

# **Canine Histiocytic Sarcoma in dogs: Genetic progress in the fight against histiocytic sarcoma last update of the genetic risk test : HS test**

Benoit HEDAN

[benoit.hedan@univ-rennes1.fr](mailto:benoit.hedan@univ-rennes1.fr)

Catherine André  
[catherine.andre@univ-rennes1.fr](mailto:catherine.andre@univ-rennes1.fr)  
Canine Genetics Team  
IGDR UMR6290 CNRS Univ.Rennes1

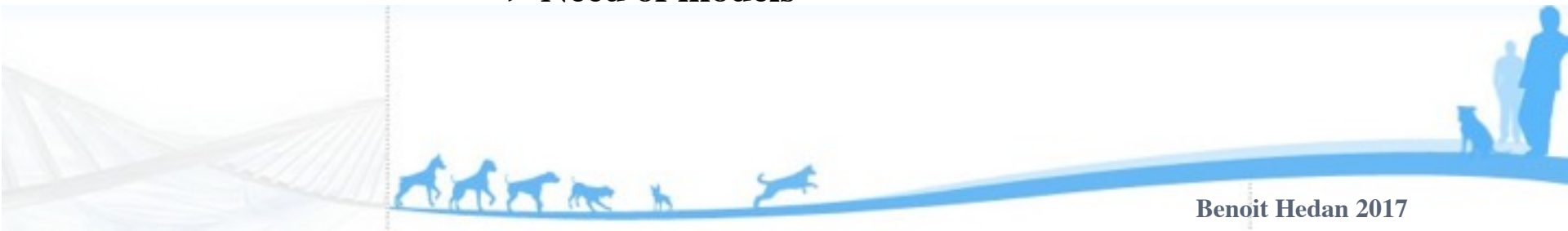
Collaboration with :  
Anne Thomas and Caroline Dufaure de Citres  
ANTAGENE



## In human

- extremely rare but aggressive tumor (1-5 cases per year in France)
- derived from monocytes/macrophage  
histiocytic markers : CD163, CD68, Lys...
- localized or disseminated ( lymph nodes, spleen, skin)
- few alterations of MAPKinase pathway (*BRAF* mutations)
- limited response to chemotherapy and high mortality.
- no consensus on prognostic factors and on standard treatments (*Takahashi et al. 2013*)

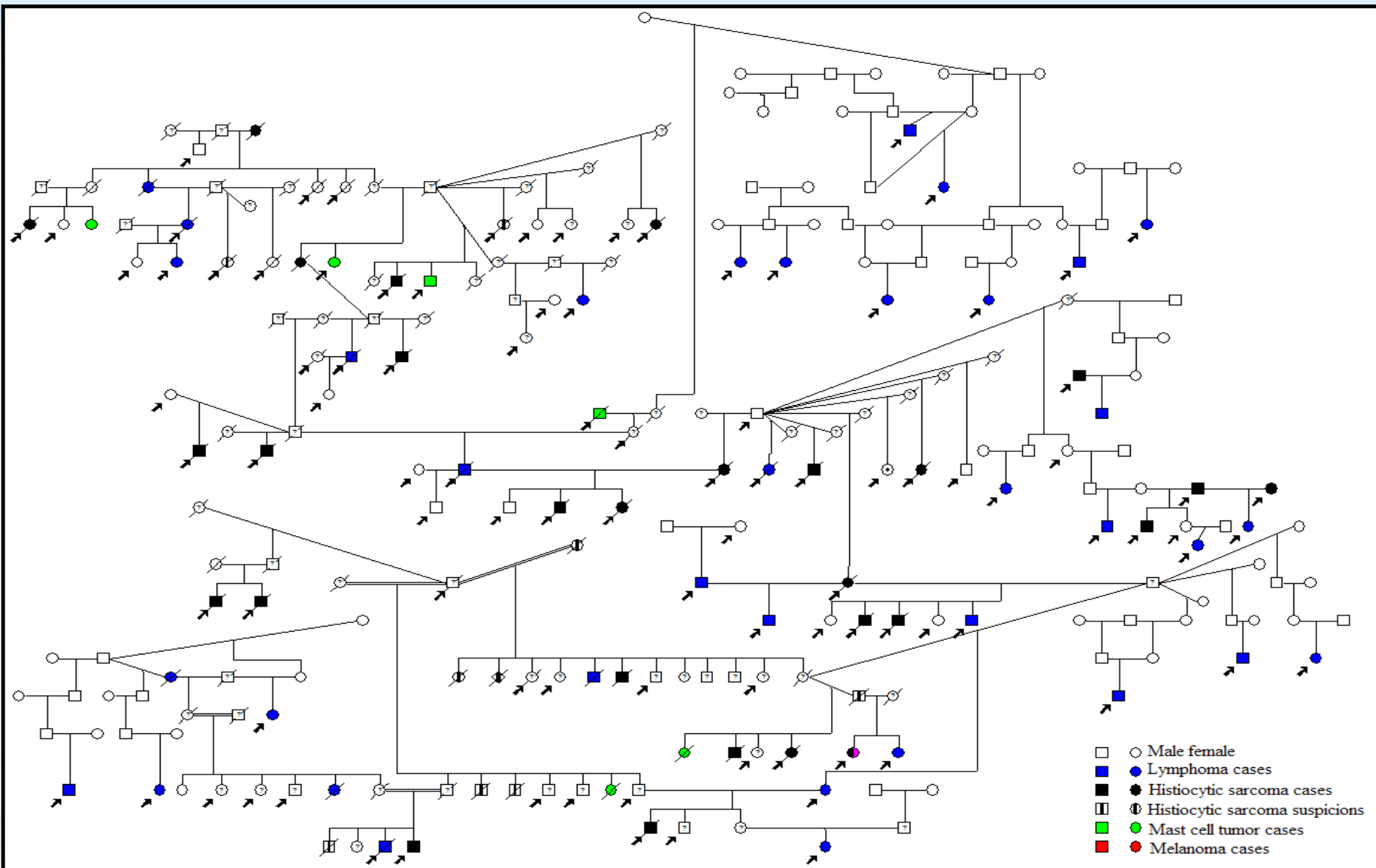
**-> Need of models**



## In dogs

- rare and highly aggressive cancer
- localized or disseminated ( lymph nodes, spleen, skin, lung, joint...)
- breed predisposition : BMD, Rottweilers, Retrievers  
familial transmission- oligogenic mode (*Abadie, Hedan et al 2009*)
- 20-25% of BMD are affected by this tumor
- expression of histiocytic markers : CD204/class A macrophage scavenger receptor+ (*Kato et al 2013*)
- aggressive disease with a poor prognosis : mean survival time : 49 days for BMD, 123 days for FCR  
(*Abadie, Hedan et al 2009*)





## In dogs

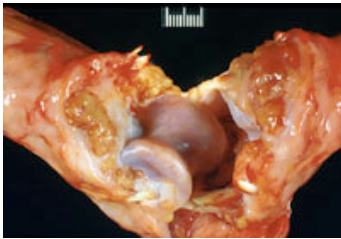
- rare and highly aggressive cancer
- localized or disseminated ( lymph nodes, spleen, skin, lung, joint...)
- breed predisposition : BMD, Rottweilers, Retrievers  
familial transmission- oligogenic mode (*Abadie, Hedan et al 2009*)
- 20-25% of BMD are affected by this tumor
- expression of histiocytic markers : CD204/class A macrophage scavenger receptor+ (*Kato et al 2013*)
- aggressive disease with a poor prognosis : mean survival time : 49 days for BMD, 123 days for FCR  
(*Abadie, Hedan et al 2009*)



## In dogs

different clinical forms

Localized forms :  
Skin/peri-articular



[www.histiocytosis.ucdavis.edu/sarcoma.html](http://www.histiocytosis.ucdavis.edu/sarcoma.html)

Retrievers



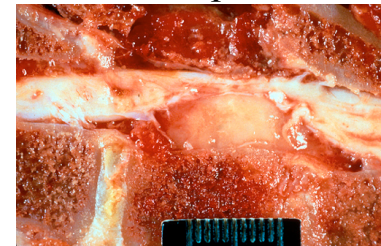
Disseminated forms :



BMD



Subdural forms :  
Cerebrum/spinal cord



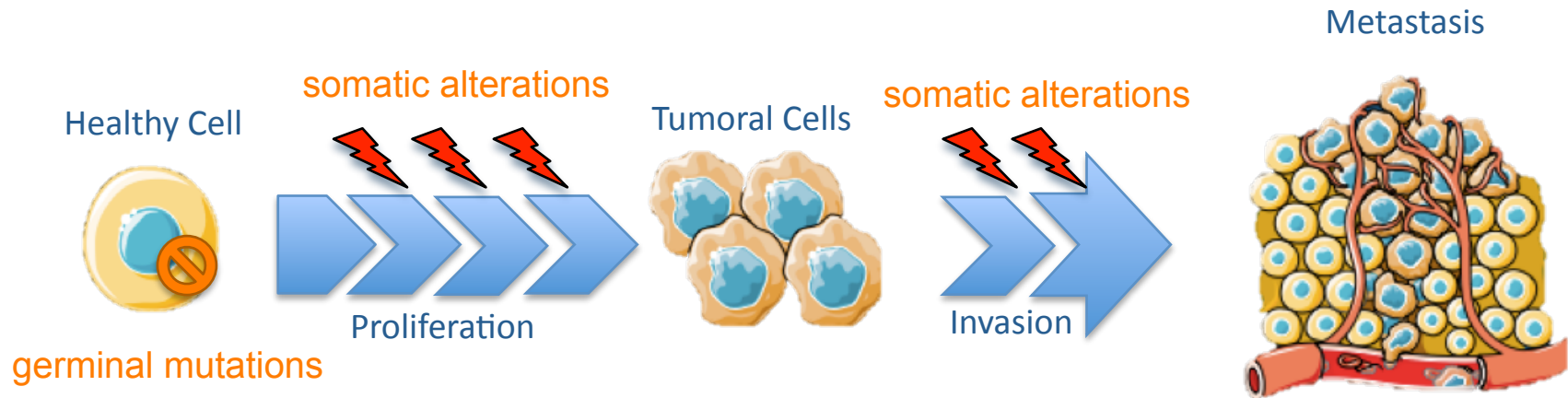
[www.histiocytosis.ucdavis.edu/sarcoma.html](http://www.histiocytosis.ucdavis.edu/sarcoma.html)

