs. All of the WGS data generated through GDG will be shared with the Dog Biomedical Variant Database Consortium (DBVDC), and specific sequences will be shared with at least 20 scientists from Europe and the USA to contribute to their research.

Keywords: Canine, Genomics and Variation, Inherited Disease

Poster 8

Single nucleotide polymorphisms in the feline ACP1 gene are associated with diabetes mellitus in lean Domestic Shorthair cats

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Diabetes mellitus (DM) in cats resembles type 2 DM in humans. A genome-wide association study of DM in Domestic Shorthair (DSH) cats identified 3 single nucleotide polymorphisms (SNPs) located at the end of chromosome A3. The ACP1 gene, coding for a protein tyrosine phosphatase involved in insulin signalling, is in close proximity to these SNPs and was selected for further investigation as a candidate diabetes susceptibility gene.

The coding sequence for the ACP1 gene was identified in the cat genome assembly and Softberry ® software was used for promoter prediction. Gene-specific primers were designed to sequence the 6 exons and putative promoter using Primer-BLAST. Genomic DNA from well-phenotyped diabetic DSH cats (≥3 months on insulin, IGF-112 years, normoglycaemic) extracted from EDTA-blood was used for PCR and subsequent Sanger sequencing. Allele frequencies for identified SNPs were compared between diabetics and controls (Chi-square; significance pC; ACP1:c.-344C>T; ACP1:c.-378G>A; ACP1:c.-420G>C; ACP1:c.-4452G>C; ACP1:c.-693T>G). A case-control study (15 diabetics, 30 controls) revealed that the A-allele of c.-227A>C (p=0.00007), G-allele of c.-378G>A (p=0.00001), G-allele of c.-420G>C (p=0.02) and G-allele of c.-452G>C (p=0.0001) were significantly associated with DM. 9/15 diabetics and 6/30 controls were homozygous for the DM-associated haplotype (p=0.007).

Given the interaction of ACP1 with the insulin receptor, the potential functional impact of SNPs within the putative promoter of the ACP1 gene warrants further investigation.

Keywords: Feline, Genomics and Variation, Inherited Disease

Poster 9

Regions associated with canine noise sensitivity and fear overlap human neuropsychiatric loci

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Introduction: Fear is an evolutionarily important emotional state that is essential for the survival of an individual. However, extreme fearfulness can cause several behavioural problems. Dogs suffer from various naturally occurring breed-specific anxieties, such as generalized anxiety disorders, different phobias, and separation anxiety. In order to better understand the genetics of fearful behaviour in dogs, we aimed to find new loci related to anxiety and fear in German Shepherds. Materials and Methods: A total of 330 German Shepherd dogs were phenotyped for two traits, noise sensitivity (NS) and fear towards novel humans and situations (fear) using our validated behavioral survey. For each dog, a quantitative score describing the severity of the phenotype was calculated. The scores for NS ranged from 0 to 60 and the scores for fear from 0 to 13.5, with 0 indicating a control in both cohorts. The dogs were genotyped using Illumina's canine HD SNP arrays and analysed for phenotype-genotype associations using both case-control (PLINK) and quantitative (GenABEL) analysis approaches.

Results: Genomic regions on CFA 20 and CFA 7 were significantly associated with NS and fear, respectively. The NS locus includes several known anxiety and hearing-related genes, such as GRM7 and OXTR that encode glutamate and oxytocin receptor genes. The fear locus was syntenic to a locus on human 18p11 that has been linked to psychiatric illnesses and includes several interesting candidate genes.

Conclusions: The findings revealed two new fear and anxiety loci in dogs. Both loci include several relevant candidate genes and regions that have been associated with human anxieties and neuropsychiatric disorders. Further replication studies and investigation of the causative variants within the loci have a potential to shed light on the biological basis of fear and anxiety in both species.

Keywords: Behaviour, Canine

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Dinner at The Rowantree

History

The Rowantree was originally called Lucky Middlesmass's Tavern and is celebrated in Robert Ferguson's much loved poem , "Cauler Oysters". It was the watering hole for the all the key figures in the Scottish Enlightenment, Rabbie Burns, David Hume, Adam Smith, Deacon Brodie, with visits from James Watt and Benjamin Franklin.

Getting There

The Rowantree
12 Niddry Street South
Edinburgh
EH1 1NS
t: +44 (0) 131 510 6969

Please plan to arrive at The Rowantree shortly after 7pm.

The Rowantree is easily reached by foot. It is a 24 minute walk (1.2 miles) from the conference centre.

