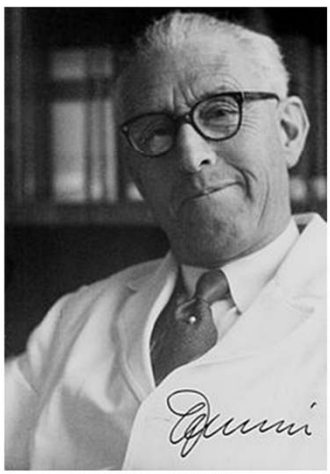
Progress in a 15 Year Search for the Genetic Causes for Fanconi Syndrome in Basenjis

Gary S. Johnson, DVM, PhD
&
Fabiana Farias
Department of Veterinary Pathobiology
University of Missouri, Columbia MO

Basenji Fanconi Syndrome

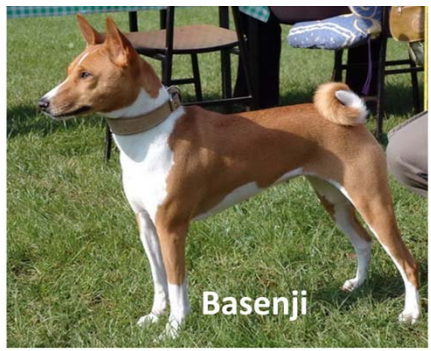
Dr. Guido Fanconi
Univ. Zurich



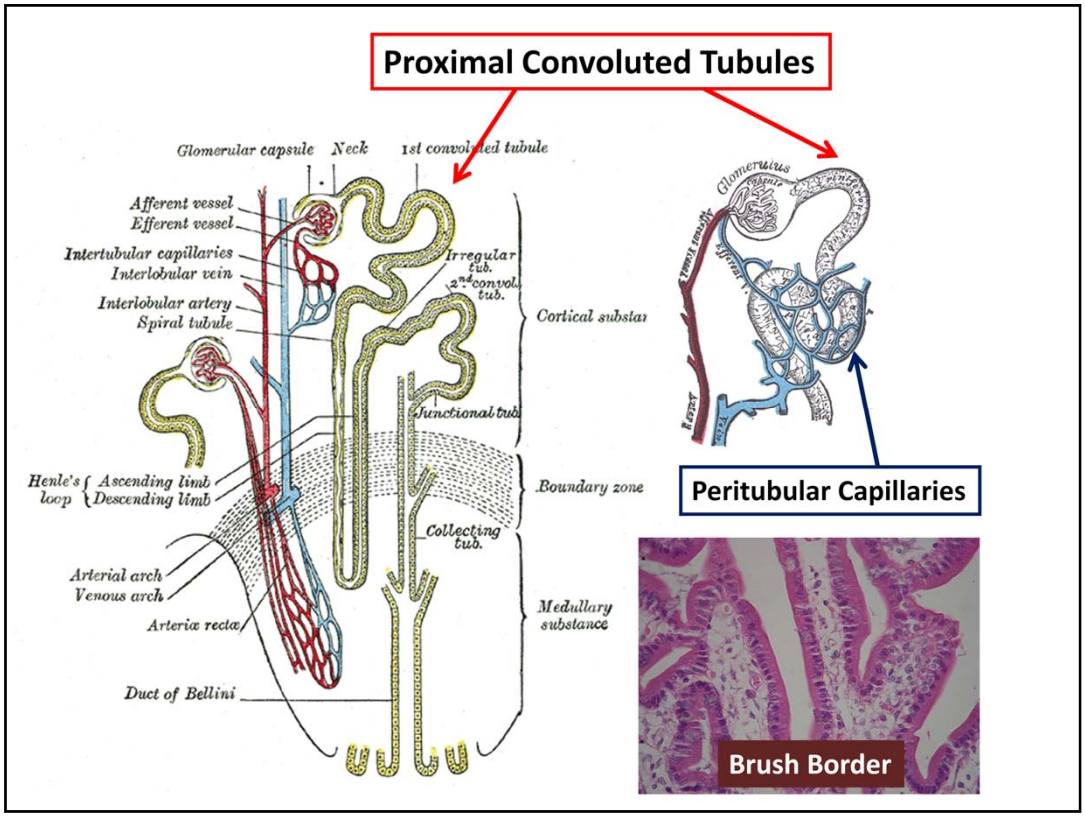
1892 - 1979

Fanconi anemia
DNA repair deficiency

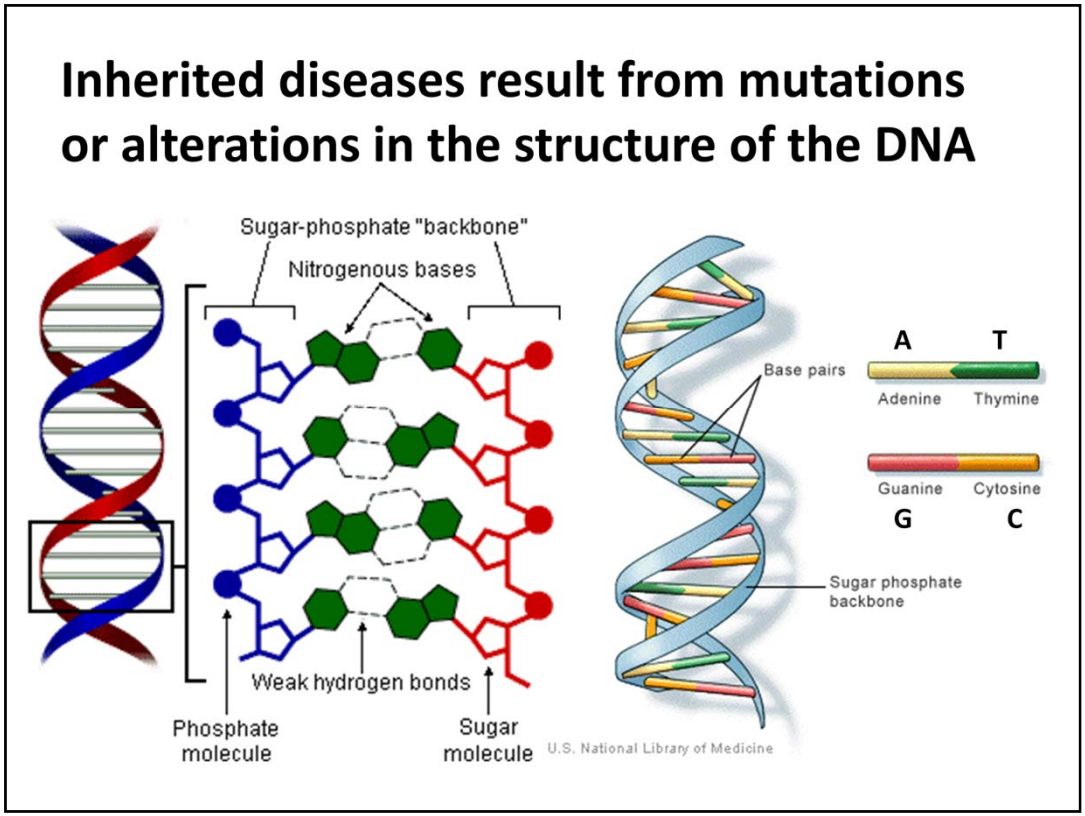
Fanconi syndrome
Renal resorbtion deficiency



In the first half of the 20th century, Dr. Guido Fanconi published detailed clinical descriptions of several heritable human diseases. Two disease syndromes were named after him: Fanconi Anemia and Fanconi Syndrome. Of the two, Fanconi Anemia is more important in human medicine. That is why it is important to refer to the Basenji disease as "Fanconi Syndrome." If you just say "Fanconi" or "Fanconi disease" many people will think you are referring to Fanconi Anemia.