Pembroke Welsh Corgi



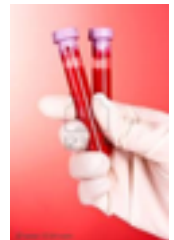


# A long term goal : Identify genetic mechanisms involved in HS



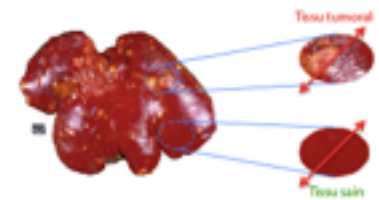
- Identify predisposing genes and risk alleles

-> Blood collection



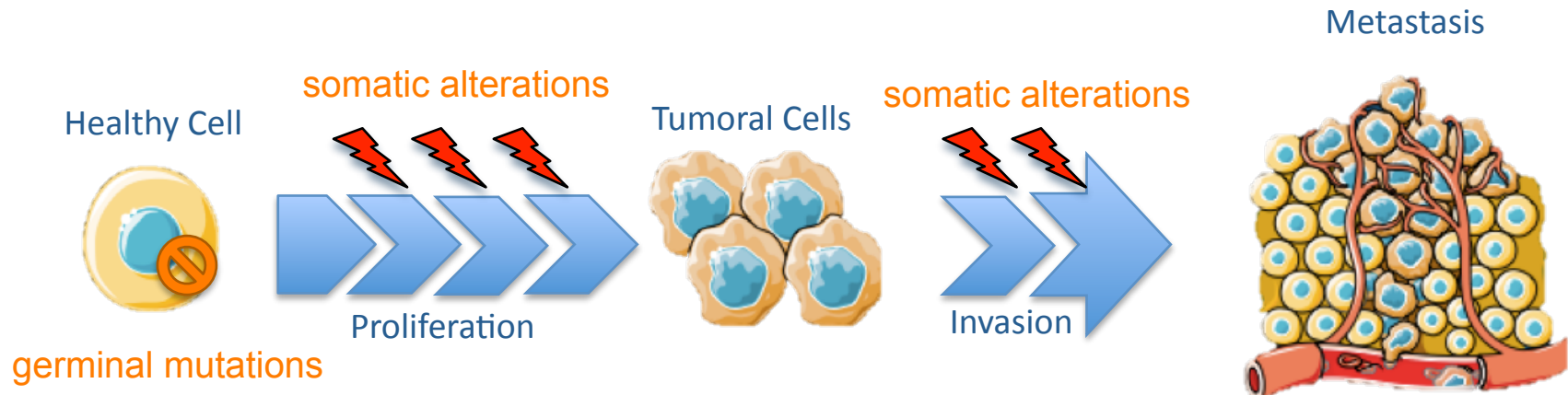
- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection



# A long term collaboration :

## Identify genetic mechanisms involved in HS



- ⊘ - Identify predisposing genes and risk alleles

E. Ostrander's lab,  
The Cancer genetics branch NIH (Bethesda, USA)

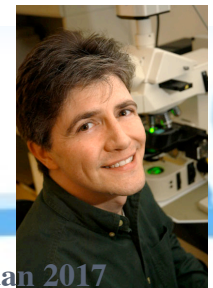
*Shearin et al. 2012*



- ⚡ - Identify recurrent somatic alterations associated with tumor progression

M. Breen's lab,  
NCSU (Raleigh, USA)

*Hedan et al. 2011*



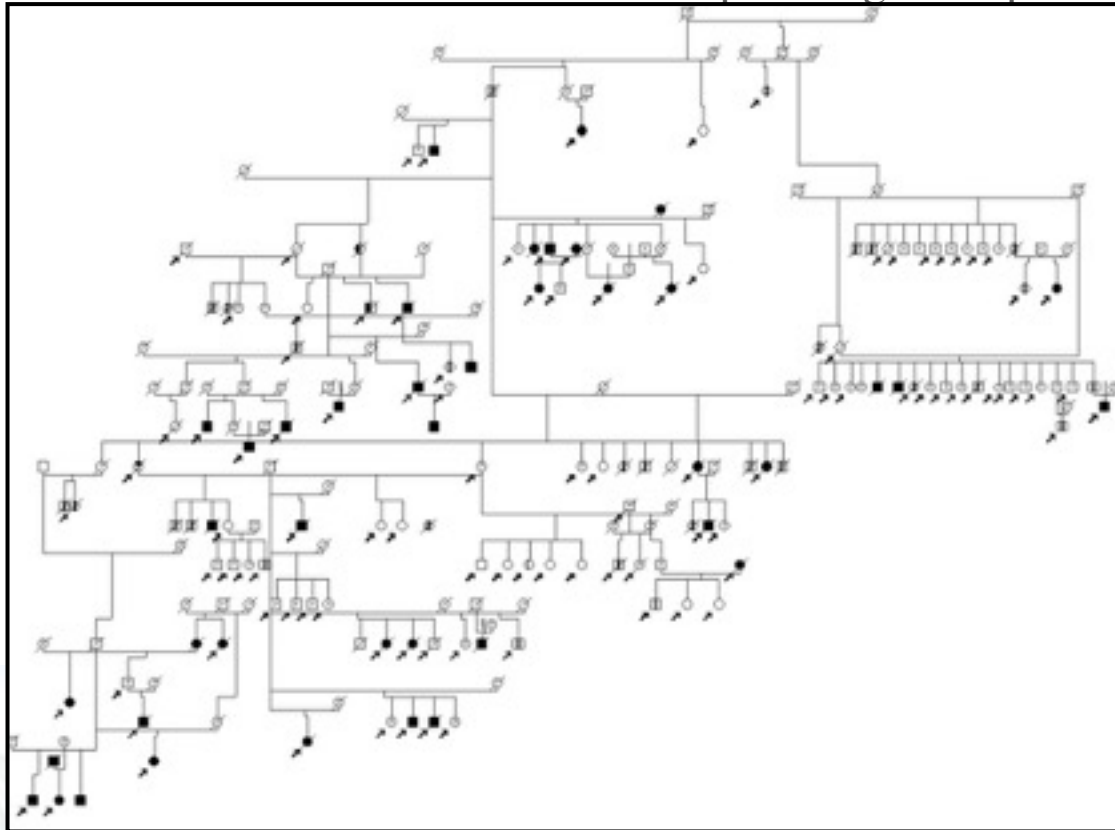
Benoit Hedan 2017



# Looking for predisposing genes: Research project

➤ Since 2002 - Cani-DNA biobank ([dog.genetics.genouest.org](http://dog.genetics.genouest.org))

- > 4000 blood samples and > 400 tissue samples
- > 450 HS cases with histopathological report



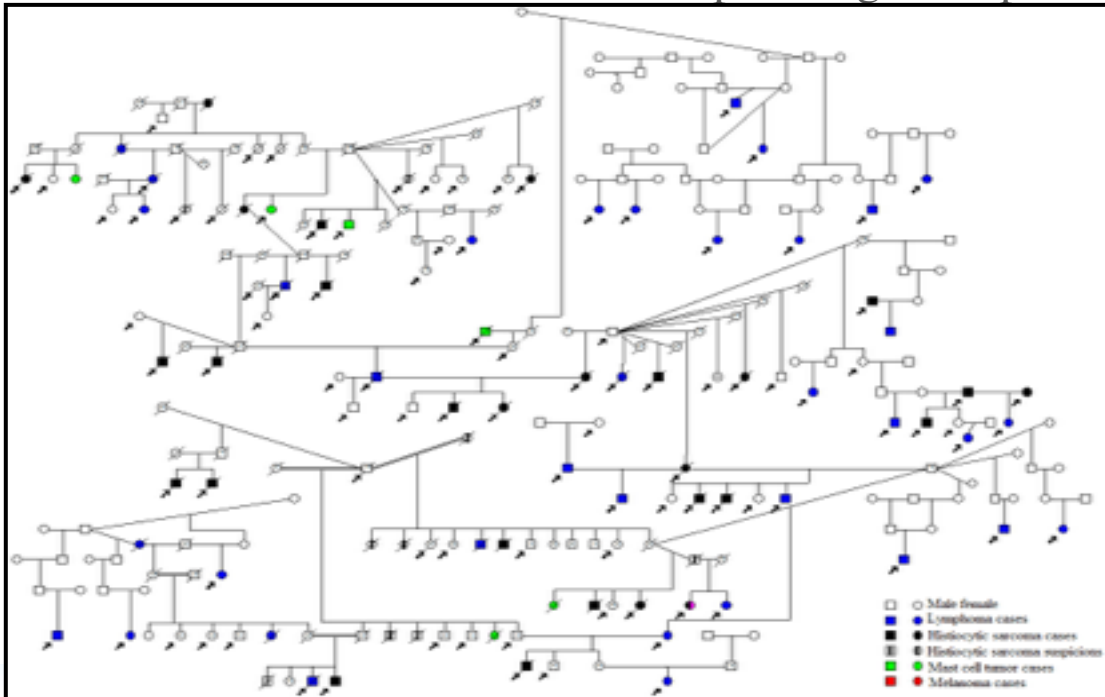
➤ Characterization of the Disease:  
*Abadie, Hedan et al. 2009*



