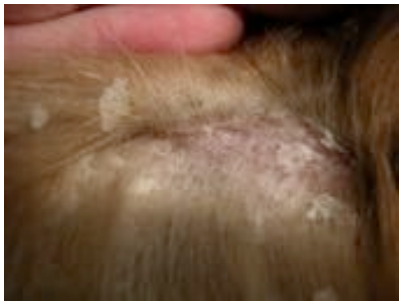
Modèles canins spontanés de maladies rares et cancers




Catherine André « Canine genetics » team

Institute of Genetics and Developpment, CNRS / University of Rennes1, France

J. Plassais, A. Grall, L. Lagoutte, N. Botherel, A.S Guillory, M. Bunel, P. Quignon,

M. Gillard, E. Cadieu, C. De Brito, T. Derrien, C. Hitte, B. Hedan

Veterinarian Collaborators

E. Guaguere, F. Degorce-Rubiales, G. Chaudieu, M. Delverdier

Jérome Abadie, P. Devauchelle, D. Lanore, M. Lagadic ….

"Human"Collaborators

E. Bourrat, J. Fischer, I. Hausser,

B. Vergier, F. Demenais, E. Maubec, T. Lesimple, M.D. Galibert, …

THE « CANINE GENETICS » TEAM

geneticists, bioinformaticians and veterinarians

Cancer genetics

Cani-DNA Biobank

Rare genetic diseases

Bioinformatics / statistics

Catherine.andre@univ-rennes1.fr,
benoit.hedan@univ-rennes1.fr,
cani-dna@univ-rennes1.fr

http://dogs.genouest.org/

The canine species:

400 breeds

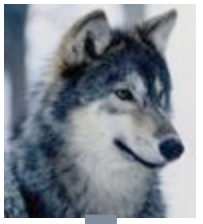
- Important variability between breeds
- Important homogeneity inside breeds

} **BREED = Genetic Isolate**

Breeding practices :

- Drastic selections
- Inbreeding
- Champions

} Co-selection of mutations Standards ⇨ **High incidence of genetic diseases**



- 15 000 years

Dog as a model for genetic diseases

Genetic diseases in dogs are:

- Spontaneously occurring
- Naturally progressing (over 10 years)
- Breed specific
- High incidences (few % to 20%)
- Homologous to human diseases
- Dogs share our environment
- Dogs have a similar physiology
- Medical attention (diagnosis)

⇨ **Dog = patient**

Objectives:

To identify predisposing genes and progression mechanisms for veterinary medicine and as natural models for the medical community

- **Identify new genes, pathways → prognostic factors, therapeutic targets**

or

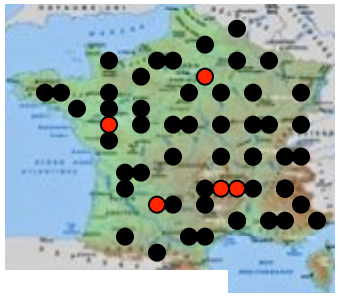
- **Demonstrate same pathways → preclinical studies, new therapies**

Specific supports : canine sample BioBank L. Lagoutte

Unique initiative in France : National Biobank

Cani-DNA

Vet network & Cani-DNA biobank



Collection of Blood and Tissues samples

12 000 canine DNA
2000 tissues (RNA)
300 breeds
Healthy / affected
100 genetic diseases

5000 Practicians, Antagene,
the 4 Vet Schools, Cancer centres,
Vet histology & biology labs,

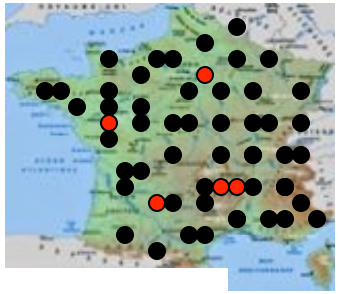
On the way :
Quality procedure ISO 9001
Human CRB database

- **2003 :** Creation and development
- **2007 :** Collaboration with Antagene
- **2010 :** Collaboration with the 4 Vet Schools : funding IBiSA, INCa
- **2012 :** Contracts CNRS with the 4 Vet Schools + Antagene
- **2013 : project « CRB-Anim » : organisation and ANR funding PIA1**

cani-dna@univ-rennes1.fr

Dedicated dog genetics tools: canine sample BioBank

Vet network & Cani-DNA biobank



Collection of
Blood and
Tissues samples

CaniDNA BioBank :
12 000 canine DNA
2000 tissues (RNA)
300 breeds
Healthy / affected
100 genetic homologous diseases
Quality procedure NF-96900

Practicians, the 4 Vet Schools
Vet histology & biology labs,
Cancer centres, Antagene,

Lagoutte et al., in prep

Database of Disease models :

Melanoma
Histiocytic sarcoma
Lymphoma
Osteosarcoma
Glioma

Cani-DNA

cani-dna@univ-rennes1.fr

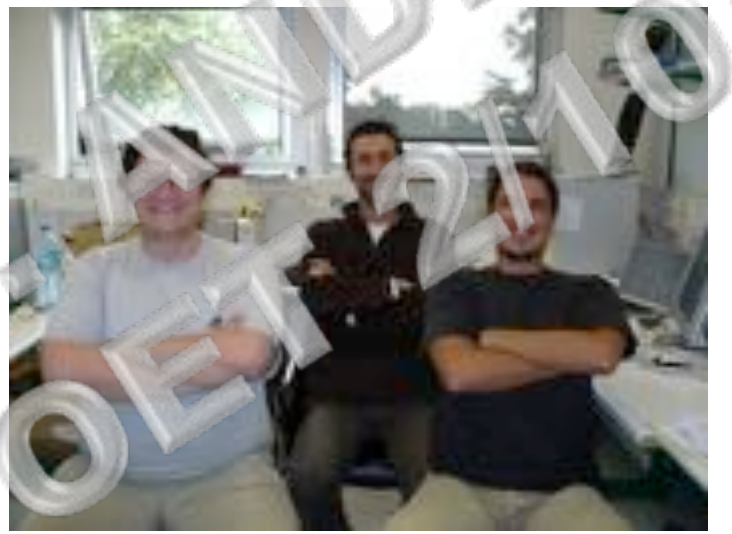
Genome analyses and bio-informatics

Christophe Hitte, Amaury Vaysse, Thomas Derrien,

Specialists of the canine genome

Identification of the CNV catalogue
(Nimbelgen array 2 million probes, 17 breeds)
(Berglund et al., 2012)

Genome re-annotation
(Derrien et al., 2007, 2008, 2010)



Genome evolution

Search for signatures of selection:
(Vaysse et al., Plos Genet. 2011)

Development of NGS analysis tools:
- Variants, fusions, expression levels for lab projects
(melanome, lymphome, sarcome, neuro)

Development of tools (FEELnc) **to annotate the Long non coding RNAs from RNAseq data :**
-> 17,000 transcript lncRNA
- -> search of correlation betweenlncRNAs and mRNA expression : functional relevance

Srategy and state of the genetic diseases projects

Work flow:

Sample collection → Clinical Histological characterization → Molecular characterization

Molecular characterization →
- **Pedigree analysis :** Transmission mode
- **Genetic studies :** Pedigree / Association → **Gene Identif. Gene function**
- **Gene expression :** Transcriptome/RNAseq

Homologies with human

Genodermatoses:

Ichthyoses In the Golden ret

Guaguere et al., 2009; Grall et al. Nature genetics, 2012 → **1 Novel gene / 1 mutation**

PNPLA1 gene : new ichthyosis gene, new function, patent + genetic test

Keratoderma In Dogues de Bx

Plassais et al., Journal of investigative Dermatology, 2014 → **1 Gene / 1 mutation**

KRT16 gene : rare human keratoderma gene, + genetic test

Neurosensoriel:

Sensitive neuropathy In hunting dog breeds

Plassais et al., in prep . → **GWAS, NGS locus sequencing One variant in a regulatory region (900 dogs)**

Exciting mechanism; new gene + genetic test

Retinopathy in Border collies

Bunel et al., in prep. → **Xlinked, WG Ssequencing of 2 dogs Analysis ongoing**

Genetic research projets

Cancers

Poster 4

Poster 3

Histiocytic sarcoma

Melanoma

Ostéosarcoma

Photo : E. Pambou

Dermatology :

Keratodermia,

Neuro-sensoriel

Epilepsy

Ichthyosis

Neuropathie périphérique

Rétinopathies

Developmental defects

Collaborations between researchers, vets, clinicians, breeders …. to analyse models of choice for human and canine medicine

Ichthyosis dog-story

In Humans,: numerous ichthyoses
Each form = rare disease


Epidermolytic Ichthyosis

KRT10 (Credille et al., 2005)

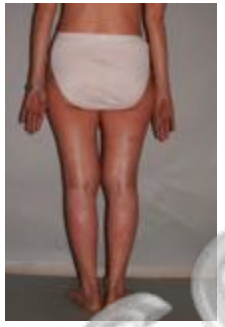
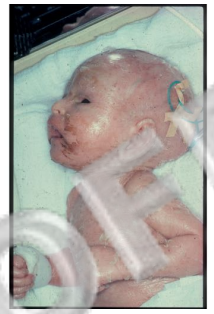

Non epidermolytic Ichthyosis

TGM1 (Credille et al., 2009)

?