Fanconi Syndrome is a kidney disease in which the beneficial small molecules in the blood that pass through the glomerular filter are not recovered by cells in the proximal tubules but instead are lost in the urine.

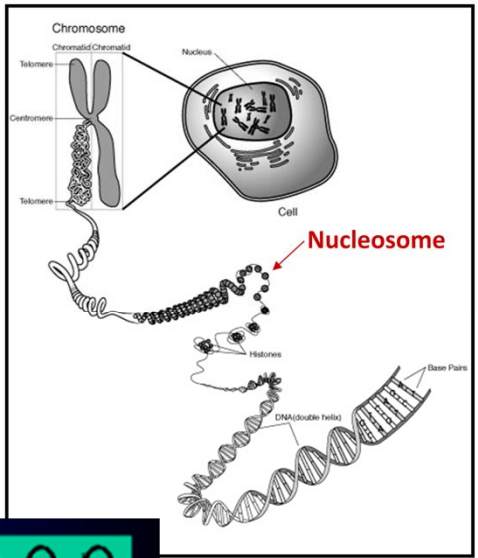


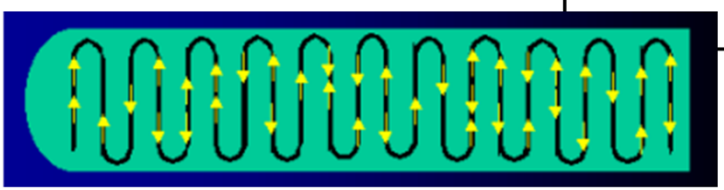
Almost all of the genetic information that is passed from one generation to the next is encoded in the order of nucleotide bases (A,C,G, or T) in the DNA.

Chromosomes: the **DNA-protein** complexes that contain the genome.

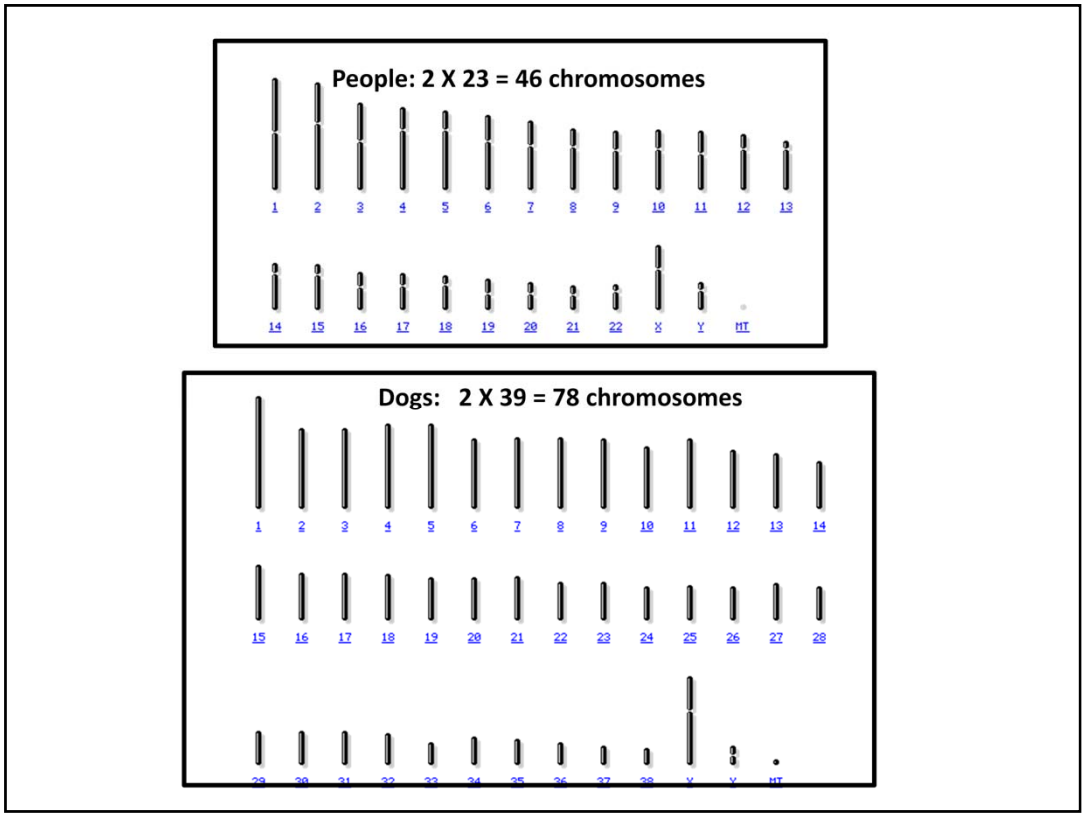
In eukaryotes, chromosomes are found in the nucleus.

A **single molecule** of coiled double-stranded DNA winds its way from one end of the chromosome to the other.



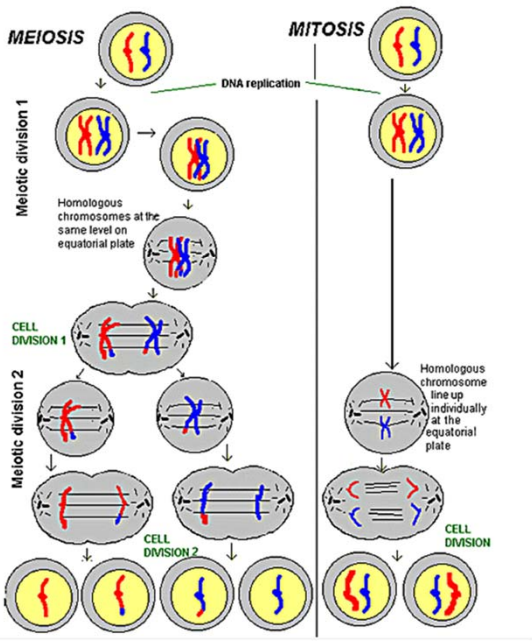


Most of the DNA occurs in chromosomes which are in the nucleus of cells. A single molecule of coiled, double-stranded DNA winds its way from one end of the chromosome to the other.