# Looking for predisposing genes: Research project

➤ Since 2002 - Cani-DNA biobank ([dog.genetics.genouest.org](http://dog.genetics.genouest.org))

- > 4000 blood samples and > 400 tissue samples
- > 450 HS cases with histopathological report



➤ Characterization of the Disease:  
*Abadie, Hedan et al. 2009*

# Looking for predisposing genes: Research project

- Since 2002 - Cani-DNA biobank ([dog.genetics.genouest.org](http://dog.genetics.genouest.org))
  - > 4000 blood samples and > 400 tissue samples
  - > 450 HS cases with histopathological report
- Characterization of the Disease:  
*Abadie, Hedan et al. 2009*
- In coll. with E. Ostrander's lab (NIH, Bethesda)
- GWAS: 232 unaffected and 244 affected BMDs (American/European)
- > Identification of main loci : CFA11, CFA14 (*Shearin, Hedan et al. 2012*)



# Looking for predisposing genes: Research project

- Work in progress to identify variants associated with HS predisposition (col. With E. Ostrander)

Sequencing of chromosomes of affected and healthy dogs

**Summary of HS research**



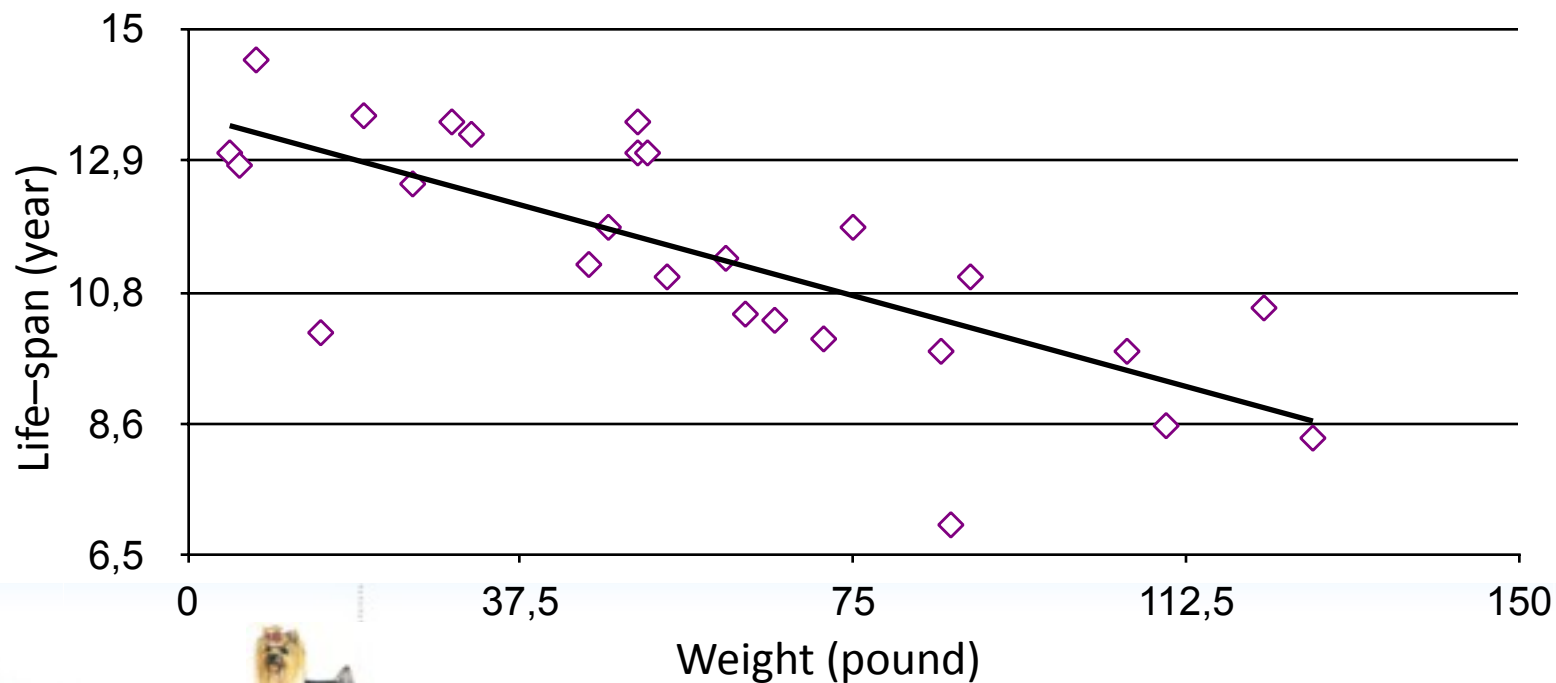
# Looking for predisposing genes: Research project

- Work in progress to identify variants associated with HS predisposition (col. With E. Ostrander)

Sequencing of chromosomes of affected and healthy dogs

## Summary of HS research

- Cancers impact BMD life span



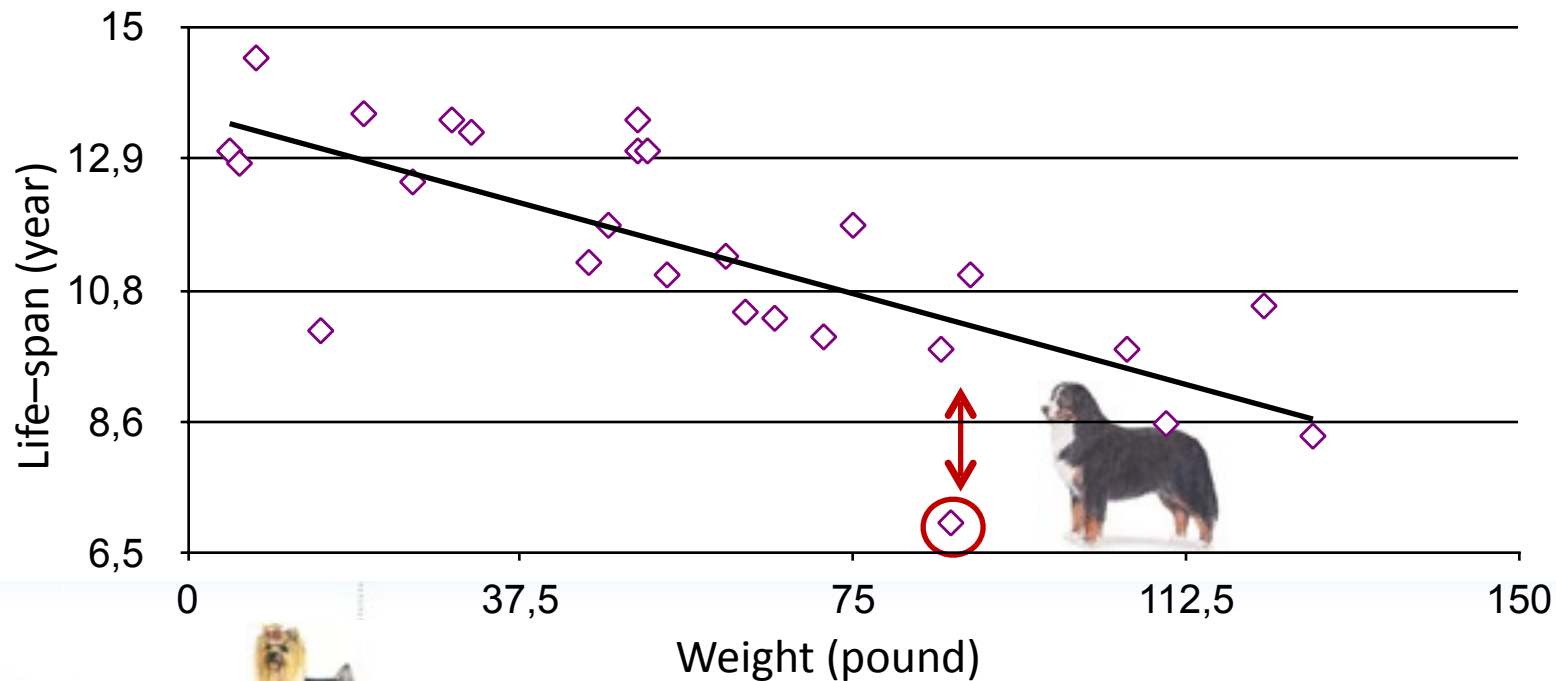
# Looking for predisposing genes: Research project

- Work in progress to identify variants associated with HS predisposition (col. With E. Ostrander)