Syndromic Ichthyosis

FAM83H (Forman et al., 2012)

ARCI

Non syndromic Ichthyosis

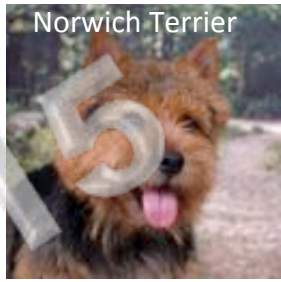
PNPLA1 (Grall et al., Nat.Genet. 2012)

Non syndromic, autosomal recessive congenital ichthyoses

Photos E. Bourrat

In dogs: several ichthyoses
Each form affect a given breed

Norwich Terrier



Jack Russel Terrier

King Charles Cavalier

Golden retriever

⋮

Clinical characteristics of ichthyoses in dogs and humans

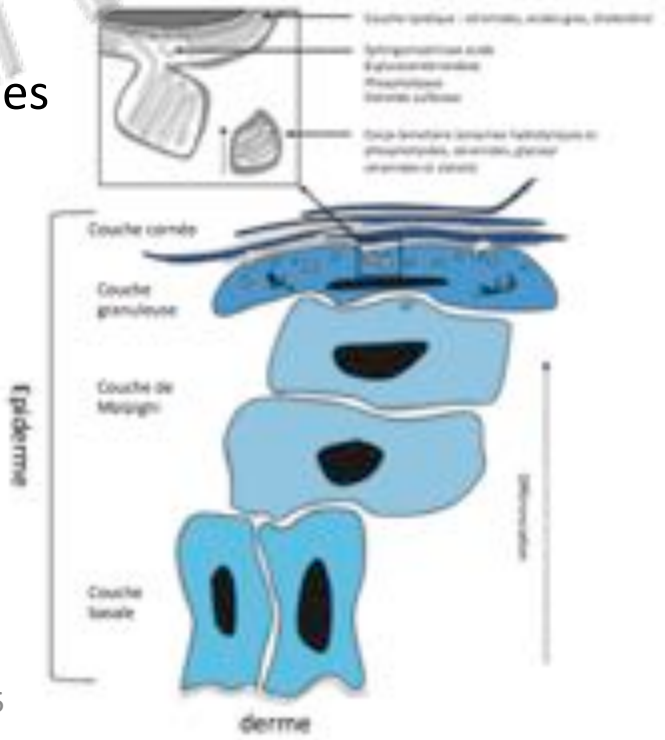
Coll E. Guaguere, F. Degorce, J.Fischer, Friburg and I. Hausser, Heidelberg, Germany

- Genodermatosis affecting epidermis differentiation
- Leads to abnormal desquamation of the whole body
- Numerous forms described in humans and dogs (classical mild forms ➔ severe forms)

Photos C. André

Photos from Oji et al., 2010

Scales

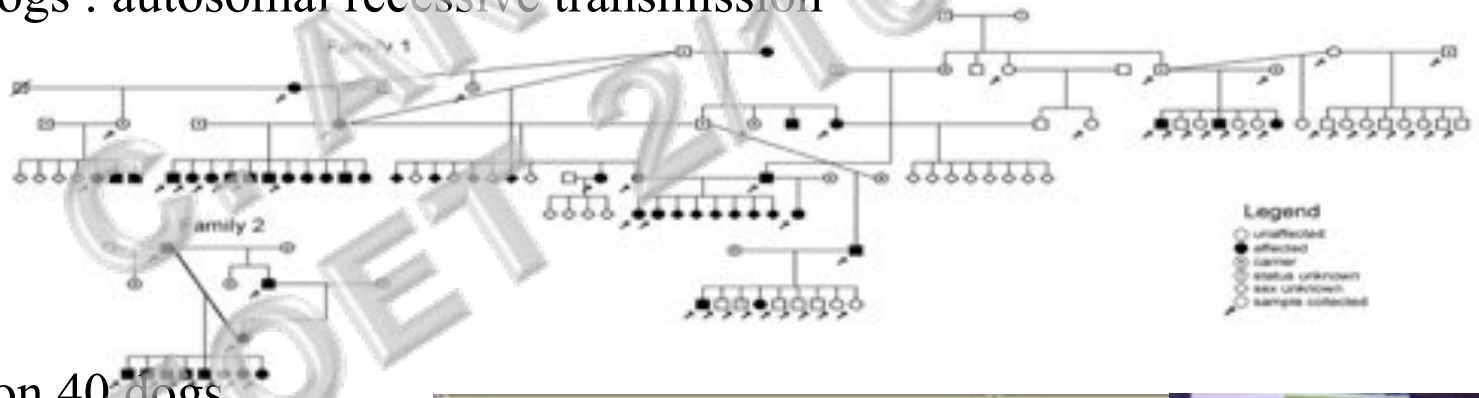


Ichthyosis in the Golden Retriever

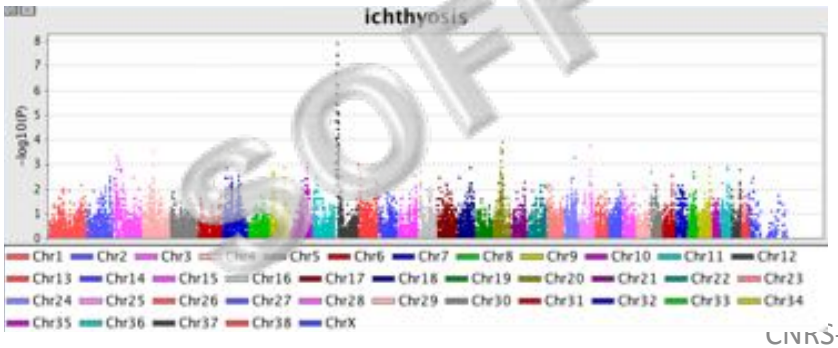
Clinical and histological data on 150 dogs



Pedigree of 150 dogs : autosomal recessive transmission



Genetic analysis on 40 dogs

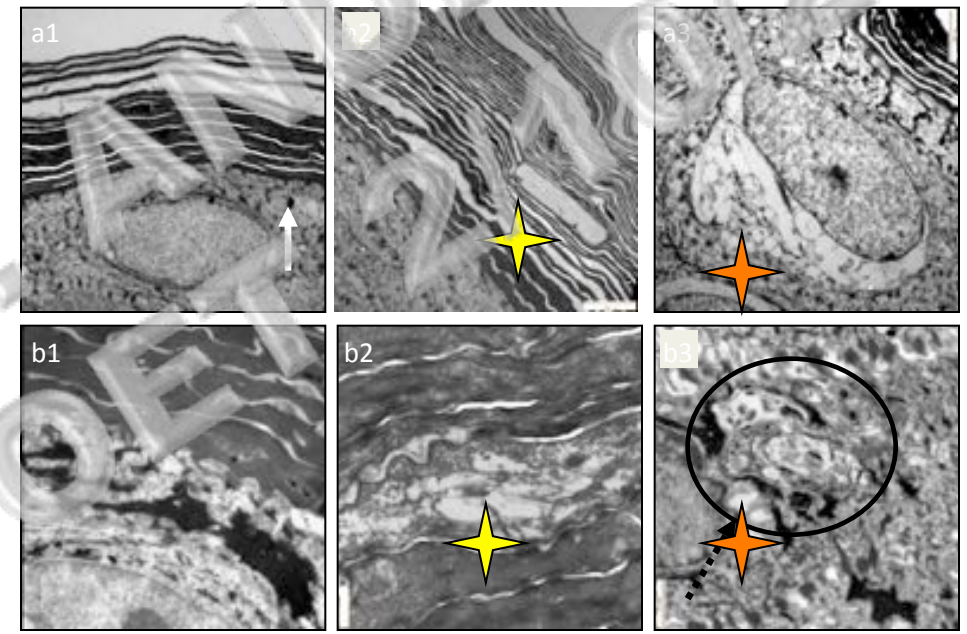


Functional Data

Coll J.Fischer, Friburg and I. Hausser, Heidelberg, Germany

-Electron microscopy in human samples
cholesterol cristals and abnormal membranes found in dogs and humans