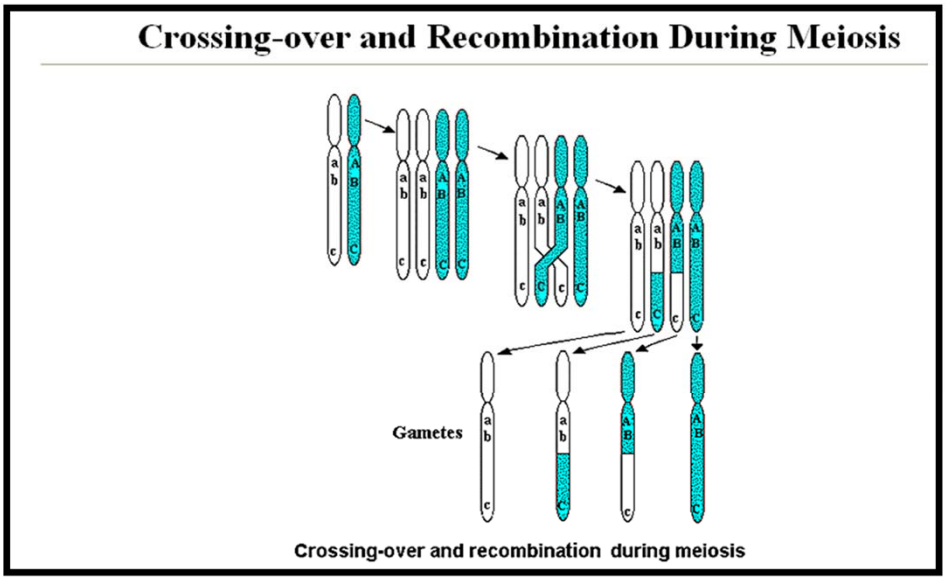


People have 23 pairs of chromosomes. Dogs have 39 pairs. The mother, or dam, contributes one chromosome to each pair. The father, or sire, contributes the other.

Meiotic Recombination



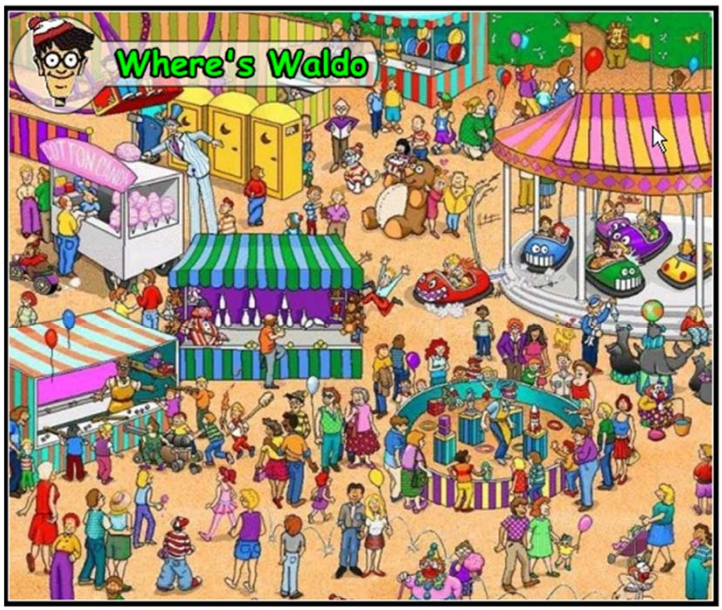
Some chromosomes are inherited intact from grandparent to offspring. Other chromosomes are inherited as a fusion of the grandmother's and the grandfather's chromosomes. The process in which segments of paired chromosomes are exchanged takes place during formation of the egg and the sperm cells and is referred to as meiotic recombination.



Recombination rarely occurs between markers that are very close together on the same chromosome but occurs more commonly between markers that are further apart .

Recombination rarely occurs between markers that are very close together on the same chromosome but occurs more commonly between markers that are further apart.

Identifying Mutations responsible for Inherited Diseases



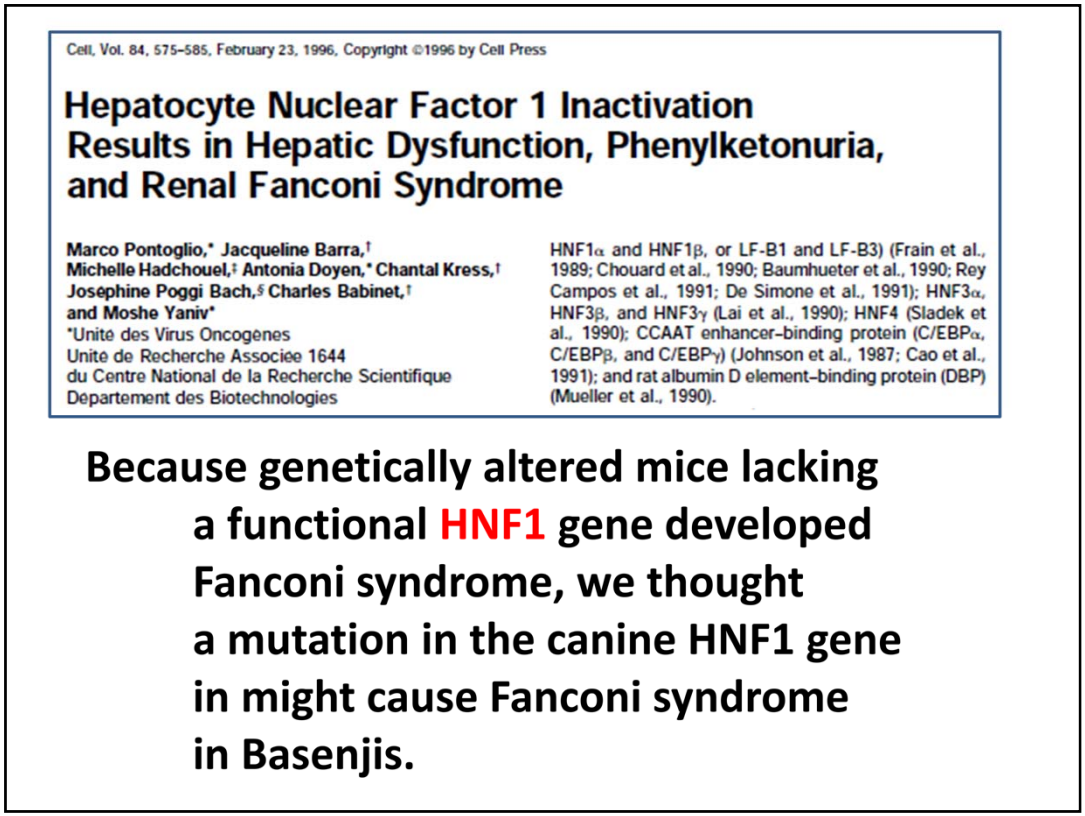
Length of the diploid genome: 6,000,000,000 bp
World population: 6,700,000,000 people

The mutation or change of a single nucleotide base in the DNA can result in an inherited disease. There are approximately 8 billion positions in a mammalian genome where such a change could occur. Coincidently, the world population is a little over 6 billion people. Thus, finding the cause of an inherited disease is like solving a giant "Where's Waldo?" game in which the entire world population is represented.