Numerous northbound Lothian Buses including the 14, 30, 33, and X33 service Dalkeith Road, the stretch of road between South Halls ("Royal Common Wealth Pool" stop) and the restaurant. Be sure to exit the bus at the "South Bridge" stop and walk to Cowgate. Cowgate passes underneath the bridge. A oneway bus fare will cost £1.70 (exact change required). Just ask the driver for an "adult single".

Use this link to view the route: https://goo.gl/maps/bByGJEMY4Ym

Of course taxis and car services (Gett and Uber) are available.

If you need assistance with your travel, please ask the organisers for help.

Transport

Edinburgh International Airport
Edinburgh Airport is located on the outskirts of the city but is well served by taxi, bus and tram services to the city centre. The journey time by car to the city centre is around 20-25 minutes unless during rush hour (07:30-09:00 and 16:00 – 18:00) when it may take an additional 15 minutes. A taxi will cost approximately £25. Alternatively, there is a regular tram and bus service from the airport to the city centre. The cost for a single journey on the tram is £5.50 and a return journey is £8.50. The cost for a single journey on the bus is £4.50 and a return journey is £7.50 and can be bought at the airport information desk, at the bus stop, from the driver or online here.

Edinburgh Waverley/Haymarket Stations
Both of Edinburgh's main rail stations, Waverley and Haymarket are located in the heart of Edinburgh's City Centre. Edinburgh Waverley (main station) is situated at the east end of Princes Street, and is a 30 minute walk from the conference venue. A taxi from Waverley will cost approximately £7 and will take approximately 10 minutes depending on traffic. The rail network connects to cities all over Scotland and the UK.

For more information visit: Edinburgh Rail Services:
www.edinburghairport.com/transport-links/trains

Edinburgh Bus Terminal
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Notes

Notes

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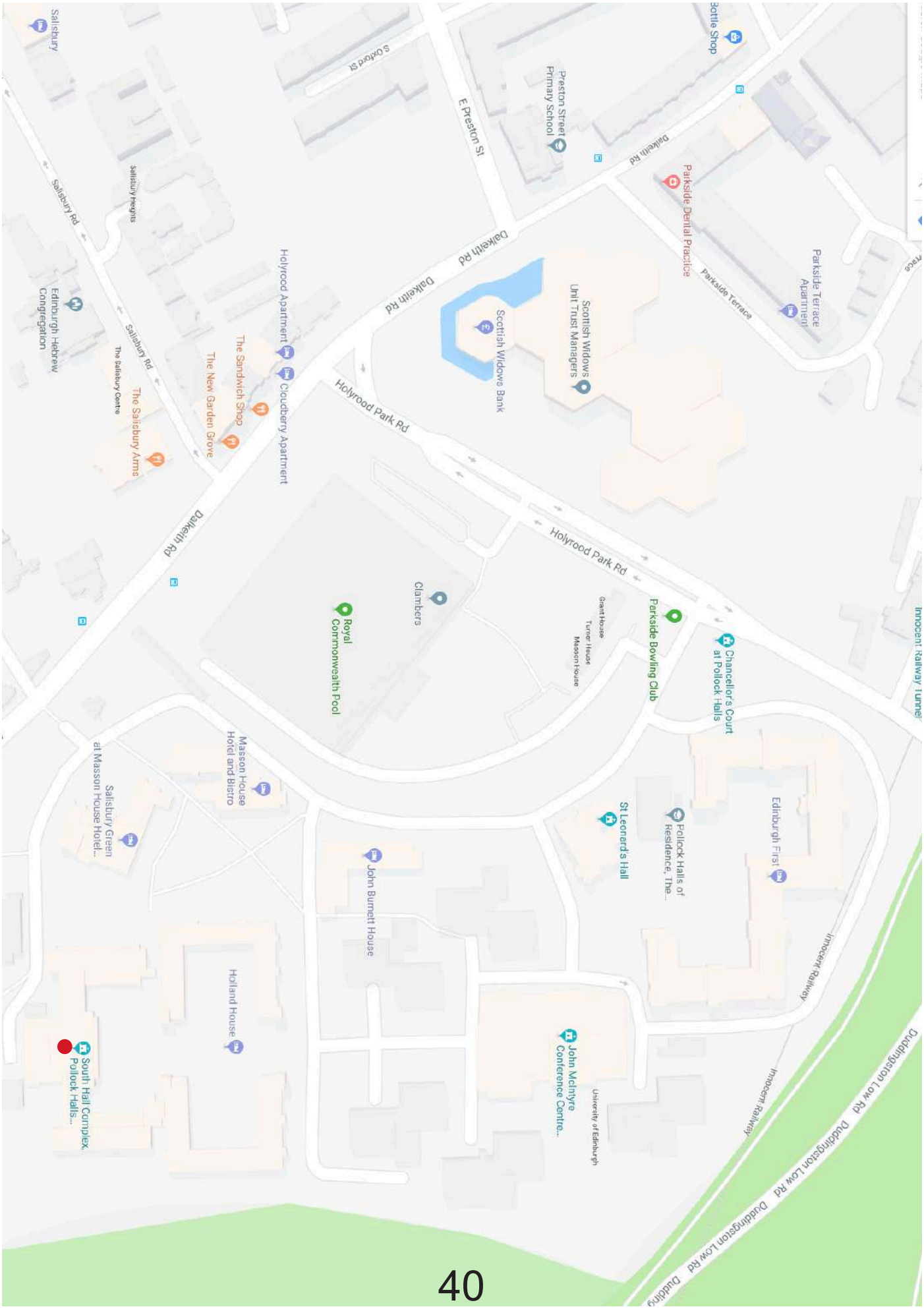