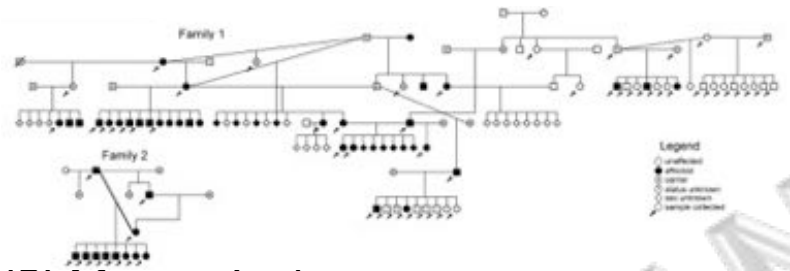
Unaffected Affected



→Similar genetic causes and functional consequences :
Impairment in membrane formation in both dogs and humans

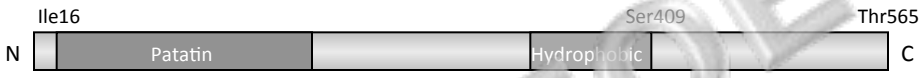
Ichthyosis in the Golden retriever breeds

Coll E. Guaguere, France

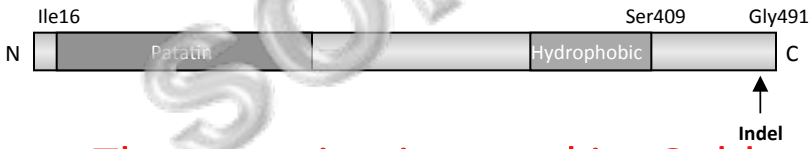


PNPLA1 gene in dog

Normal protein in dog



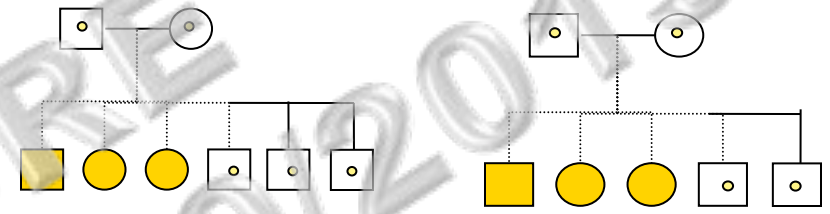
Mutated protein (74 amino acid shorter)



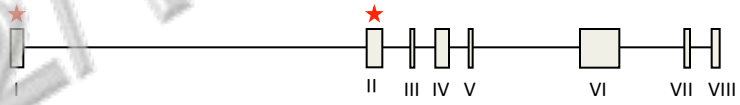
---> The mutation is causal in Golden retrievers and in Humans