At the University of Missouri we offer the following DNA tests:

Disease	Breed and Species	Gene	Type of Test
Protoporphyria	Limosin cattle	*FECH*	Direct
Ceroid Lipofuscinosis	American Bulldog	*CTSD*	Direct
Ceroid Lipofuscinosis	Tibetan Terrier	*ATP13A2*	Direct
Fanconi Syndrome	Basenji dog	Unknown	Linked marker
Neonatal encephalopathy	Standard Poodle dog	*ATF2*	Direct
Degenerative myopathy	Several dog breeds	*SOD1*	Direct
Primary Lens Luxation	Several Terrier Breeds	*ADAMTS17*	Direct
Neonatal Cerebellar ataxia	Coton de Tulear	*GRM1*	Direct

Although finding the cause of an inherited disease is difficult, it can be done. This slide shows some of the mutations discovered at the University of Missouri that are the basis of DNA tests we offer for a fee. Note that a direct test was offered for all diseases except Basenji Fanconi Syndrome. Prior to 29 August 2011, we offered an indirect test for this disease because we had not yet identified the mutation that caused the Fanconi Syndrome.



Cell, Vol. 84, 575–585, February 23, 1996, Copyright ©1996 by Cell Press

Hepatocyte Nuclear Factor 1 Inactivation Results in Hepatic Dysfunction, Phenylketonuria, and Renal Fanconi Syndrome

Marco Pontoglio,* Jacqueline Barra,† Michelle Hadchouel,‡ Antonia Doyen,* Chantal Kress,† Josephine Poggi Bach,§ Charles Babinet,† and Moshe Yaniv*
*Unité des Virus Oncogènes
Unité de Recherche Associée 1644
du Centre National de la Recherche Scientifique
Département des Biotechnologies

HNF1α and HNF1β, or LF-B1 and LF-B3) (Frain et al., 1989; Chouard et al., 1990; Baumhueter et al., 1990; Rey Campos et al., 1991; De Simone et al., 1991); HNF3α, HNF3β, and HNF3γ (Lai et al., 1990); HNF4 (Sladek et al., 1990); CCAAT enhancer–binding protein (C/EBPα, C/EBPβ, and C/EBPγ) (Johnson et al., 1987; Cao et al., 1991); and rat albumin D element–binding protein (DBP) (Mueller et al., 1990).

Because genetically altered mice lacking a functional HNF1 gene developed Fanconi syndrome, we thought a mutation in the canine HNF1 gene in might cause Fanconi syndrome in Basenjis.

We began our search for the mutation responsible for Basenji Fanconi Syndrome back in the 1990s when we learned that a gene called HNF1 could cause Fanconi Syndrome in mice.

Canine Health Foundation Grant

0001445: Evaluation of the Canine HNF1 and HNF2 Genes as Candidates for the Locus Causing Fanconi Syndrome in Basenjis