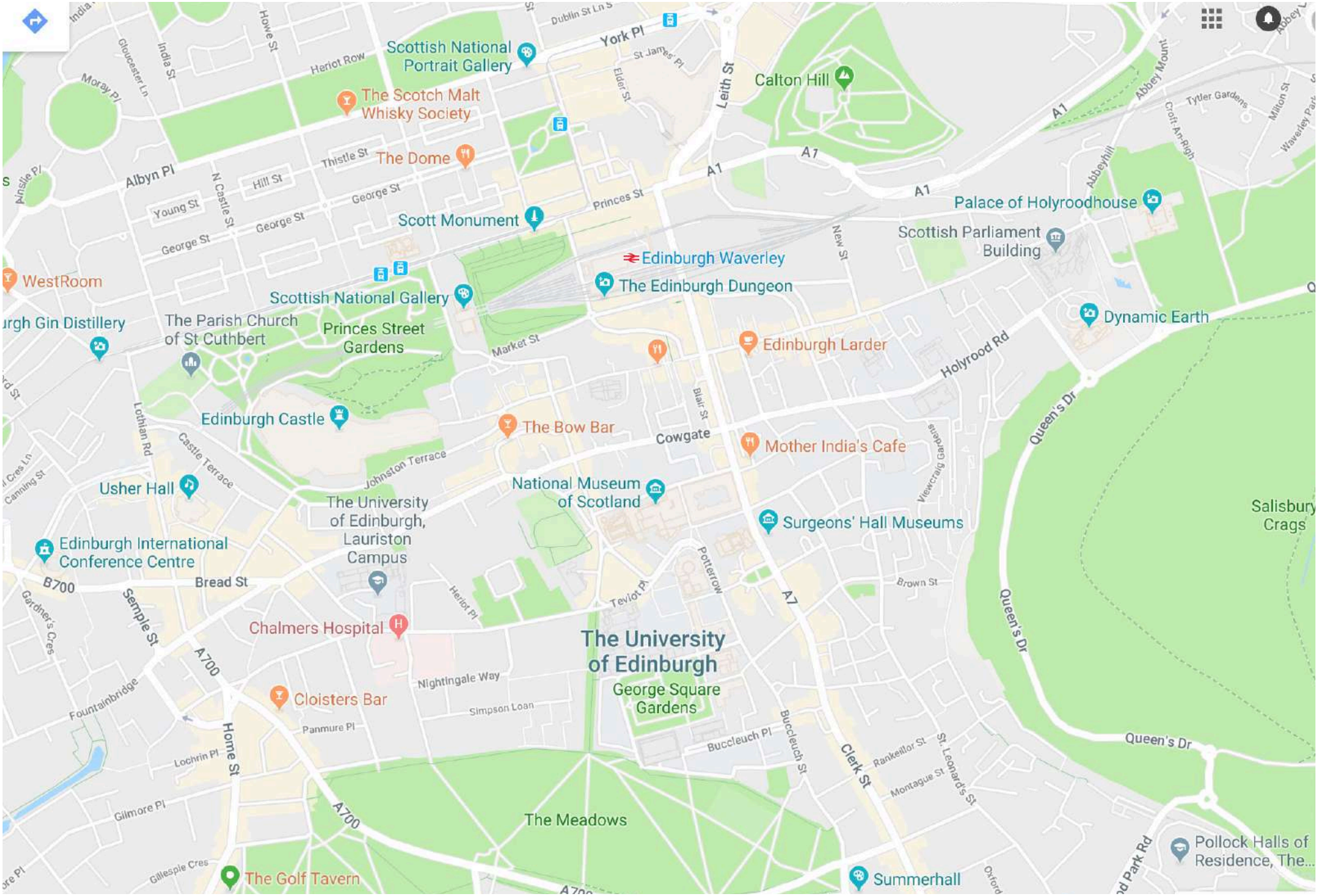
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