Ichthyosis in 2 Human families

Coll J.Fischer, Freiburg Germany

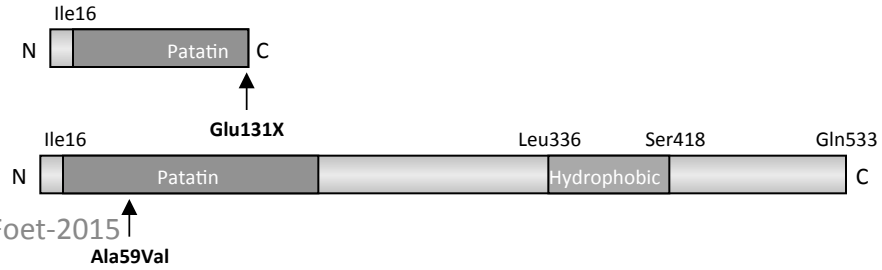


PNPLA1 gene in human



Normal protein in Family 1 and 2

Mutated proteins in Family 1 and 2

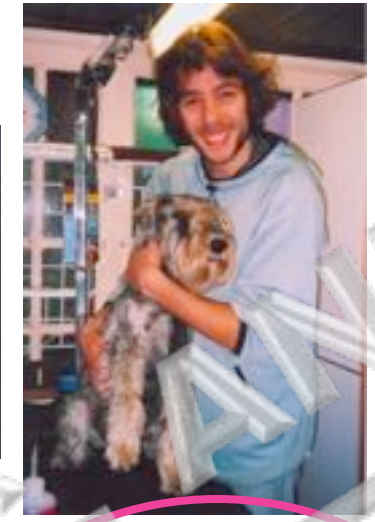


Genetic research projets

Cancers

Histiocytic sarcoma

Mélanoma



Ostéosarcoma

Photo : E. Pambou

Neuro-sensoriel

Epilepsy

Dermatology : Keratodermia, Ichthyosis

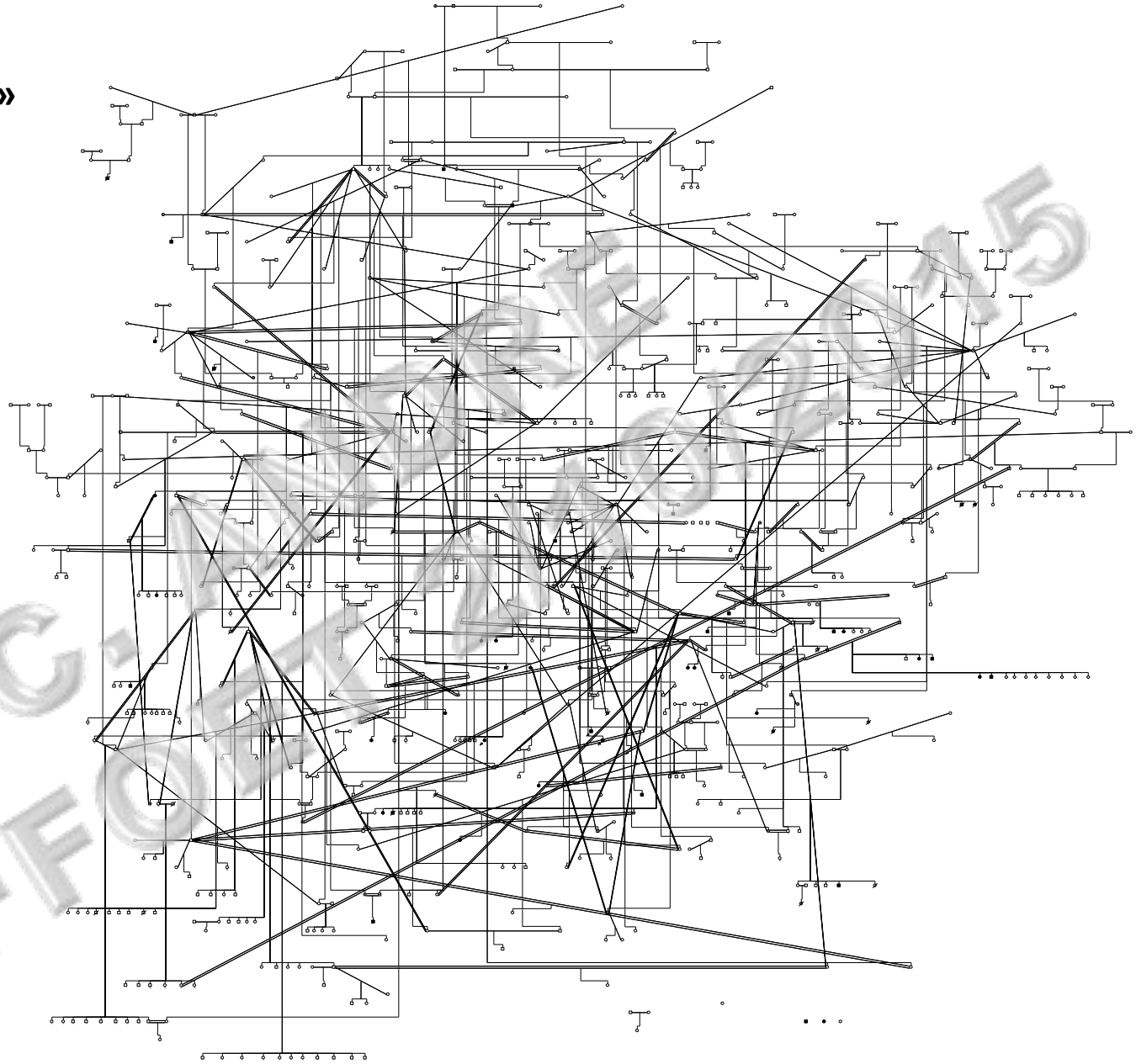
Acral Mutilation

Rétinopathies

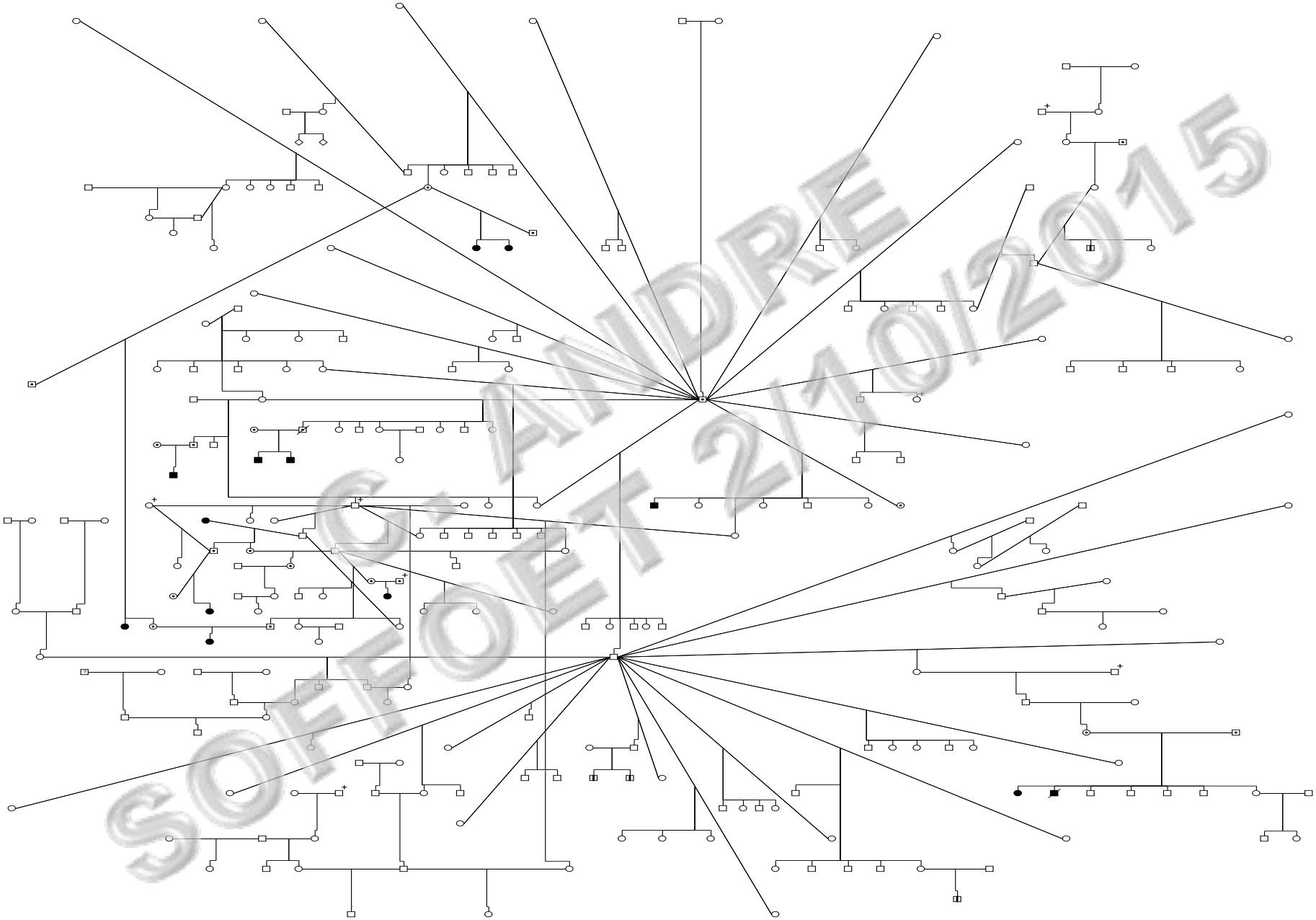
Developmental defects

Collaborations between researchers, vets, clinicians, breeders …. to analyse models of choice for human and canine medicine

The « Breed pedigree »
With over 1000
Dogues de bordeaux



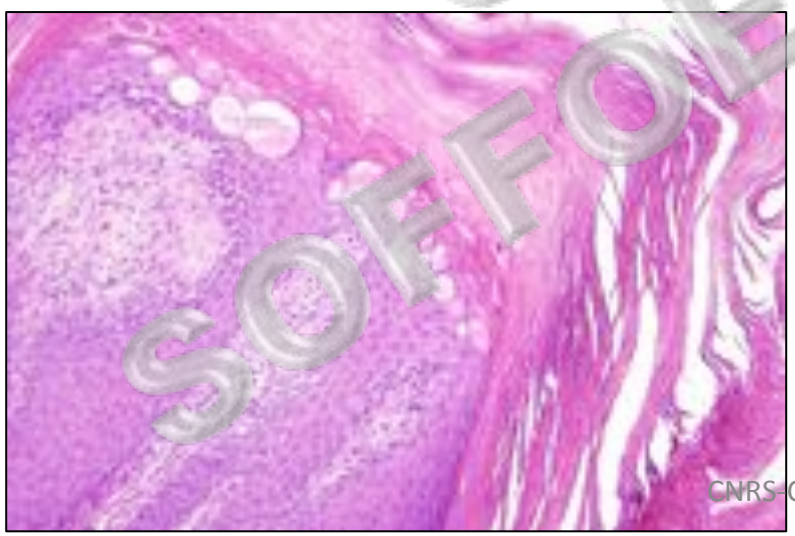
Rre-construct a more analysable pedigree



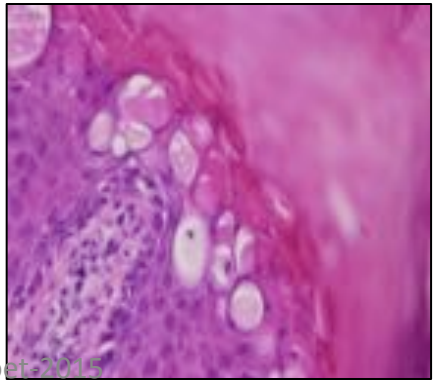
Photos: Club AFBS, E. Guaguere, X. Langon

Histological findings: F. Degorce (LAPVSO Toulouse)

Diffuse and papillary orthokératosticHyperkeratosis

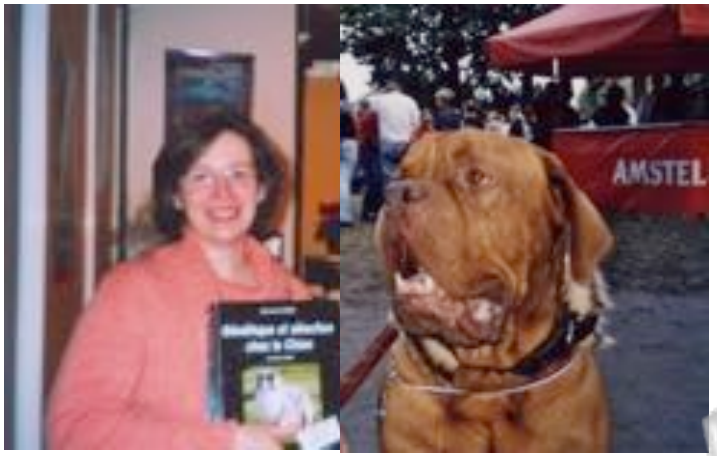


Vacuolisation of superfical keratinocytes



Keratoderma in Dogue de Bordeaux

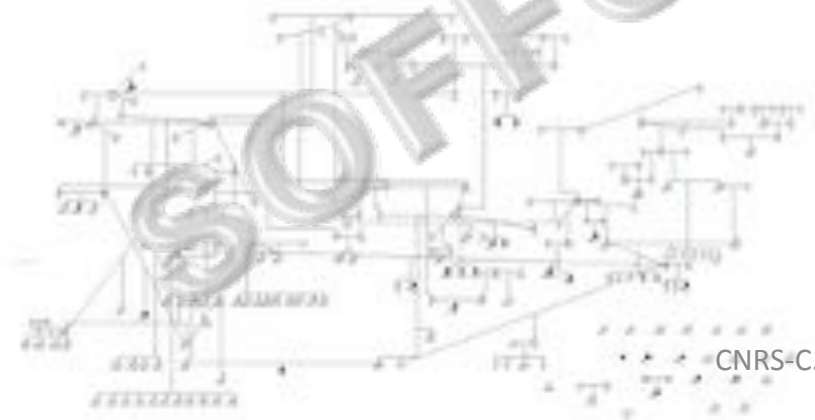
(Coll. E. Guaguere, E. Bensignor, F. Degorce, A. Thomas, E. Bourrat)