Grant Status: Closed

Grant Amount: $16,000

Dr. Gary S. Johnson, DVM PhD, University of Missouri, Columbia

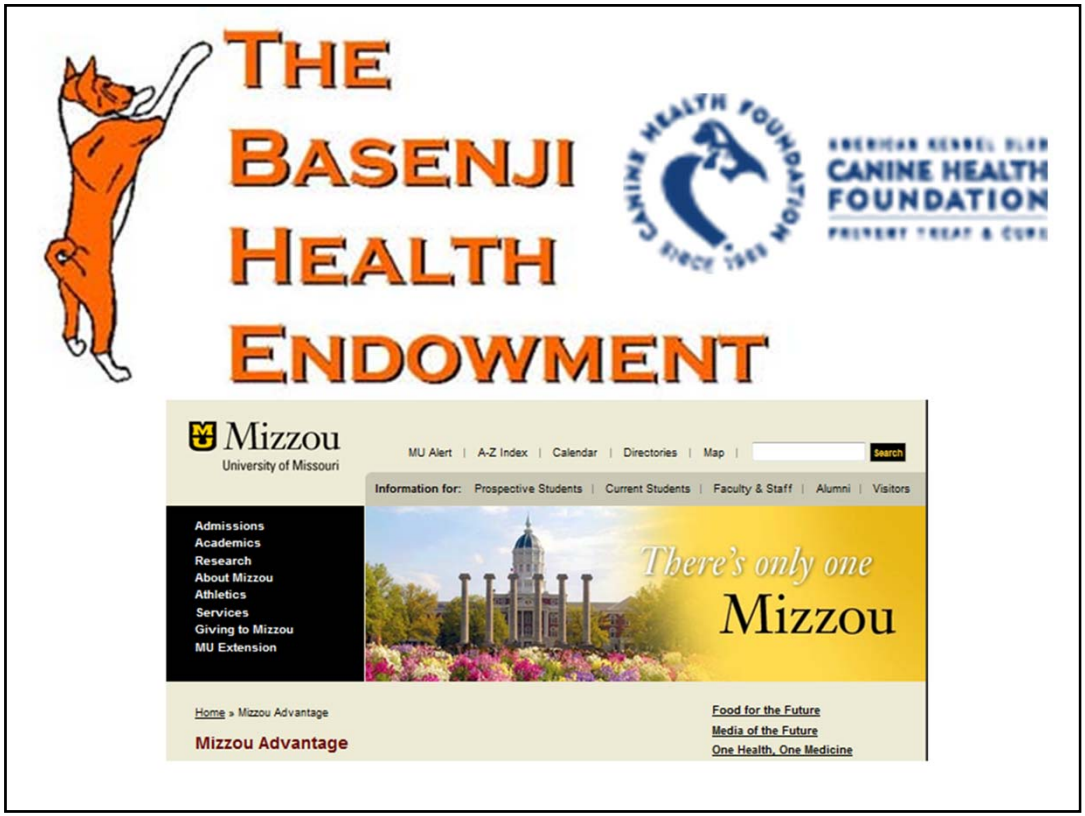
May 19, 1997 - May 18, 1998

Sponsor(s): Basenji Club of America, Inc. & Basenji Health Endowment

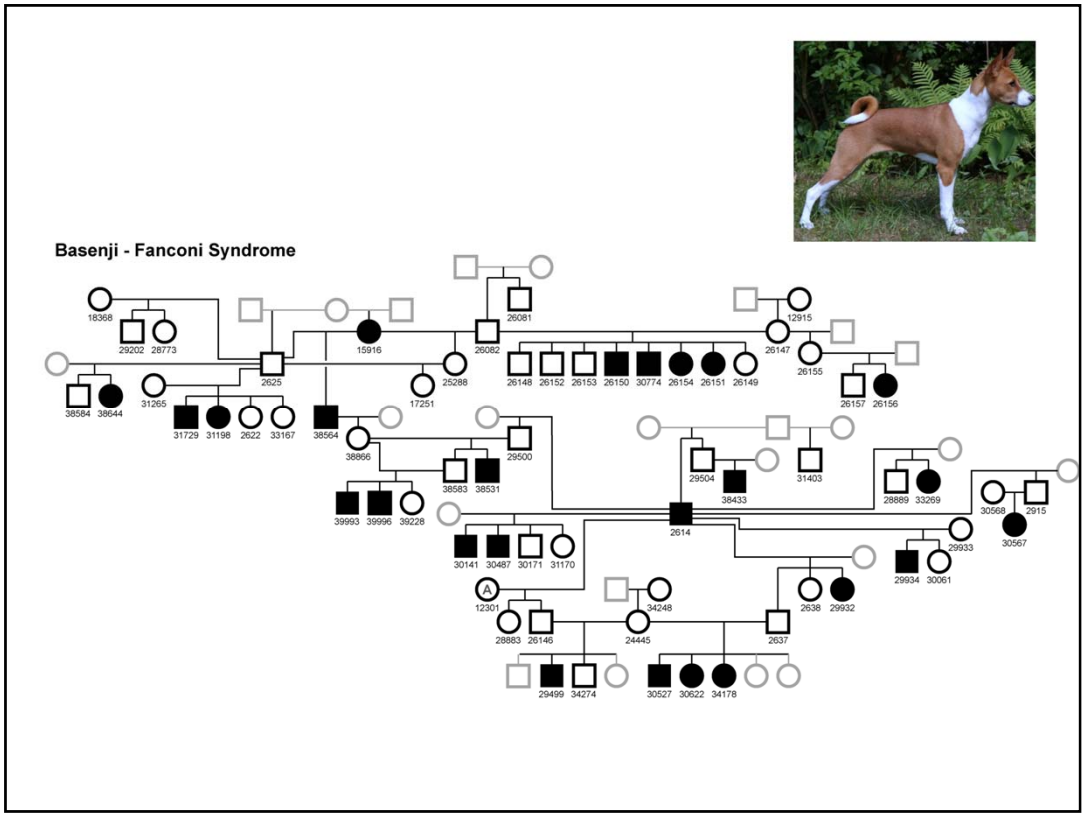
Breed(s): Basenji

Disease(s): Fanconi Syndrome

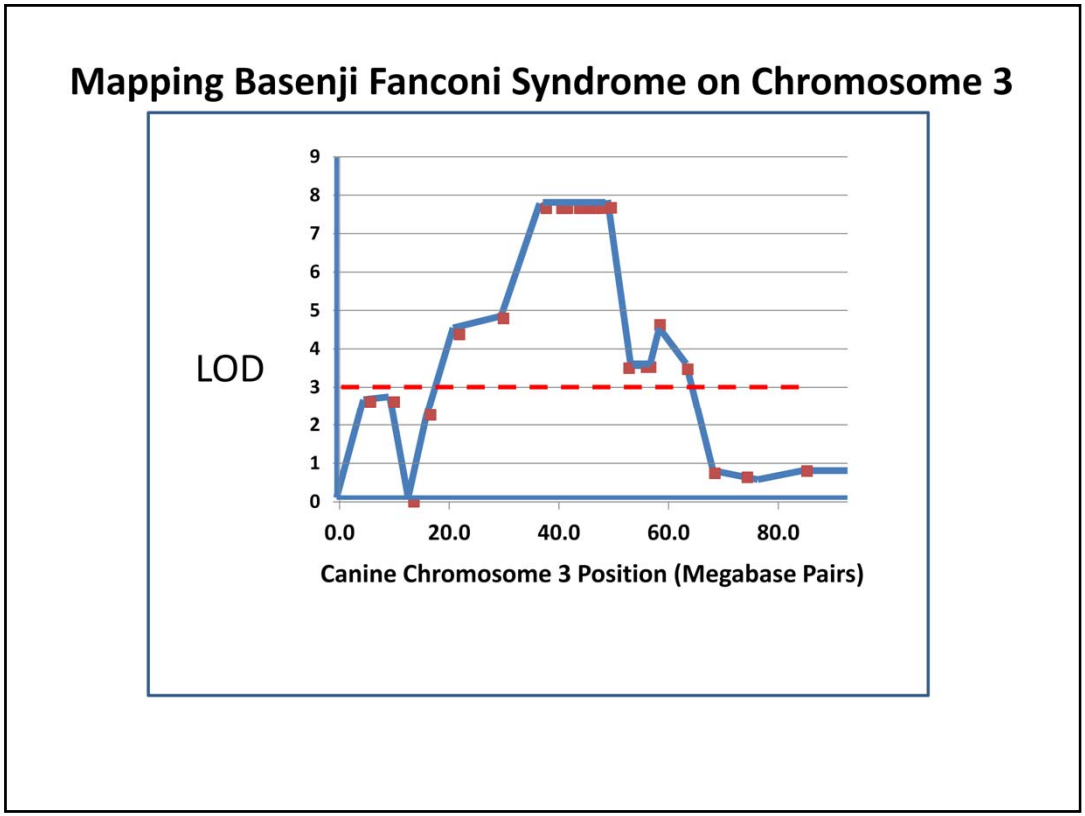
Our first grant was from the Canine Health Foundation with matching funds from the Basenji Club of America and the Basenji Health Endowment.