Sequencing of chromosomes of affected and healthy dogs

## Summary of HS research

- Cancers impact BMD life span





# Looking for predisposing genes: Research project

- Work in progress to identify variants associated with HS predisposition (col. With E. Ostrander)

Sequencing of chromosomes of affected and healthy dogs

## Summary of HS research

- Cancers impact BMD life span
- Cancer : polyfactorial disease -> involving genetic and environmental factors
- Many regions are involved in HS predisposition of which CFA11
  - > Needs of research to better understand development of this cancer to prevent and treat it

**BUT data produced by research could be useful for breeders to help selection!**



# How these data could be useful for breeders?

In 2012 , in collaboration with Antagene , we decided to develop a genetic risk test

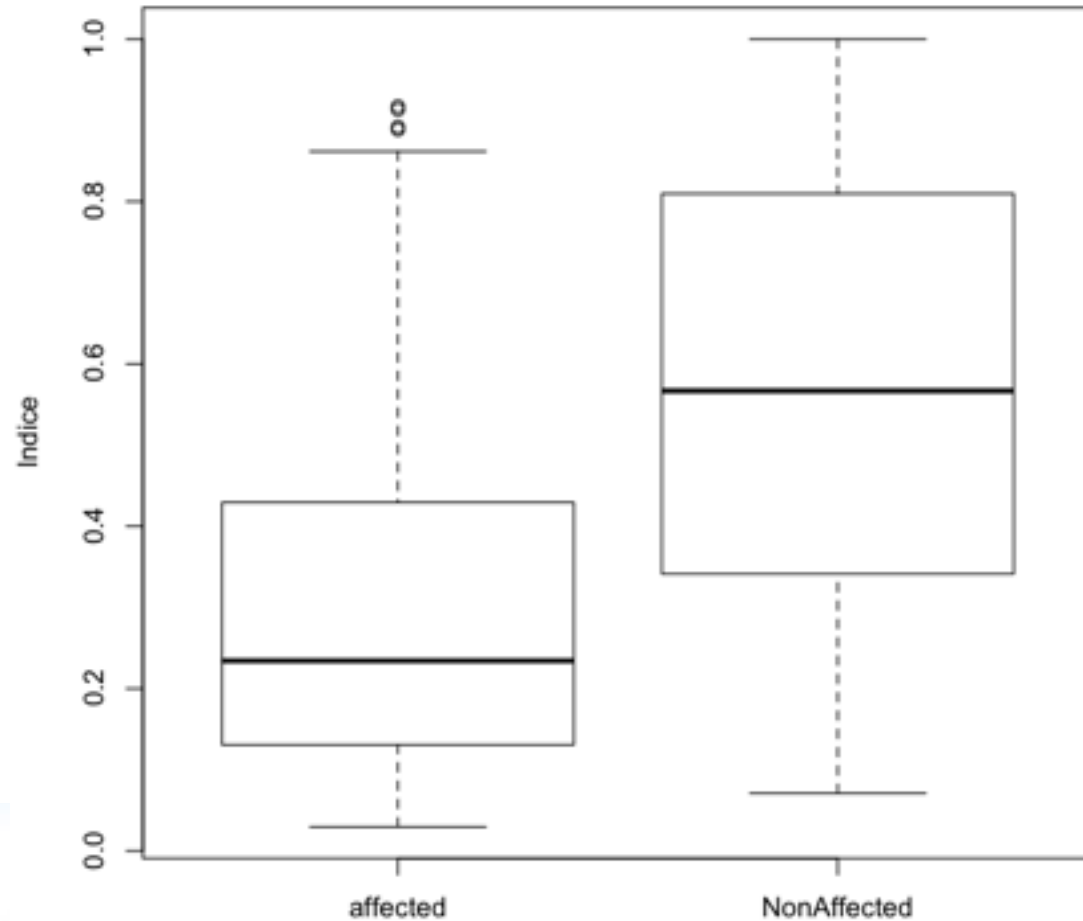
- Based on our first GWAS data, selection of 140 SNPs associated with HS
- Genotyping of 1081 French BMDs ( 256 affected dogs + 165 unaffected dogs)
  - > selection of the 9 SNPs more significantly associated with risk to develop HS
- Estimation of risk to be unaffected (GLM model) :

$$\text{Risk} = \frac{\exp(\sum^n (\alpha_i \text{SNP}_i))}{1 + \exp(\sum (\alpha_i \text{SNP}_i))}$$

Index

# Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

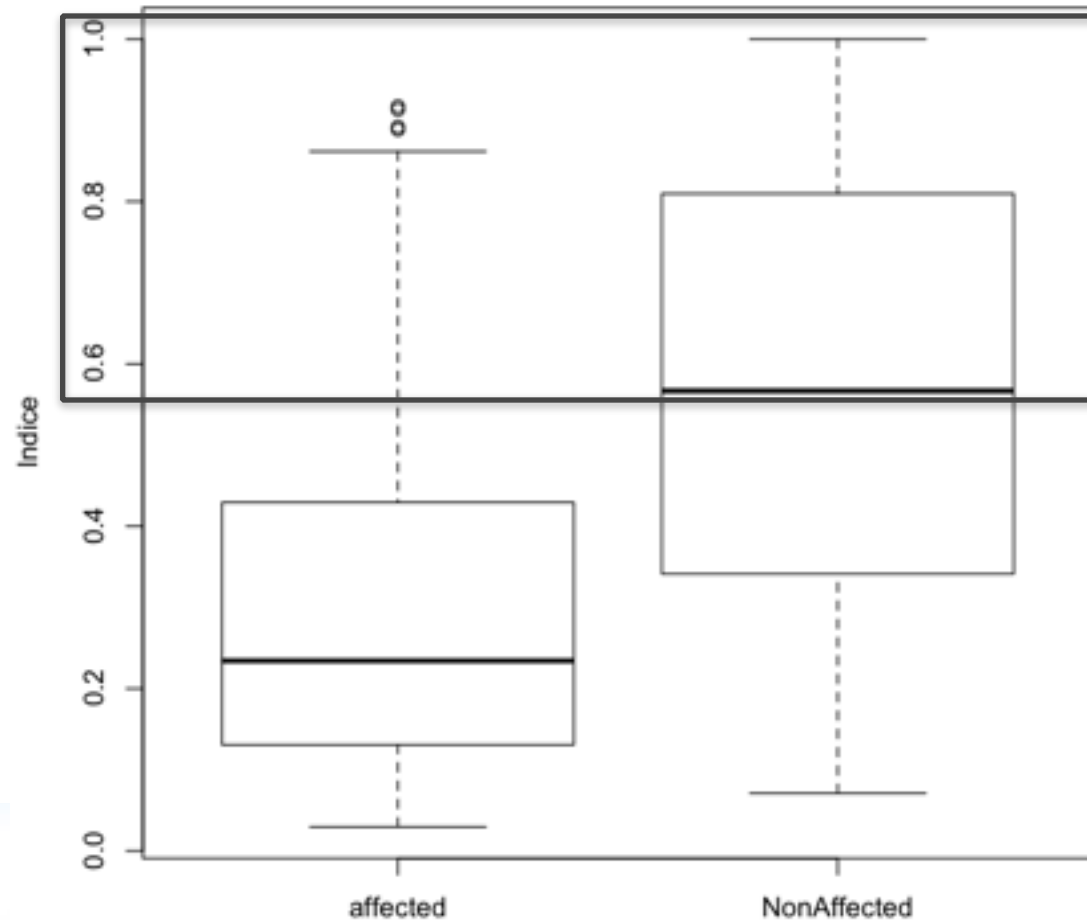


p-value :  $1.754 \times 10^{-16}$  (Mann Whitney test)

# Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

Category A



47 % of healthy  
10% of affected

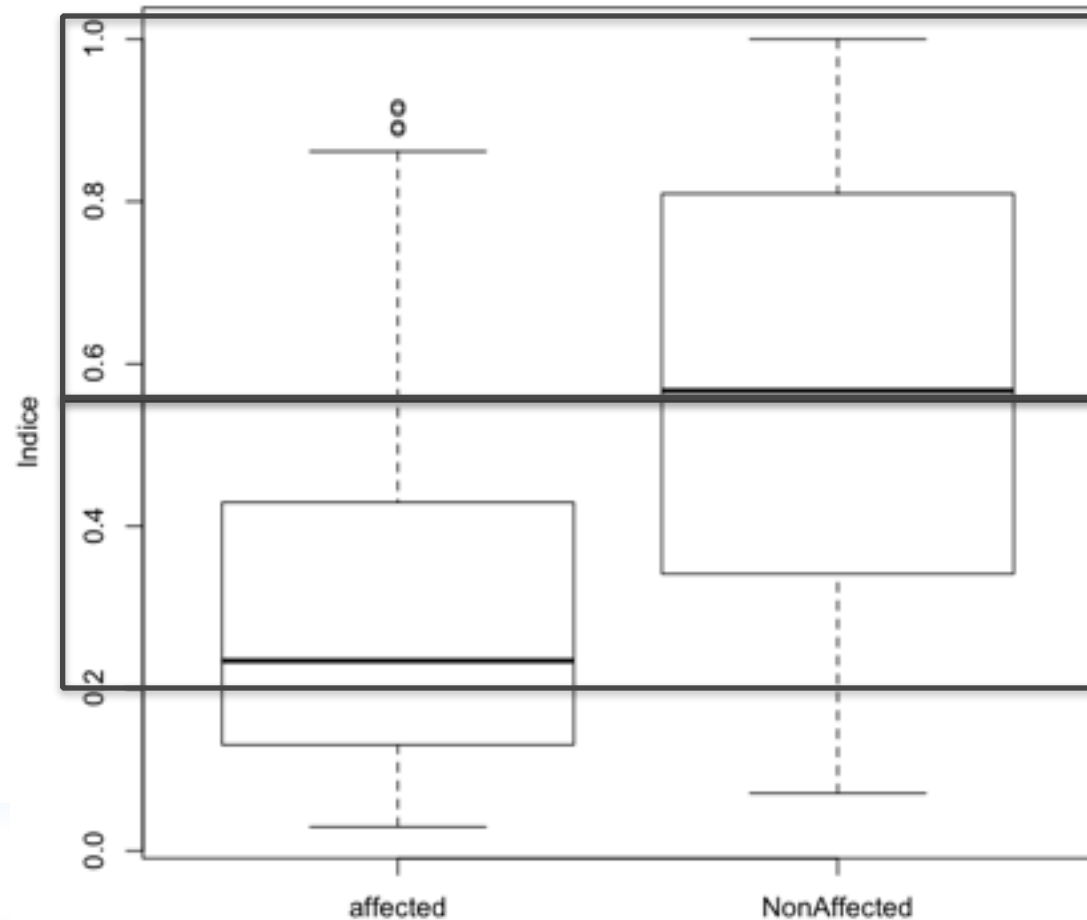
p-value :  $1.754 \times 10^{-16}$  (Mann Whitney test)

# Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

Category A

Category B



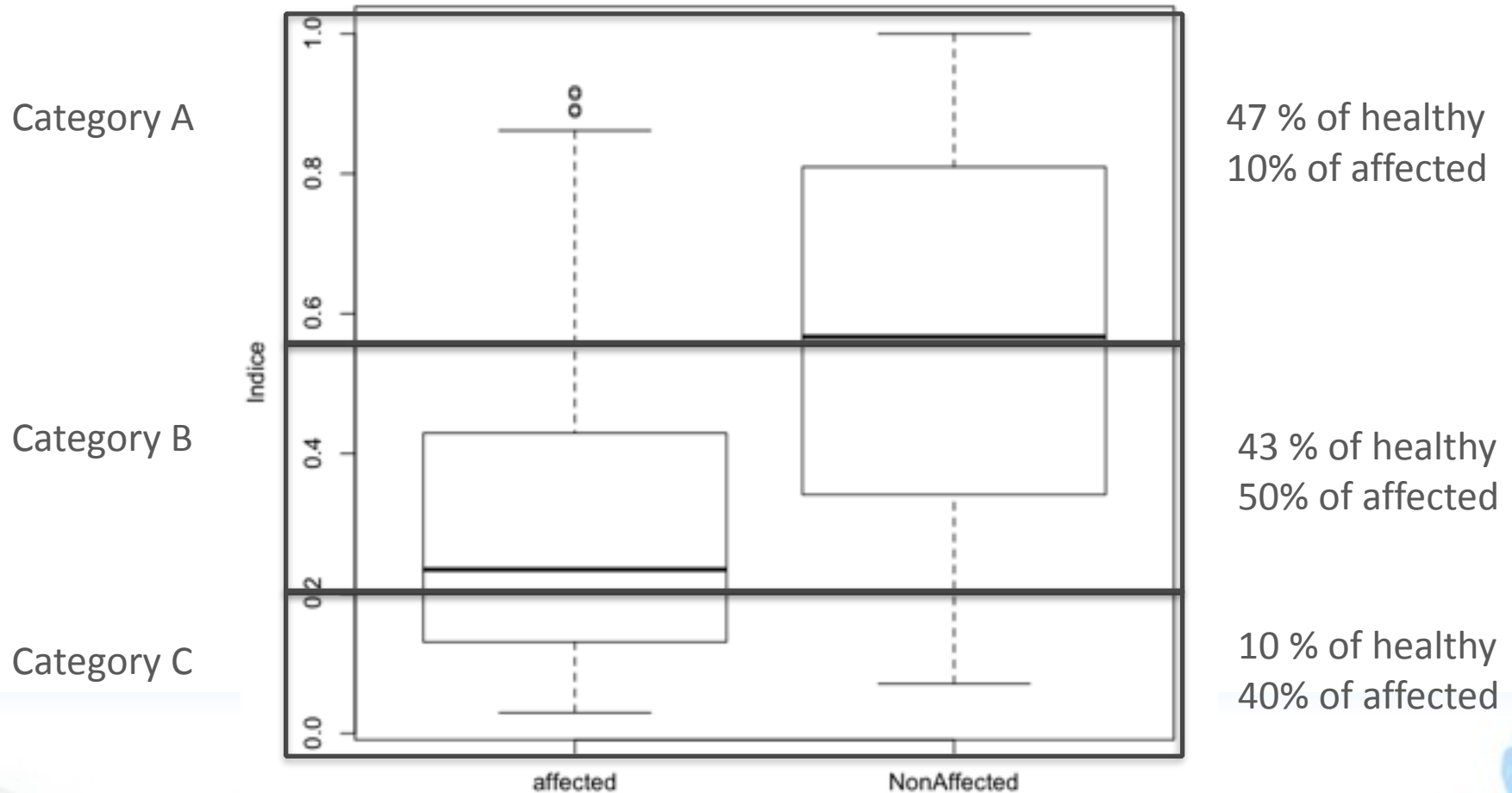
47 % of healthy  
10% of affected

43 % of healthy  
50% of affected

p-value :  $1.754 \times 10^{-16}$  (Mann Whitney test)

# Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected



p-value :  $1.754 \times 10^{-16}$  (Mann Whitney test)



# Validation of index: On European population

In collaboration with the European breeder clubs, validation of the pre-test on European BMDs (Belgium, Danemark, Finland, Germany, Ireland, Italia, Poland, Portugal, Spain, Sweden, Switzerland) :

- 80 dogs with Histiocytic Sarcoma
- 64 unaffected dogs (> 10 years )

|                        | A             | B             | C             |
|------------------------|---------------|---------------|---------------|
| <i><b>Affected</b></i> | <b>6.25%</b>  | <b>33.75%</b> | <b>60%</b>    |
| <i><b>Healthy</b></i>  | <b>30.16%</b> | <b>47.62%</b> | <b>22.22%</b> |



# Validation of index: On European population

In collaboration with the European breeder clubs, validation of the pre-test on European BMDs (Belgium, Danemark, Finland, Germany, Ireland, Italia, Poland, Portugal, Spain, Sweden, Switzerland) :

- 80 dogs with Histiocytic Sarcoma
- 64 unaffected dogs (> 10 years )

|                 | A      | B      | C      |
|-----------------|--------|--------|--------|
| <i>Affected</i> | 6.25%  | 33.75% | 60%    |
| <i>Healthy</i>  | 30.16% | 47.62% | 22.22% |

77%

- Pre test validated for European BMDs

# Validation of index: On American population

In collaboration with the Berner Garde and Dr Vilma Yuzbasiyan-Gurkan (Michigan State University)

- 54 dogs with Histiocytic Sarcoma
- 37 unaffected dogs (> 10 years )

|                        | A             | B             | C             |
|------------------------|---------------|---------------|---------------|
| <i><b>Affected</b></i> | <b>9.26%</b>  | <b>50%</b>    | <b>40.74%</b> |
| <i><b>Healthy</b></i>  | <b>35.14%</b> | <b>48.65%</b> | <b>16.22%</b> |

- Pre test validated for American BMDs :

# Validation of index: On American population

In collaboration with the Berner Garde and Dr Vilma Yuzbasiyan-Gurkan (Michigan State University)

- 54 dogs with Histiocytic Sarcoma
- 37 unaffected dogs (> 10 years )

|                 | A      | B      | C      |
|-----------------|--------|--------|--------|
| <i>Affected</i> | 9.26%  | 50%    | 40.74% |
| <i>Healthy</i>  | 35.14% | 48.65% | 16.22% |

83%

- Pre test validated for American BMDs : -> test name has been changed to HS test

# Summary of Index Validation

In total, **718** dogs tested for development and validation of the pre-test

- 415 dogs with Histiocytic Sarcoma
- 303 unaffected dogs (> 10 years )

|                        | A            | B            | C            |
|------------------------|--------------|--------------|--------------|
| <i><b>Affected</b></i> | <b>14%</b>   | <b>43.1%</b> | <b>42.9%</b> |
| <i><b>Healthy</b></i>  | <b>41.5%</b> | <b>46.5%</b> | <b>11.9%</b> |

➤ **Statistics :**

88%

Index A to detect healthy dogs :

Specificity is **probability** that an affected dog is not A : 86%

Index C to detect affected dogs :

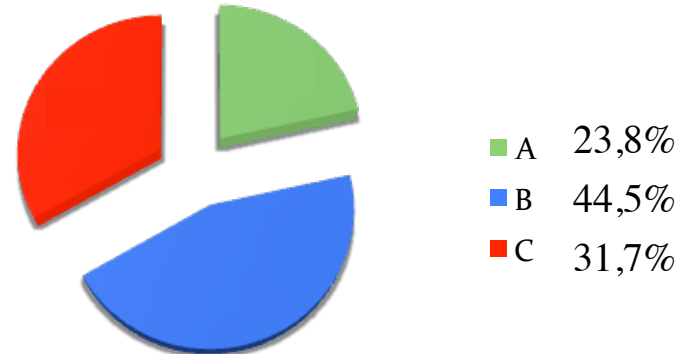
Specificity is **probability** that a healthy dog is not C : 88%