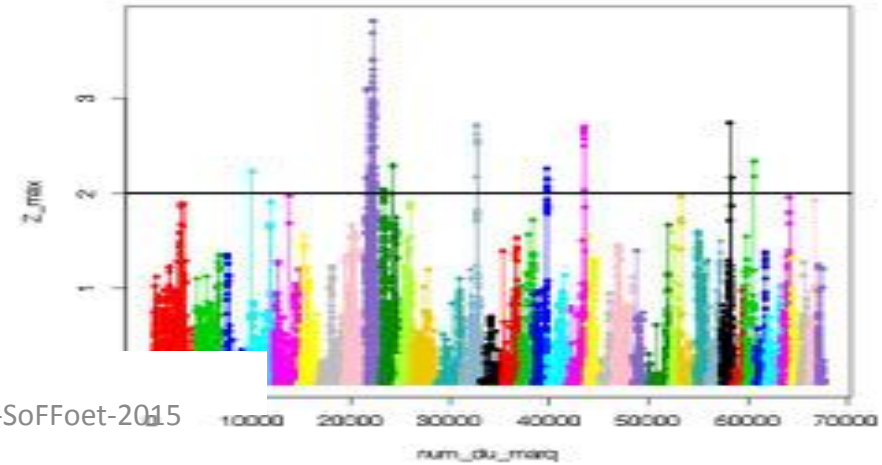


Photos of E. Guaguere

Photos of X. Langon

1. Collection of 300 blood samples + 15 tissus in RNAlater (2005-now !)
2. Genetic analysis : Family of 200 Dogues → autosomal recessive
2. GWAS and genetic linkage analyses on 20 affected + 30 healthy
4. Identification of a locus on the Keratin cluster of chromosome 9 (HSA17)





Locus of 20 Mbases on CFA9

Sequencing of candidates keratines

Identification of a complex mutation on canine chromosome 9 : Keratine 16

Screening of 350 Dogues de Bordeaux :

-28 affected (28 homozygous mutated)

-322 unaffected: 68 (hétérozygous)

254 healthy (homozygotes sains)

Sreening of dogs from other breeds (Boxer, bouledogue,…) 400 dogs

- the mtation was never found

This mutation is specific of the disease and of the Dogues de Bordeaux

Monogenique Recessive Keratoderma

Keratoderma in Dogue de Bordeaux

Plassais et al., JID Dec 2014

→ IDENTIFICATION OF THE CAUSAL GENE AND MUTATION ! KERATINE 16

A Keratin mutation, responsible in humans of a rare keratoderma : Pachyonita congenita

D del

Photos
E. Bourrat

Tests génétiques : HFH-B

Hereditary Footpad hyperkeratosis-B

ANTAGENE

In Dogues de Bordeaux

DNA extraction

Genetic test to detect the mutation

Keratoderma dog-story

In Humans,: numerous keratoderma
Each form is a rare disease

In dogs: several keratoderma
Each form affect a given breed

Labrador retriever

Hereditary nasal Parakertosis

SUV39H2 gene (Jagannathan et al. 2013)

?

Human keratoderma ?

Irish terrier

Hereditary Footpad hyperkeratosis

FAM83G (Drögemüller *et al*. 2014)

?

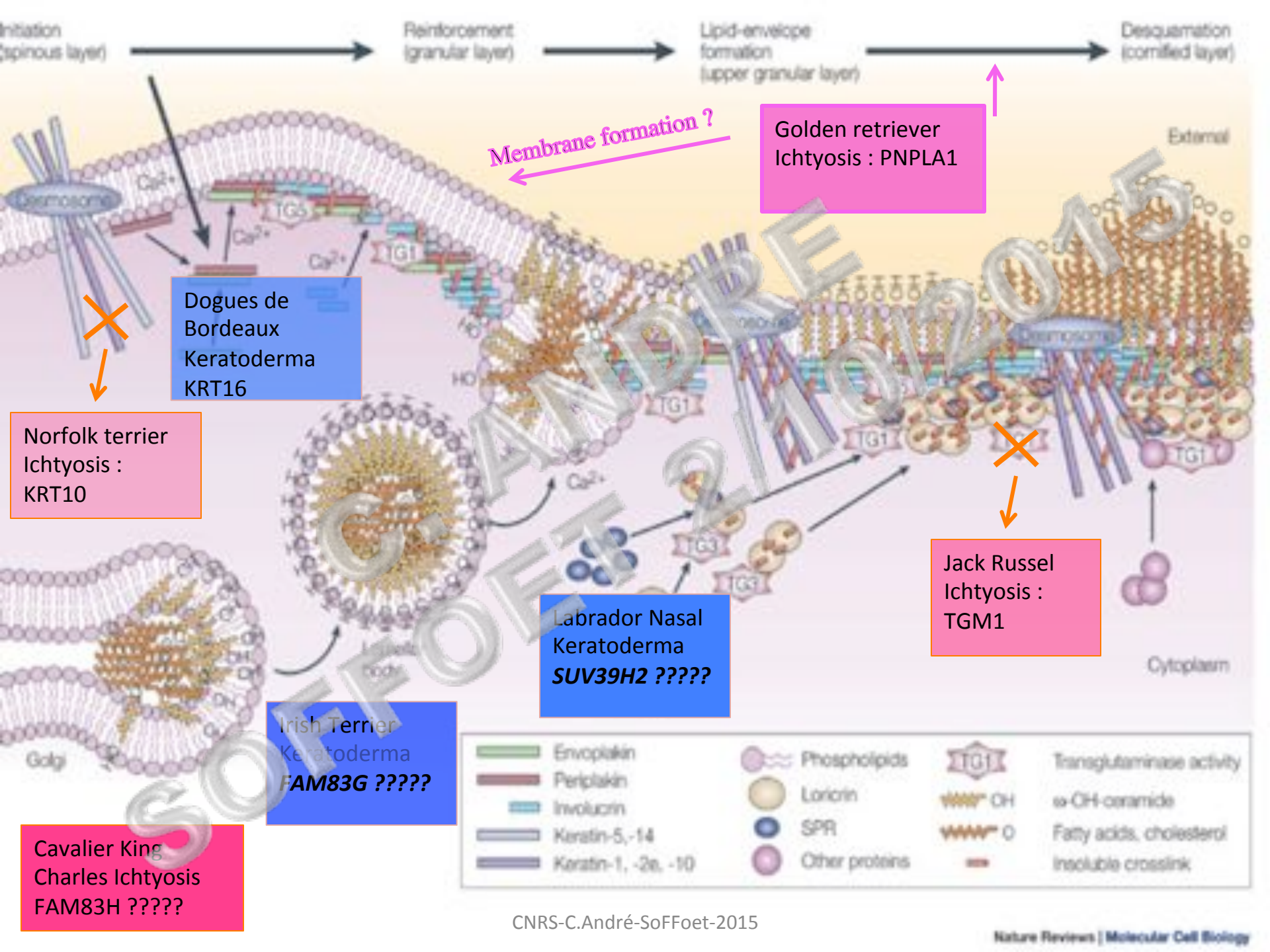
Human FNEPPK keratoderma:
Focal Non Epidermolytic
Palmo Plantar Keratoderma

Dogue de Bordeaux

Hereditary Foodpad Hyperkeratosis

(Plassais et al., JID, 2015)

...



Strategy for Cancer genetic projects

Work Flow : translational medicine

Sample collection → Clinical Histological characterization → Molecular characterization

- **Genetic studies :** Pedigree / Association
- **Gene expression :** Transcriptome/RNAseq → **Gene identif. Gene function**
- **Chromos. alterations:** CGH, CNV

Transfer to Human Translational research

Homologies with Human cancers

Cancers :

Melanoma

Gillard et al., 2014

500 DNA samples from affected, + controls → GWAS
100 matched tumour/healthy tissues → 30 RNAseq

→ 2 predisposing Loci (oral mel)
+ Expression profiles (RNAseq))
+ Somatic alterations

Histiocytic sarcoma

Abadie, Hédan et al., 2009; Hédan et al., 2011;
Shearin, Hedan et al.,2012

1200 DNA samples, 300 affected → Linkage + GWAS
500 matched tumour/healthy tissues → 20 RNAseq

→ 3 major predisposing Loci
+ Expression profiles and
Somatic alterations (RNAseq)
+ CGH analyses