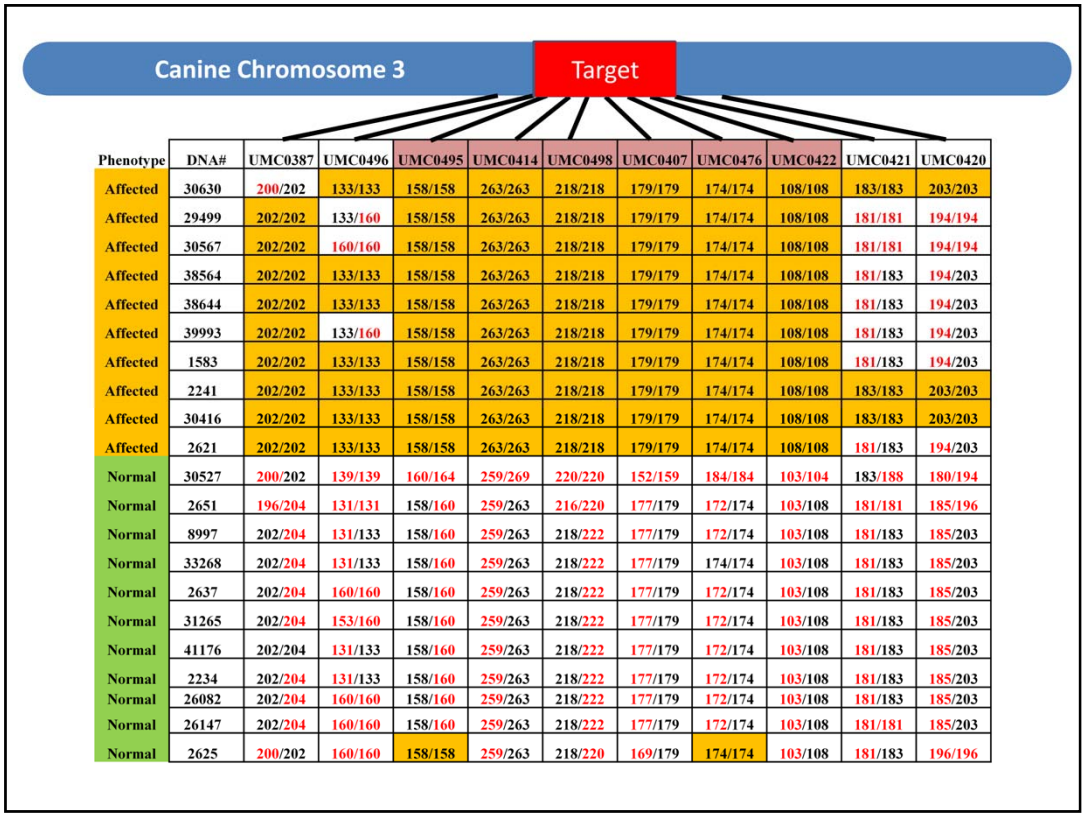
Since then we have received additional support from the Basenji Health Endowment and the AKC Canine Health Foundation. Most recently we obtained funds to complete the project from the University of Missouri in the form of a Mizzou Advantage Grant.



Although it turned out that the HNF1 had nothing to do with Basenji Fanconi Syndrome, that initial study allowed us to accumulate DNA samples from related Basenjis, some that had Fanconi Syndrome and some that remained normal when they reached old age. We used these samples to determine the chromosomal location of the mutation. This was done with a panel of approximately 300 marker assays representing locations scattered throughout all of the canine chromosomes. We looked for marker results that had patterns of distribution that were similar to the distribution of the disease in the Basenji pedigree.

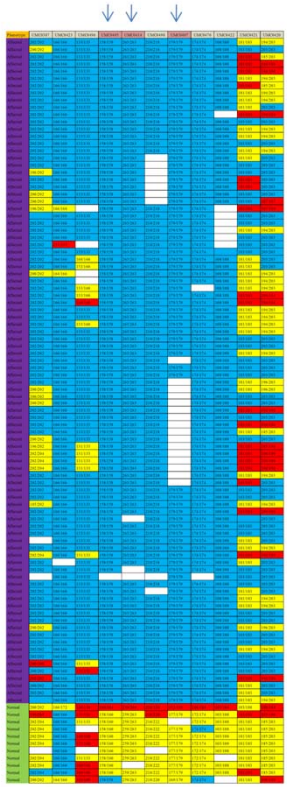


All of the marker test results with patterns of distribution that were similar to the distribution of the disease in the Basenji pedigree represented locations on Canine Chromosome 3.



Phenotype	DNA#	UMC0387	UMC0496	UMC0495	UMC0414	UMC0498	UMC0407	UMC0476	UMC0422	UMC0421	UMC0420
Affected	30630	200/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	183/183	203/203
Affected	29499	202/202	133/160	158/158	263/263	218/218	179/179	174/174	108/108	181/181	194/194
Affected	30567	202/202	160/160	158/158	263/263	218/218	179/179	174/174	108/108	181/181	194/194
Affected	38564	202/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	181/183	194/203
Affected	38644	202/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	181/183	194/203
Affected	39993	202/202	133/160	158/158	263/263	218/218	179/179	174/174	108/108	181/183	194/203
Affected	1583	202/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	181/183	194/203
Affected	2241	202/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	183/183	203/203
Affected	30416	202/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	183/183	203/203
Affected	2621	202/202	133/133	158/158	263/263	218/218	179/179	174/174	108/108	181/183	194/203
Normal	30527	200/202	139/139	160/164	259/269	220/220	152/159	184/184	103/104	183/188	180/194
Normal	2651	196/204	131/131	158/160	259/263	216/220	177/179	172/174	103/108	181/181	185/196
Normal	8997	202/204	131/133	158/160	259/263	218/222	177/179	172/174	103/108	181/183	185/203
Normal	33268	202/204	131/133	158/160	259/263	218/222	177/179	174/174	103/108	181/183	185/203
Normal	2637	202/204	160/160	158/160	259/263	218/222	177/179	172/174	103/108	181/183	185/203
Normal	31265	202/204	153/160	158/160	259/263	218/222	177/179	172/174	103/108	181/183	185/203
Normal	41176	202/204	131/133	158/160	259/263	218/222	177/179	172/174	103/108	181/183	185/203
Normal	2234	202/204	131/133	158/160	259/263	218/222	177/179	172/174	103/108	181/183	185/203
Normal	26082	202/204	160/160	158/160	259/263	218/222	177/179	172/174	103/108	181/183	185/203
Normal	26147	202/204	160/160	158/160	259/263	218/222	177/179	172/174	103/108	181/181	185/203
Normal	2625	200/202	160/160	158/158	259/263	218/220	169/179	174/174	103/108	181/183	196/196

To narrow down the target region, we developed many more Chromosome 3 markers. Six consecutive markers on Chromosome 3 gave identical test results for all afflicted Basenjis, which defined our target region.