# Summary of tests performed by Antagene



Since 2012 , Antagene has tested 1846 dogs (unknown phenotypes):

distribution of index in this population:



of which 64 dogs (>10 years) : 30 A (46,8%) , 24 B (37,5%) et 10 C (15,6%)

(  $p\text{-value} : 2.79 \times 10^{-5}$  test Chi2)

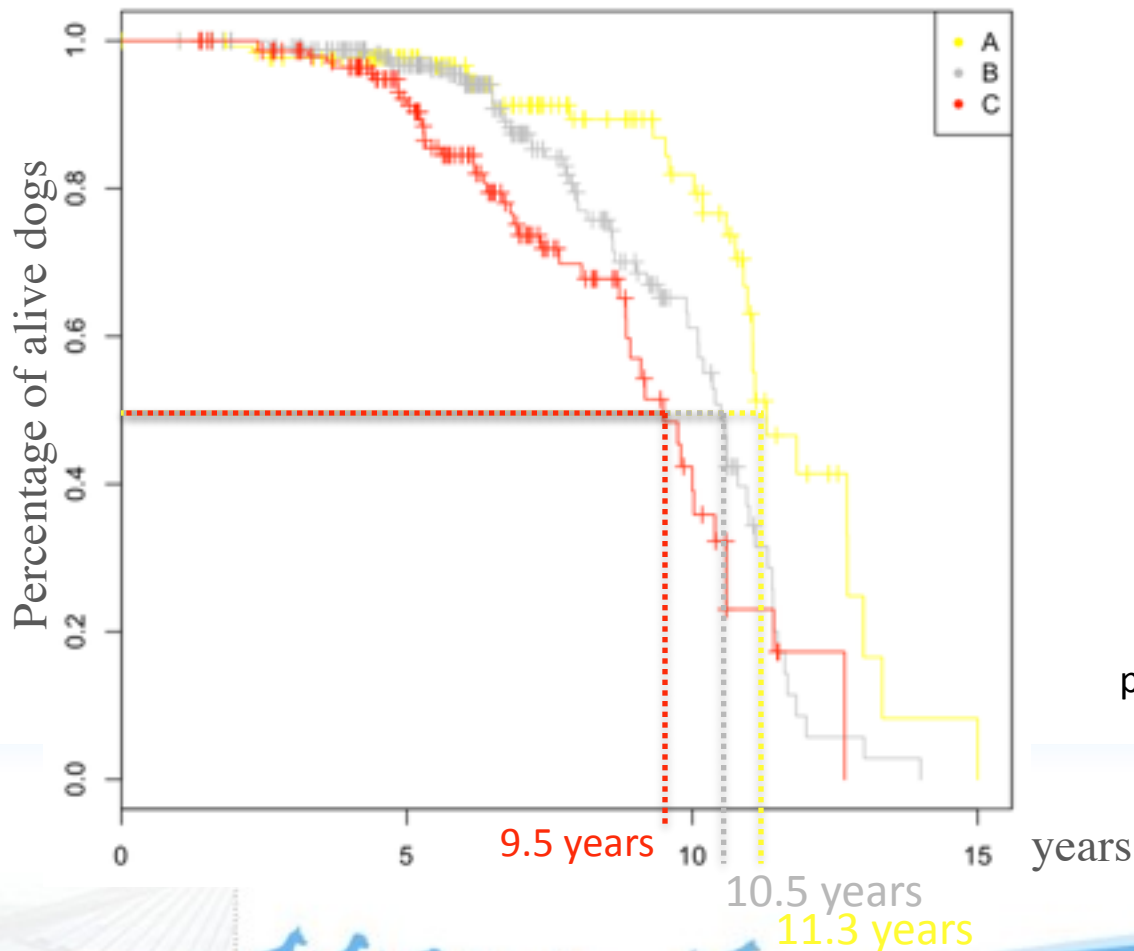
- good correlation of the index and clinical status

- be improved with new markers following research advances



# Summary of tests performed by Antagene

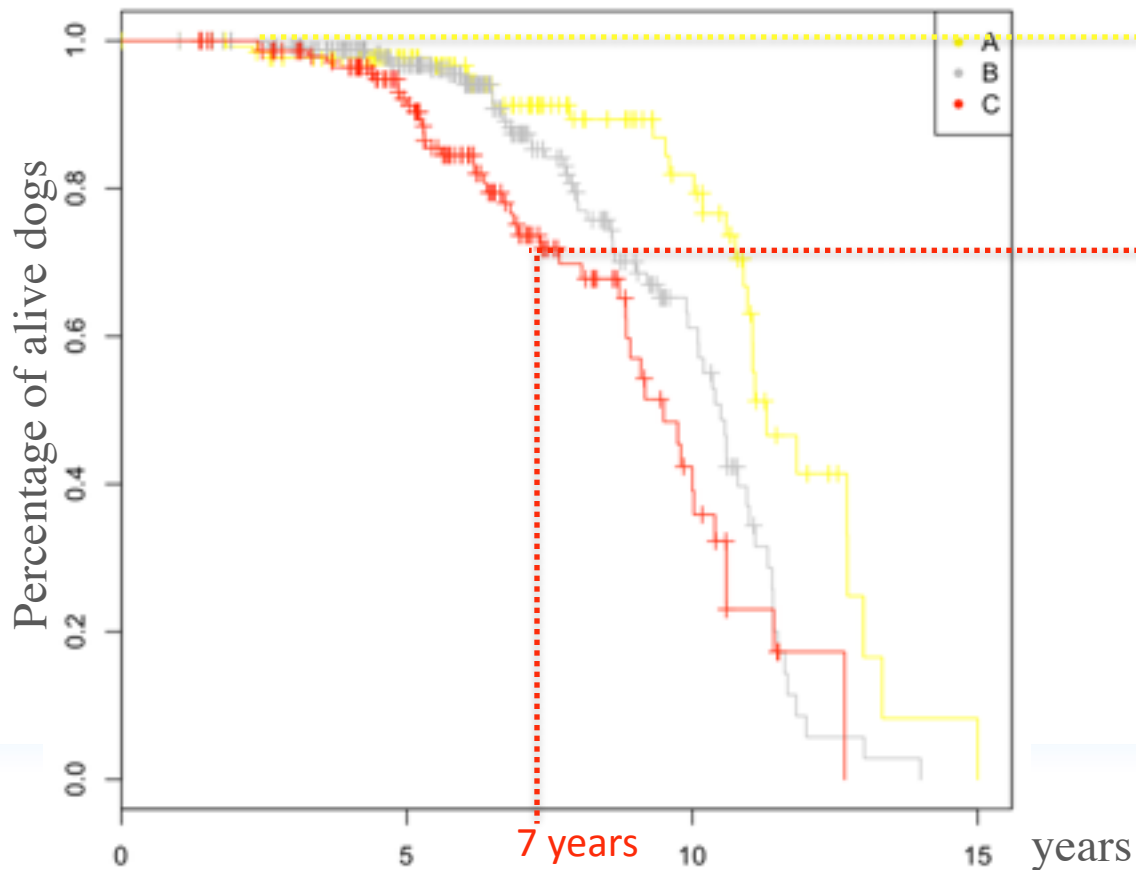
Since 2012 , Antagene has tested 1846 dogs (unknown phenotypes):  
follow up of >5 years old dogs (Eléonore Thiery)



pval = 3.791686e-06

# Summary of tests performed by Antagene

Since 2012 , Antagene has tested 1846 dogs (unknown phenotypes):  
follow up of >5 years old dogs (Eléonore Thiery)



A: 15,1% of deaths

B: 35,8% of deaths

C: 49,1% of deaths

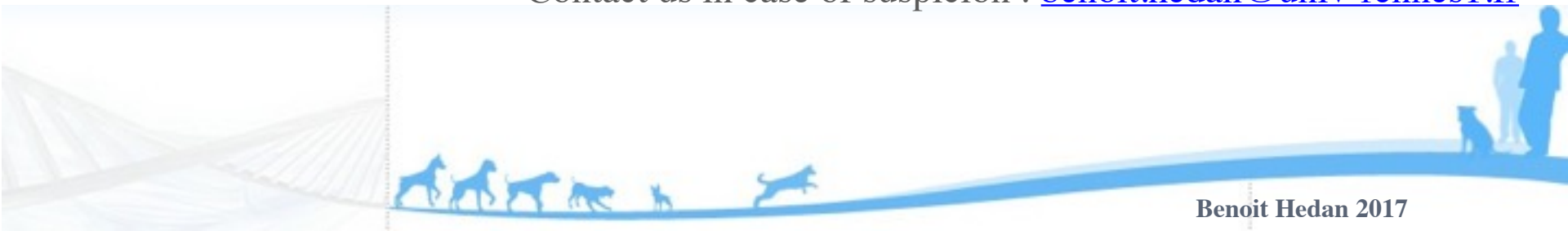
# Summary of tests performed by Antagene

Since 2012 , Antagene has tested 1846 dogs (unknown phenotypes):  
follow up of >5 years old dogs (Eléonore Thiery)