Lmphoma

→ Sampling and Analyses ongoing

Glioma

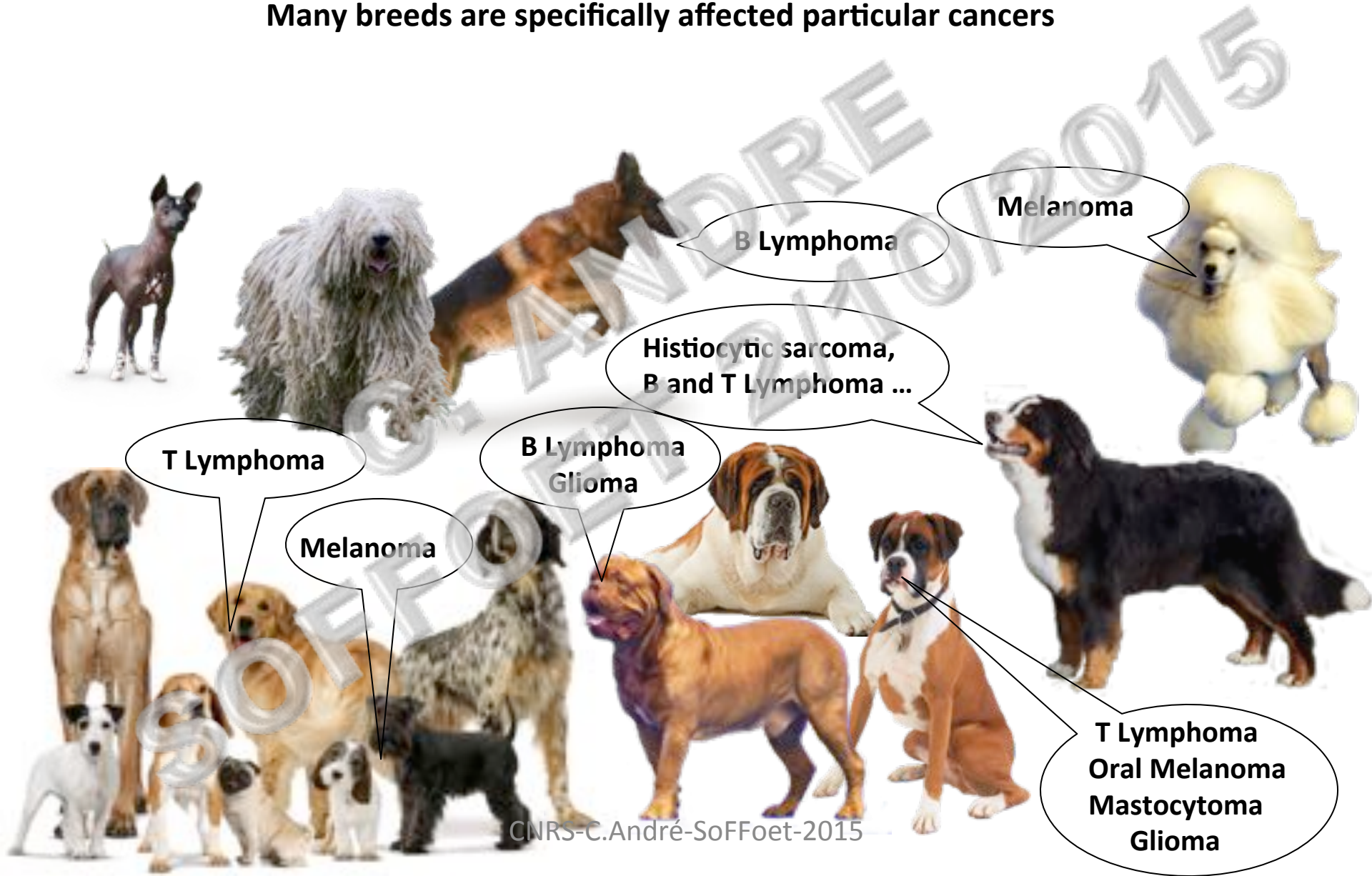
200 DNA samples from affected, + controls → GWAS
50 tumour tissues → 10 RNAseq

Osteosarcoma

→ Sampling cases/controls and familly + characterization ongoing

Naturally occuring cancers in Dog breeds

Many breeds are specifically affected particular cancers



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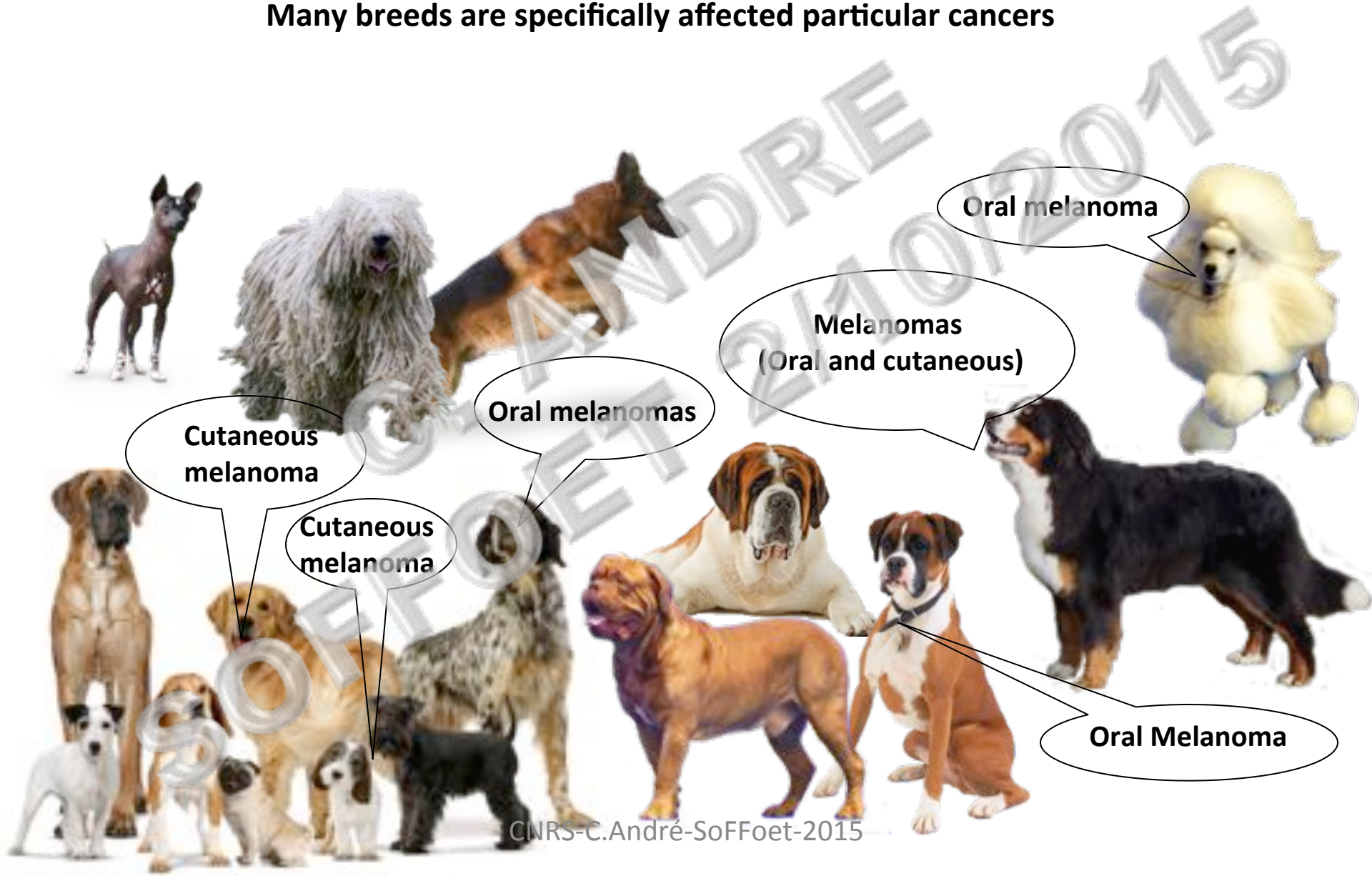


ESPCR 2015

M. Gillard, PhD

I. Characterization of canine melanomas

- 2350 canine melanoma : characterization ; epidemiology

II. Comparison with Human melanomas

- Sample Collection : 500 cases (from ~10 breeds)

-> 300 cases : Comparative histology + Clinics

(In collaboration with B. Vergier, CHU Bordeaux
and J. Abadie, Oniris, Vet School Nantes)

E. Cadieu, Post Doc

C. de Brito, Vet

III. Genetic approaches

- Predisposing mutations – GWAS (70 cases + 100 controls on oral melanomas)
(In collaboration with F. Demenais INSERM-Paris)