The Linked Marker Test for Fanconi Syndrome

	Markers		
Reported Result	UMC0495	UMC0414	UMC0407
Affected	M/M	M/M	M/M
Carrier	M/N	M/N	M/N
Carrier	M/N	M/N	M/M
Carrier	M/N	M/M	M/N
Carrier	M/M	M/N	M/N
Carrier	M/M	M/M	M/N
Carrier	M/N	M/M	M/M
Carrier	M/M	M/N	M/M
Normal	N/N	N/N	N/N
Normal	N/N	N/N	M/M
Normal	N/N	M/M	N/N
Normal	M/M	N/N	N/N
Normal	N/N	N/N	M/N
Normal	N/N	M/N	N/N
Normal	M/N	N/N	N/N
Indeterminate	M/M	M/N	N/N
Indeterminate	N/N	M/M	M/N
Indeterminate	N/N	M/N	M/M

In an effort to further narrow the target region we tested over 80 afflicted Basenjis, but all 80 gave consistent results for all six markers. At that time we were asked if we could offer something for Basenji breeders for the 2007 breeding season. Based on this data, we offered a linked marker test that estimated the disease status based on the genetic background determined by three of the six markers. Since each marker produces two test results (one from Chromosome 3 inherited from the dam and one from Chromosome 3 inherited from the sire), there are six test results to interpret. If all six results were from the afflicted genetic background, we reported a test result of 'Probably Affected.' If none of the six results were from the afflicted genetic background, we reported a test result of 'Probably Normal.' If the results indicated that a mutant background and a normal background were represented at each of the three markers, we reported the test result of 'Probably Carrier.' When the three markers were not in complete agreement, we reported the most likely interpretation. When one marker indicated that the dog had only the afflicted genetic background, another marker indicated that the dog had both normal and afflicted backgrounds, and the third marker only the normal background, we didn't know what was the most likely interpretation and we reported a test result of 'Indeterminate.'

Linked Marker Test Results

	Markers		
Reported Result	UMC0495	UMC0414	UMC0407
Affected	M/M	M/M	M/M
Carrier	M/N	M/N	M/N
Carrier	M/N	M/N	M/M
Carrier	M/N	M/M	M/N
Carrier	M/M	M/N	M/N
Carrier	M/M	M/M	M/N
Carrier	M/N	M/M	M/M
Carrier	M/M	M/N	M/M
Normal	N/N	N/N	N/N
Normal	N/N	N/N	M/M
Normal	N/N	M/M	N/N
Normal	M/M	N/N	N/N
Normal	N/N	N/N	M/N
Normal	N/N	M/N	N/N
Normal	M/N	N/N	N/N
Indeterminate	M/M	M/N	N/N
Indeterminate	N/N	M/M	M/N
Indeterminate	N/N	M/N	M/M

Year	Normal	Carrier	Affected	Indeter-minate	Total	% Affected
2007	791	584	112	60	1547	7.2%
2008	460	309	43	60	872	4.9%
2009	391	236	39	50	716	5.4%
2010	383	258	29	21	691	4.7%
2011	279	178	16	18	491	3.2%
Total	2304	1565	239	209	4317	5.5%

We had hoped that the linked marker test would soon be replaced by a direct test; however, that did not happen. In the last four years we tested over 2,300 Basenjis. Note that the percentage of “Probably Affected” Basenjis dropped from 7.2% in 2007 to 3.2% in 2011. While we do not know how well the tested population of Basenjis represents the entire population, we believe that this drop indicates that many Basenji breeders have used the linked marker test to guide their breeding strategies to reduce the likelihood that Fanconi Syndrome will occur in the Basenjis they breed.

Costs for Whole-Genome Sequencing

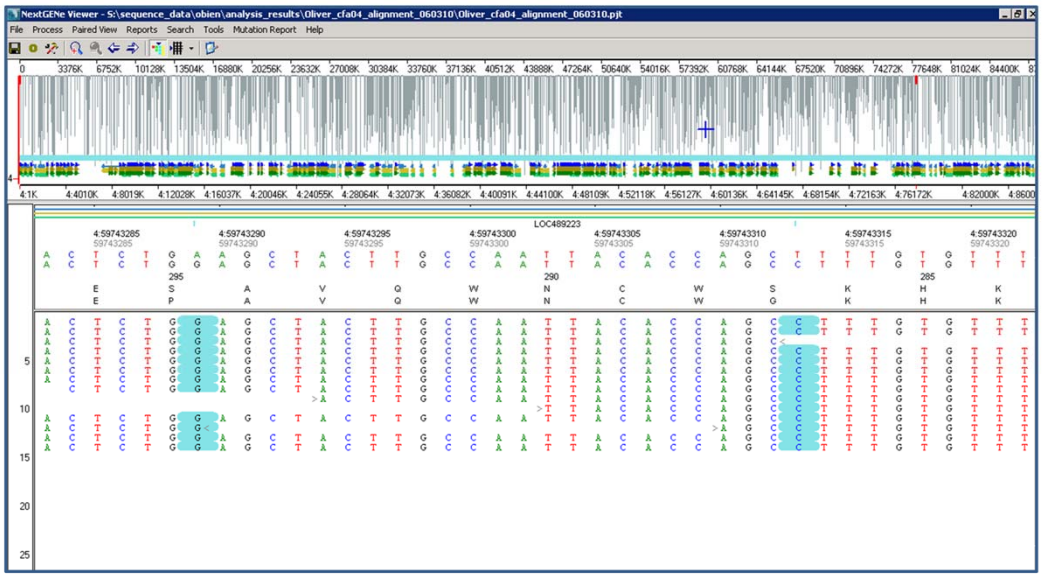
Cost of the Human Genome (2001)
Sequence -- $3,000,000,000

Cost of the Dog Genome (2005)
Sequence -- $50,000,000

Cost of the Rhesus Macaque Genome (2007)
Sequence – $25,000,000

Current cost for re-sequencing the genome of an individual dog -- $6,000

Our next strategy was to produce a whole genome sequence from an afflicted Basenji. This was possible because of spectacular advances in DNA sequencing technology which greatly reduced the cost.



NextGene software shows reads from the Illumina sequencer aligned to the canine genome reference sequence

The sequences from an afflicted Basenji were aligned to a previously assembled Boxer sequence, producing approximately 8 million chromosomal positions where the sequences differ.

Mission accomplished:

Basenji Syndrome is caused by a 370 base pair deletion on canine chromosome 3.

WE FOUND WALDO. Inspection of the entire target region with data from the whole genome revealed that Basenji Fanconi Syndrome is caused by a 370 base pair deletion on Canine Chromosome 3.

We have used Illumina NextGeneration technology to generate whole-genome sequences for 10 individual dogs each with a different disease.