Needs of follow up :

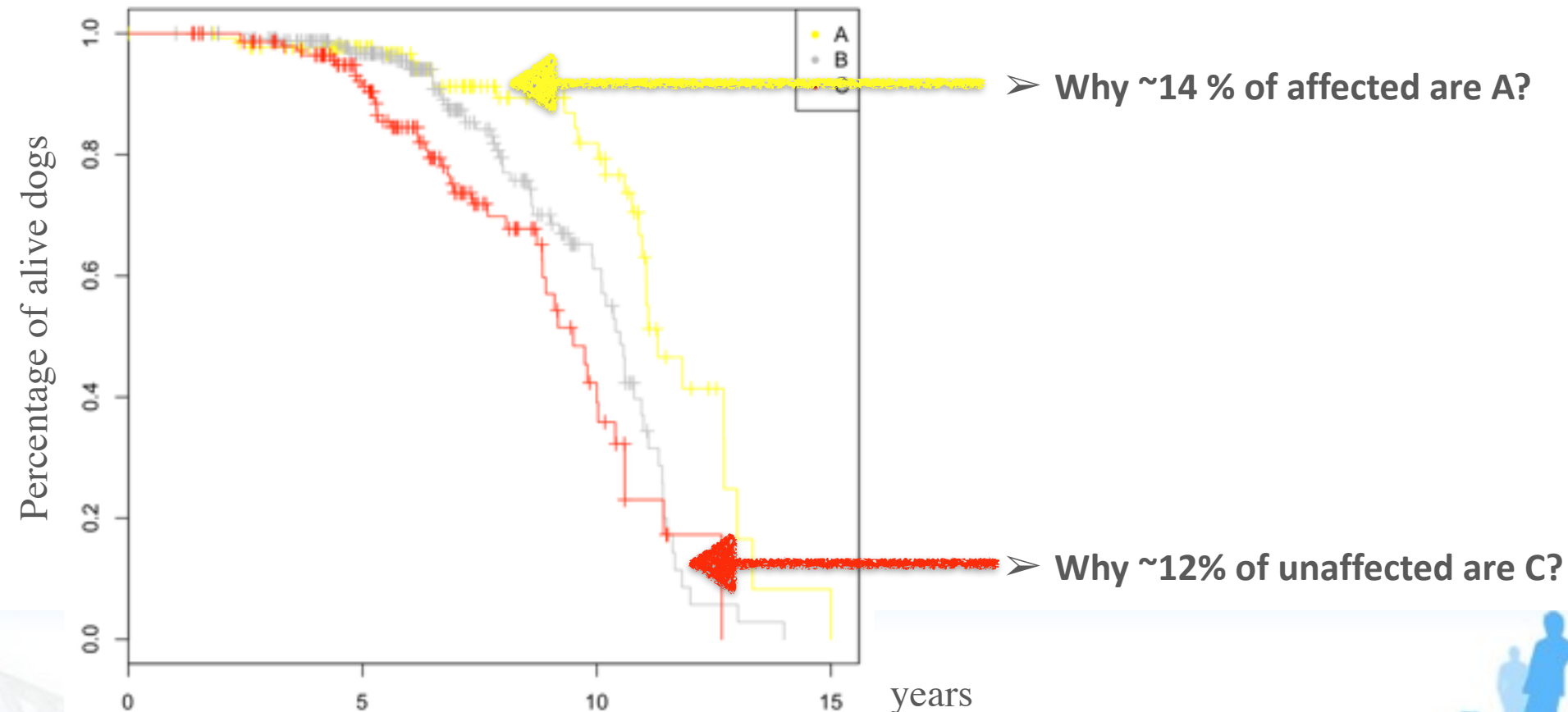
- > inform Antagene of dogs' outcome  
online clinical follow-up questionnaire :  
<http://www.antagene.com/en/contact/histiocytic-sarcoma-questionnaire/>
- > perform histological investigation

Contact us in case of suspicion : [benoit.hedan@univ-rennes1.fr](mailto:benoit.hedan@univ-rennes1.fr)



# Summary of tests performed by Antagene

Since 2012 , Antagene has tested 1846 dogs (unknown phenotypes):  
follow up of >5 years old dogs (Eléonore Thiery)



# Summary of Index Validation

**What explains the differences? -> Histiocytic sarcoma = polyfactorial disease**

➤ Other involved chromosomes

HS test based on 9 known markers but others regions involved  
-> work on discordant dogs (GWAS/sequencing)



# Summary of Index Validation

**What explains the differences? -> Histiocytic sarcoma = polyfactorial disease**

➤ Other involved chromosomes

HS test based on 9 known markers but others regions involved

-> work on discordant dogs (GWAS/sequencing)

➤ environnemental factors :

-sexual hormones

-inflammation

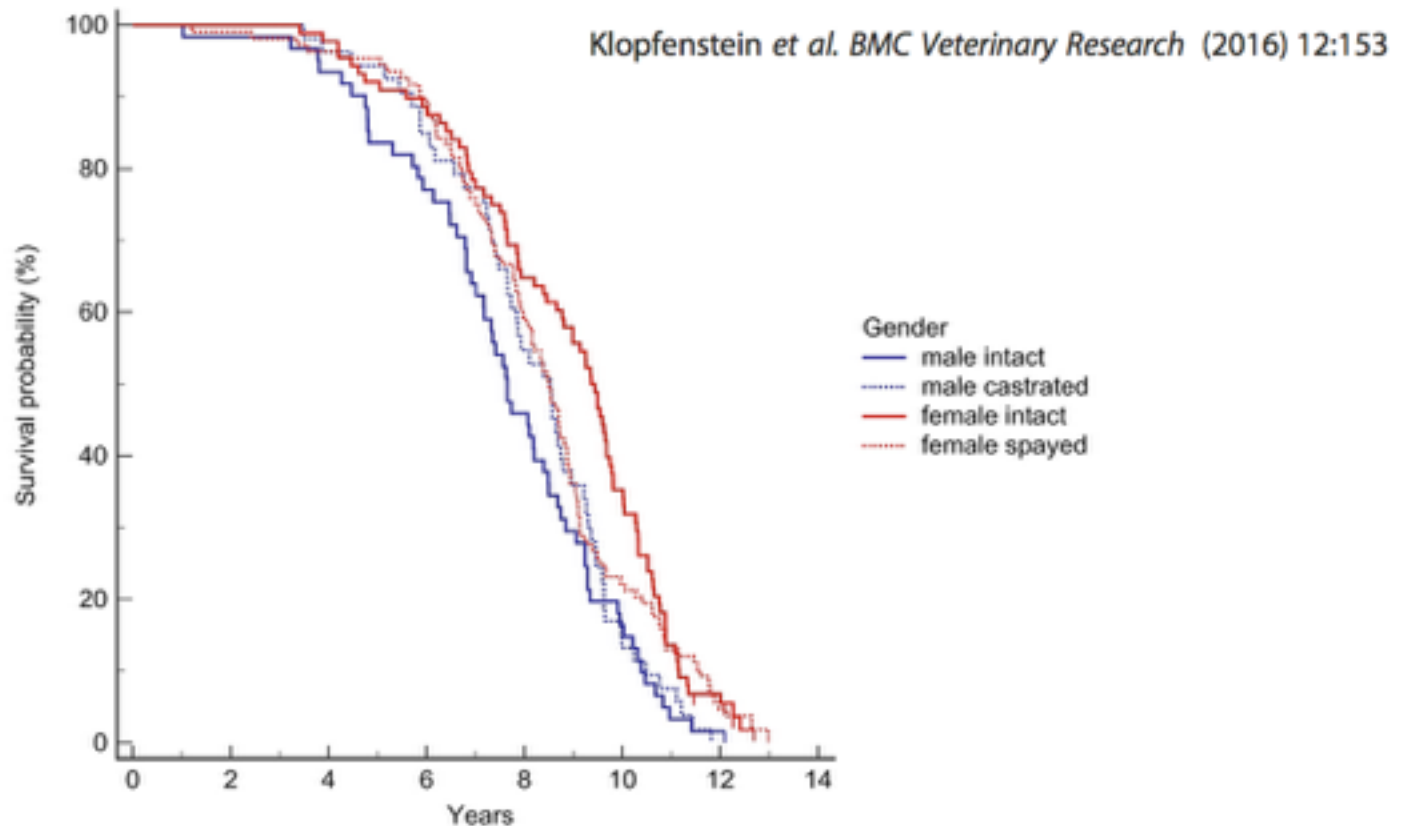
-treatments





# Summary of Index Validation

What explains the differences? -> Histiocytic sarcoma = polyfactorial disease



**Fig. 1** Kaplan-Meier survival curves for Bernese mountain dogs based on sex



# Summary of Index Validation

**What explains the differences? -> Histiocytic sarcoma = polyfactorial disease**

➤ Other involved chromosomes

HS test based on 9 known markers but others regions involved

-> work on discordant dogs (GWAS/sequencing)

➤ environnemental factors :