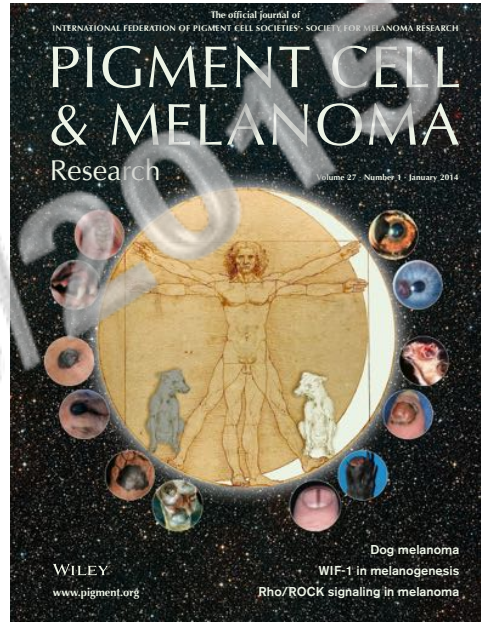
- Gene expression – RNAseq (DE, gene fusions, somatic variations on 20 cases)

-Landscape of somatic alterations : exome-sequencing of 75 cases

(In collaboration with K. Lindblad-Toh, Upsalla University, Sweeden/Broad Institute, Boston, US)

ESPCR 2015

Gillard et al., PCMR 2014



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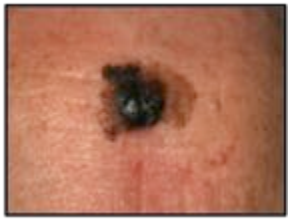
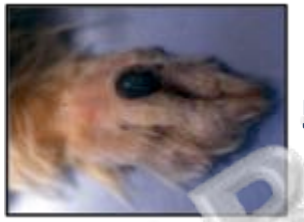
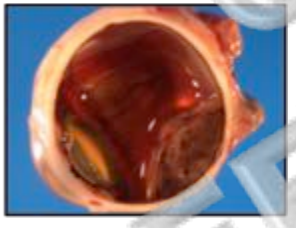
Characterization of canine melanomas

	HUMAN		DOG			
Localization		Frequency		Frequency	Severity	
Cutaneous		90%		52%	++	 Rottweilers Schnauzer
Oral		1-5%		45%	+++	 Poodle Labrador Golden Retriever
Ocular		1-5%		3%	+	 Labrador

→ High risk breeds ---> genetic predispositions

Ramos-Vara et al., 2000
Smith et al., 2002
Bergman, 2007

Comparative histology :

Double blind analysis of **150 melanoma cases**

B. Vergier (CHU Bordeaux) ; J. Abadie (Vet School of Nantes)

- **Most canine melanoma are intra-dermal:**

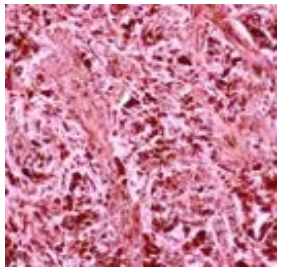
Comparative histology :

Double blind analysis of **150 melanoma cases**

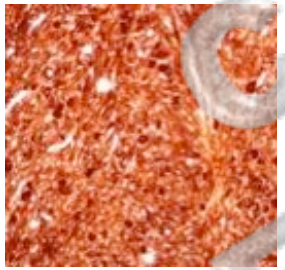
B. Vergier (CHU Bordeaux) ; J. Abadie (Vet School of Nantes)

- **Most canine melanoma are intra-dermal:**
- **Three major morphological types corresponding to Human melanoma types**

« Animal type »

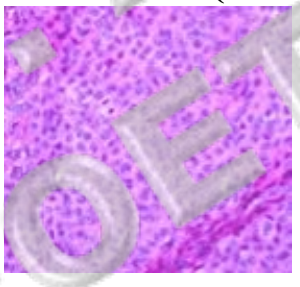


Human

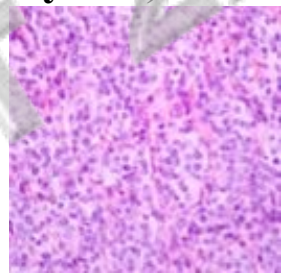


(Ringo)

« Congenital type » (nevocytoid)

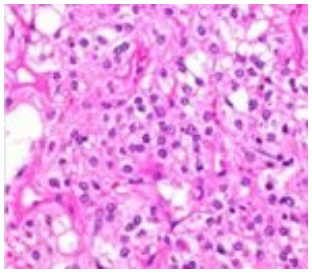


Human

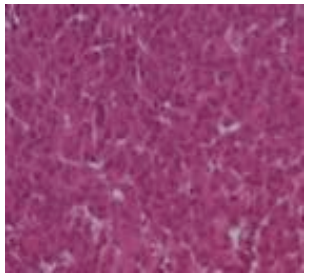


(Rouki)

« Epithelioid type » ALM ou MLM



Human



Dog

Photos – B. Vergier

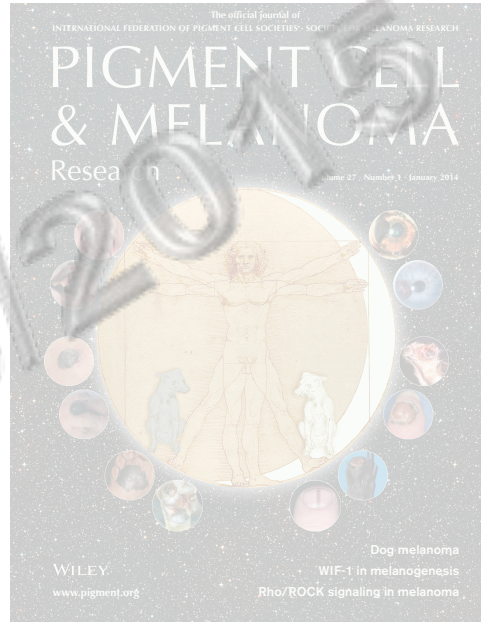
Dog melanomas are non sun-exposed :

- oral localizations and skin covered with fur / over-representation of black dogs

Canine melanomas can mostly inform on non-UV dependant pathways

ESPCR 2015

Gillard et al., PCMR 2014



I. Characterization of canine melanomas

- 2350 canine melanoma : characterization ; epidemiology

II. Comparison with Human melanomas

- Sample Collection : 500 cases (from ~10 breeds)

-> 300 cases : Comparative histology + Clinics

(In collaboration with B. Vergier, CHU Bordeaux
and J. Abadie, Oniris, Vet School Nantes)

III. Genetic approaches

- Predisposing mutations – GWAS (70 cases + 100 controls on oral melanomas)

(In collaboration with F. Demenais INSERM-Paris)

- Gene expression – RNAseq (DE, gene fusions, somatic variations on 30 cases)

-Landscape of somatic alterations : exome-sequencing on 70 cases

(In collaboration with K. Lindblad-Toh, Upsalla University, Sweeden/Broad Institute, Boston, US)

Genetic approaches of canine melanomas

I- Somatic mutations in canine oral melanoma tumors

Study design:

- ✓ 75 dogs matched tumor -vs- normal samples (150 Exomes)
- ✓ 3 breeds with oral melanoma (25 cases each)


poodle


golden


labrador

Canine Exome:

- ✓ NimbleGen (Broeckx et al., 2014)
- ✓ 53 Mb ; ~20,000 coding protein genes ; ~200,000 exons

Stastistical analysis (Broad Institute pipeline):

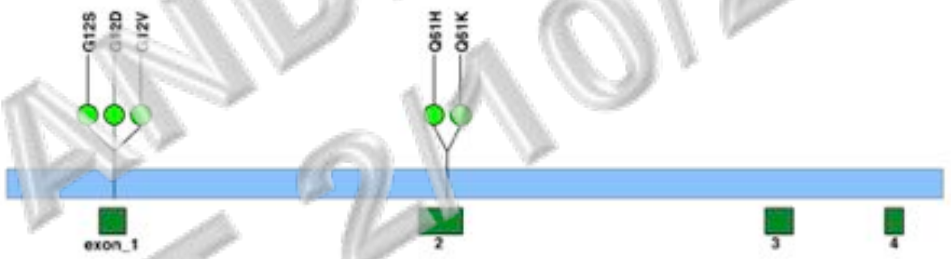
1. Comparison of the tumour DNA versus the blood DNA :
   → mean of X000 variants per sample
2. BWA (mapping), Mutect (SNV, Indel) & MuSiC (Statistical significancy)

Genetic approaches of canine melanomas

3- Identification of Driver genes with somatic mutations:

- Common SMGs with human : *NRAS, PTEN, TP53*
- Absence of BRAF mutations
- Novel SMGs

G12 S, D and V **Q61 H and K**



NRAS - [Oncogene]

Same hotspots than human

4- Patterns of mutual exclusive mutations

- molecular classification of **mucosal** melanomas in sub-types
(like the TCGA Human subtypes : MAPK, BRAF, RAS, NF1 : The Gene Cancer Atlas Network, Cell 2015)
Comparisons to Furney et al., 2013; Krauthammer et al., 2015)
- breed specificity of some relevant genes

Comparison with human melanoma: molecular aspects

Based on Whiteman *et al.*, 2011

UV linked

Non UV linked

Non UV linked N.UV l.

Human

1. Cutaneous

Somatic mutations — Histology

CSD (chronic sun damage) 15%

-**Lentiginous Melanoma** (Dubreuilh)

-**Nodular Melanoma**

BRAF V600 — NRAS Q61 — CKIT Ex 11 — Epidermal

Non CSD 70 %

-**SSM**: Superficial spreading melanoma

BRAF V600 — NRAS Q61 — Epidermal

2. Rare cutaneous 10%

-**Acral melanoma**

CKIT Ex 11 — NRAS Q61 — Epidermal

Blue naevus (Ota,Ito)

Giant congenital naevus

GNAQ — GNA11 — Dermal

3. Uveal 2.5%

GNAQ — GNA11 — Dermal

4. Mucosal 2.5%

CKIT Ex 11 — NRAS Q61 — PTEN G251 — Epidermal

Dog

1. Cutaneous

Somatic mutations — Histology

Rarely observed

Only 1 case of SSM / 153 melanoma

2a. Cutaneous (Digit and ungual) 5-10%

dermal

Rare (-) but severe (+++)

Predisposed breeds (black dogs: schnauzer, rott)

2b. Cutaneous 27%

dermal

Less severe (+)

Predisposed breeds

(black dogs: golden,Labrador, rottweiler, beauceron)

3. Ocular 1%

dermal

Benign

4. Mucosal 62%

Frequent (+++) and severe (+++)

NRAS Q61H — PTEN G251 — NOVEL GENES

dermal

Predisposed breeds (black dogs (poodle))

Comparison with human melanoma: histogenetic data

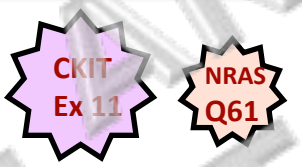
Based on Whiteman *et al.*, 2011

Dog breeds are relevant models for Non-UV Linked pathways of human melanoma

Non UV linked

2. Rare cutaneous 10%

-Acral melanoma



Epidermal

Blue naevus (Ota,Ito)

Giant congenital naevus

Dermal

2a. Cutaneous (Digit and ungual) 5-10%

Rare (-) but severe (+++)

dermal

Predisposed breeds (black dog(schnauzer, rott)

2b. Cutaneous 27%

Less severe (+)

dermal

Predisposed breeds
(black dogs: golden,Labrador, rottweiler, beauceron)

Non UV linked

4. Mucosal 2.5%

Epidermal

4. Mucosal 62%

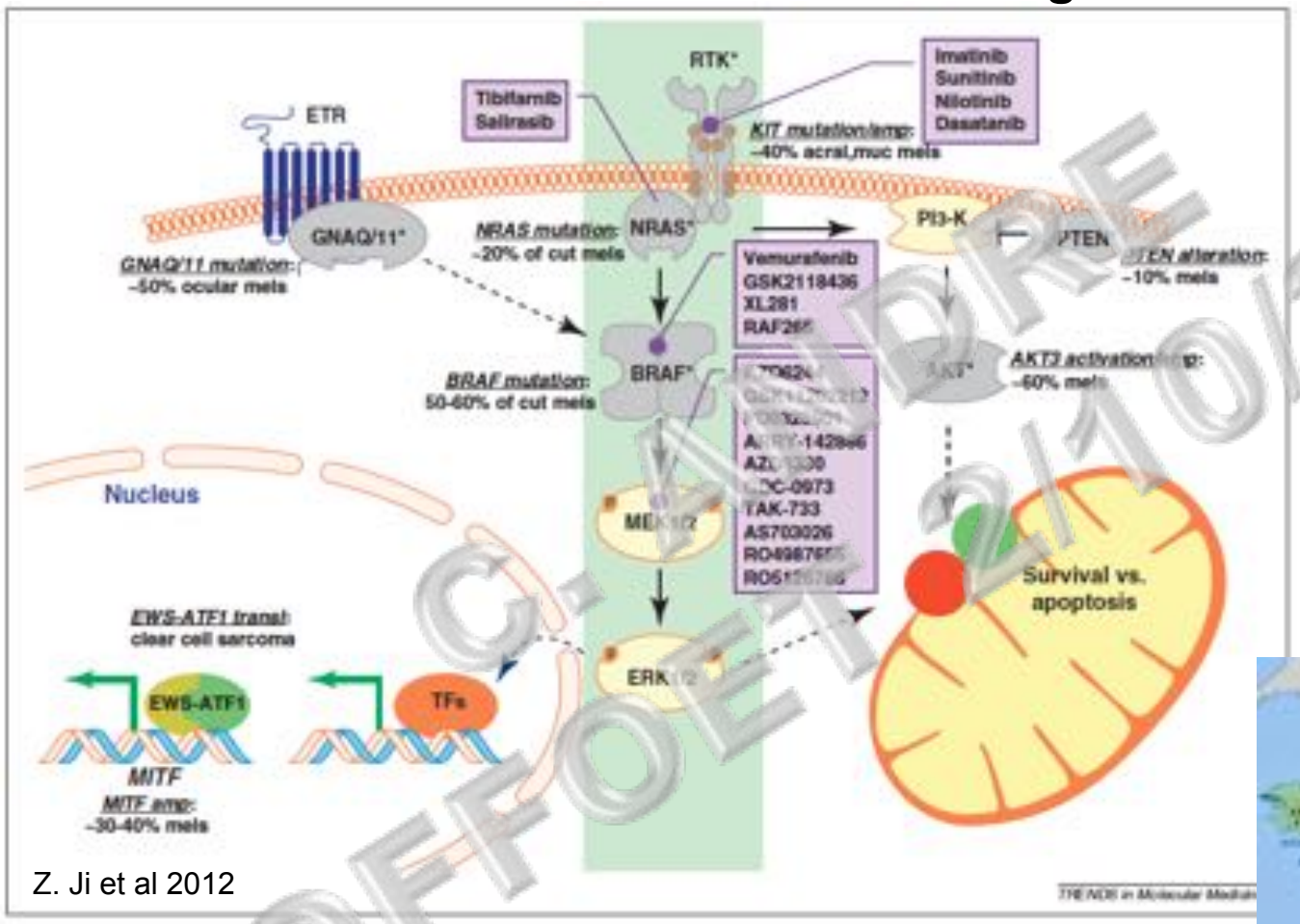
Frequent (+++)
and severe (+++)

dermal

Predisposed breeds (black dogs (poodle))

André-CNRS- SoFFoet 2015

THERAPIES in dogs for phase 1 trials:
For a mutual benefit for dogs and humans



Z. Ji et al 2012

Test of drugs in melanoma affected dogs :
In Veterinary Oncology Centres/ Vet Schools

Conclusion

Dog melanoma are good models for human melanomas:

- Acral and mucosal melanomas (less investigated in humans)
- Rare cutaneous melanomas of dermal origin (blue nevus, Giant congenital nevus)

Ongoing work :

- Predisposing and somatic alterations (NRAS, PTEN, P53 …)

-Replication + Functional validation of SMGs

-SiRNA in cell lines (10 canine mucosal & 1 human mucosal) + Human melanoma collections

Opportunity to decipher:

- «non UV pathways» of melanomas
- “Cocktail” of pigmentation genes in melanoma

Translational oncology:

- Pre-clinical studies on dog patients and humans

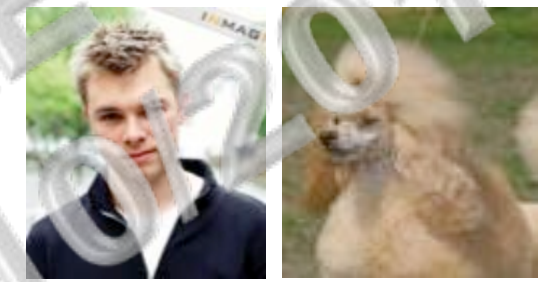
Correlation with the phototypes:

gene pigmentation variants are similar « tel chien, tel maître »

MC1R, B defensin WT

MC1R or aMSH variants

MC1R variants + Dilution gene



Trp 1 variants

MC1R WT, Trp1 variants

MC1R variants

→ **Knowledge of the gene pigmentation variants « cocktail » is important**

Acknowledgements

To the « dog genetics team »
IGDR, Rennes, France
Veterinarians, Vet schools, Breeders, owners
Histopathology labs : LAPVSO and IDEXX

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