-sexual hormones

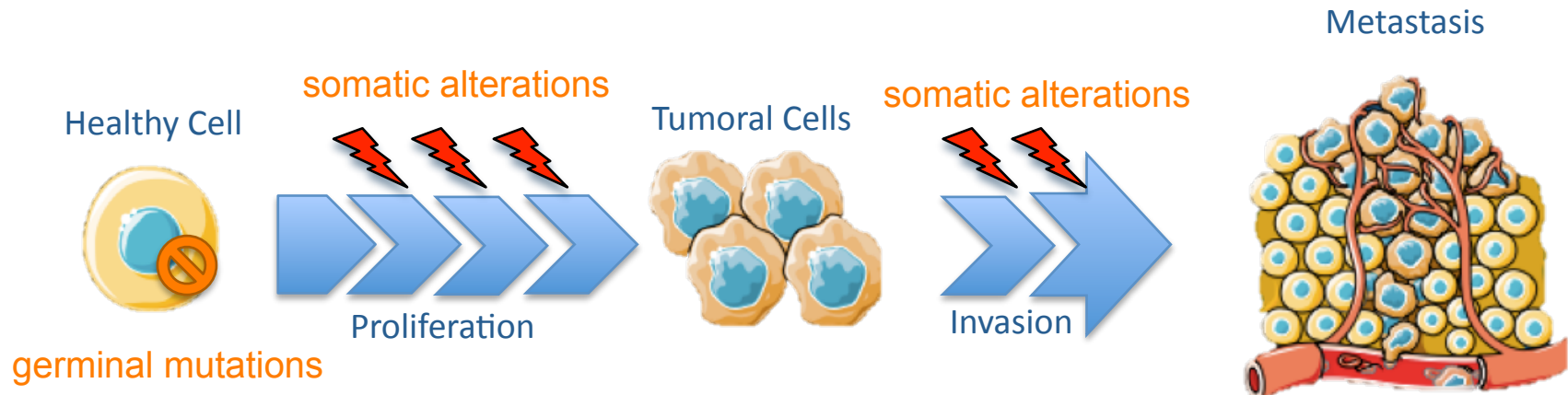
-inflammation : joint diseases...

(Manor et al. 2017, Kujik et al. 2013, Ruple et al 2016)

-treatments : anti-inflammatory medications (Ruple et al. 2016)

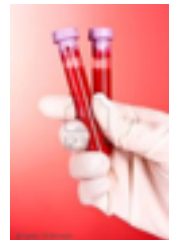


# A long term goal : Identify genetic mechanisms involved in HS



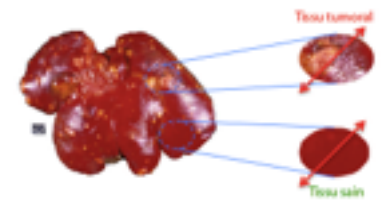
- Identify predisposing genes and risk alleles

-> Blood collection



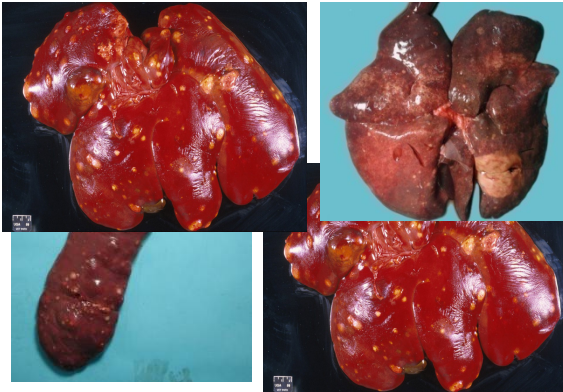
- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection



# Identification of somatic mutations associated with tumor progression

## Identification of key somatic mutations in HS



detected



not detected



# Identification of somatic mutations associated with tumor progression

Sequencing of 102 tumors from different breeds ( BMD, Rottweiler, Retrievers,...) and different organs (Spleen, liver, lung, kidney, skin, lymph nodes)

-> *a specific gene* found mutated in 53% (55) tumors

**-> driver event**

|              | Mutated | WT |
|--------------|---------|----|
| BMD          | 48      | 36 |
| other breeds | 6       | 12 |

$p\text{-value} = 0.07$  (Exact Fisher test)

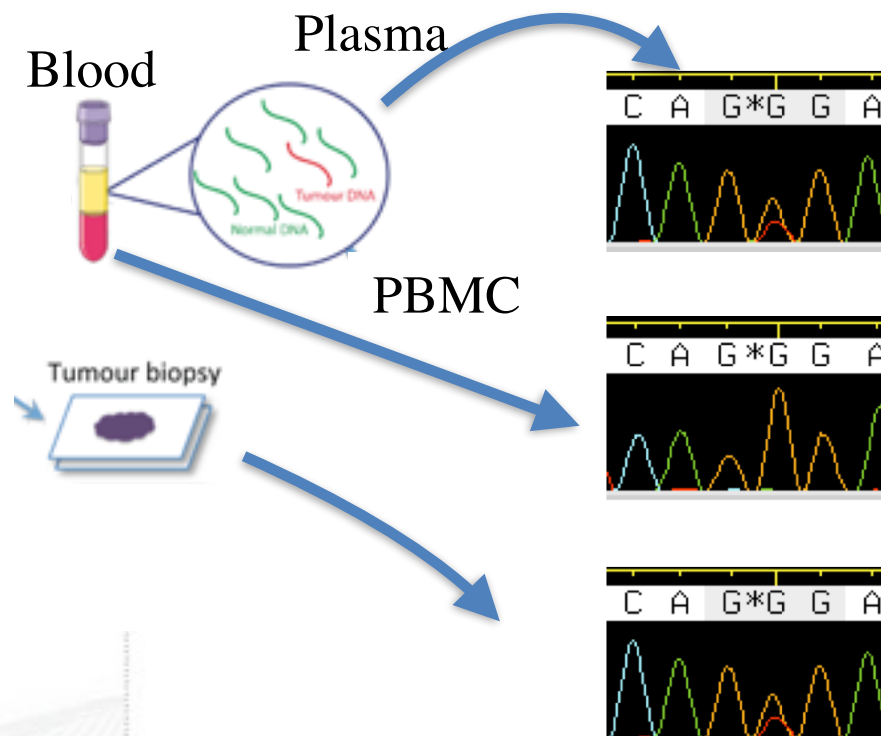
|          | Mutated | WT |
|----------|---------|----|
| internal | 42      | 25 |
| external | 5       | 20 |

$p\text{-value} = 0.00035$  (Exact Fisher test)

# Canine histiocytic sarcoma: Somatic mutations = Therapeutic and diagnostic tools

Somatic mutation is :

- specific of HS ( validated on 102 HS and 21 lymphomas)
- detectable in Plasma of affected dogs (6 cases: Allele Frequencies 1-59%)



# Canine histiocytic sarcoma: Somatic mutations = Diagnostic tools

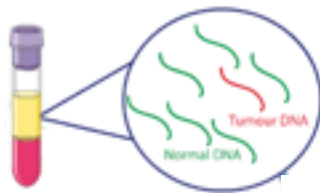
Somatic mutation is :

- specific of HS ( validated on 102 HS and 21 lymphomas)
- detectable in Plasma of affected dogs (6 cases: Allele Frequencies 1-59%)

-> Collection of samples on suspicion and confirmed cases

Plasma

Blood



-> to explore interest of these markers for an earlier diagnosis

Tumor



## Test HS : first genetic risk test for dogs

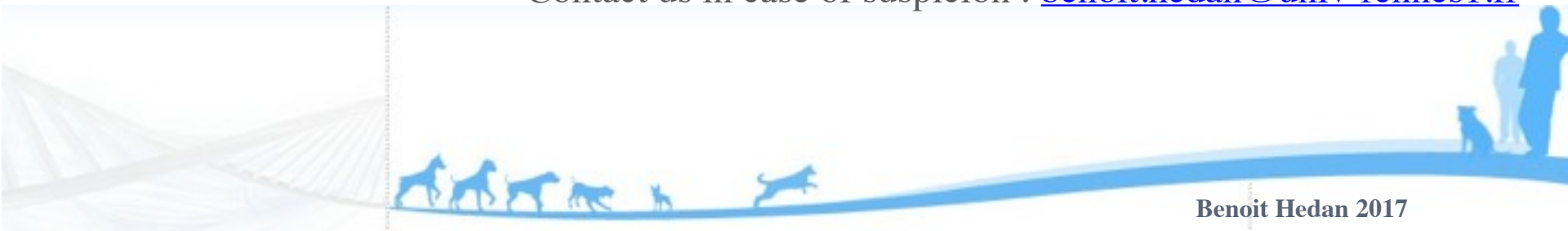
- > validated for all BMD origins
- > follow up of dogs confirm interest of this test for selection
- > research is still needed to improve this test!

## Mutations in tumors

- > Useful markers for an earlier diagnosis?  
for treatment

**Looking for samples (suspicion/cases...) to pursue research!**

Contact us in case of suspicion : [benoit.hedan@univ-rennes1.fr](mailto:benoit.hedan@univ-rennes1.fr)







## Canine Genetics Team

Catherine Andre et al.  
CNRS Rennes France

## Antagene

Anne Thomas  
Caroline Dufaure de Citres  
Lyon France



## The Cancer genetics branch NIH

Elaine Ostrander

## French Vet Schools

Jerome Abadie  
Patrick Devauchelle



## NCSU Vet School

Matthew Breen

## Owners, breeders,

## MSU Vet School

Vilma Yuzbasiyan-Gurkan

## All clubs, Berner Garde,... who sent samples

**Fundings** CNRS, AKC, INCA,  
BMD clubs : AFBS, SIBB, DCBS...