Name	Breed	Coverage	Disease	Inheritance
Jasper	Chinese Crested	20.08	Multiple system degeneration	AR
Oliver	Standard Poodle	13.85	Polymicrogyria	AR
Skittles	Airedale	15.90	Cerebellar ataxia	AR
Miranda	Basenji	12.75	Fanconi syndrome	AR
Maggie	Soft Coated Wheaten Terrier	19.68	Paroxysmal dyskinesia	AR
Alistair	Kerry Blue Terrier	23.06	Cerebellar ataxia	AR
Clifford	English Coker Spaniel	10.54	Degenerative myelopathy	AR
Katie	Miniature Schnauzer	7.61	Neuronal ceroid lipofuscinosis	AR
Dandy	Labrador Retriever	11.28	Laryngeal paralysis	Unknown
Tansis	Chinook	9.06	Dyskinesia/epilepsy	Unknown

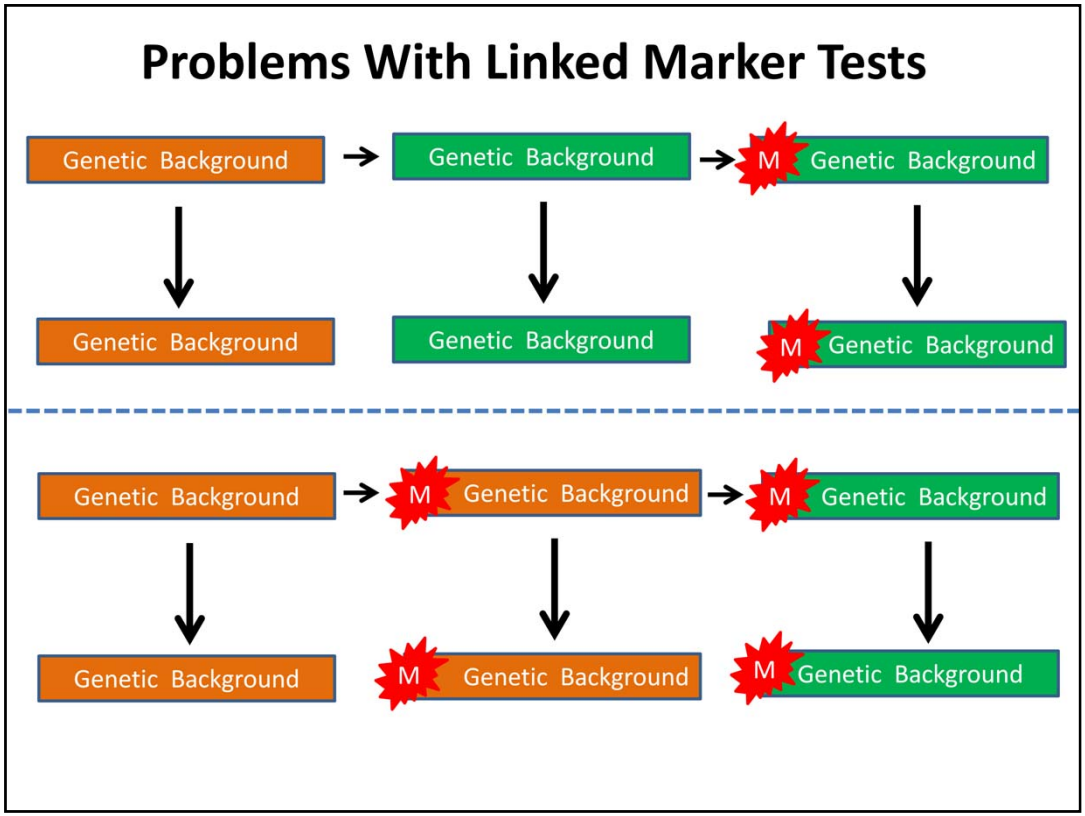
We have used this data to identify the causal mutation for Basenji Fanconi Syndrome and are currently evaluating a plausible sequence variant that may be responsible for dyskinesia/epilepsy of Chinooks

The Basenji whole genome sequence is one of 10 we have generated at the University of Missouri. It is the first one we have solved and so far was we know, it is the first heritable dog disease solved by whole-genome sequencing.

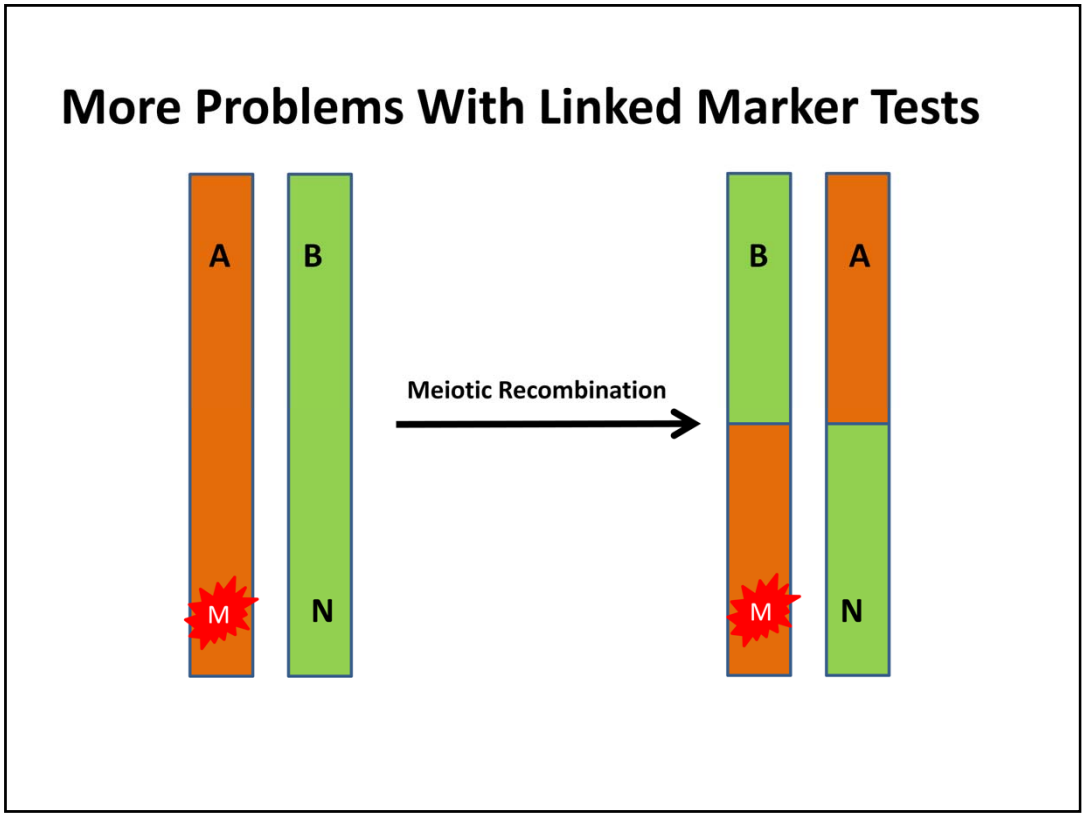
At the University of Missouri we offer the following DNA tests:

Disease	Breed and Species	Gene	Type of Test
Protoporphyria	Limosin cattle	*FECH*	Direct
Ceroid Lipofuscinosis	American Bulldog	*CTSD*	Direct
Ceroid Lipofuscinosis	Tibetan Terrier	*ATP13A2*	Direct
Fanconi Syndrome	Basenji dog	Censored	Direct
Neonatal encephalopathy	Standard Poodle dog	*ATF2*	Direct
Degenerative myopathy	Several dog breeds	*SOD1*	Direct
Primary Lens Luxation	Several Terrier Breeds	*ADAMTS17*	Direct
Neonatal Cerebellar ataxia	Coton de Tulear	*GRM1*	Direct

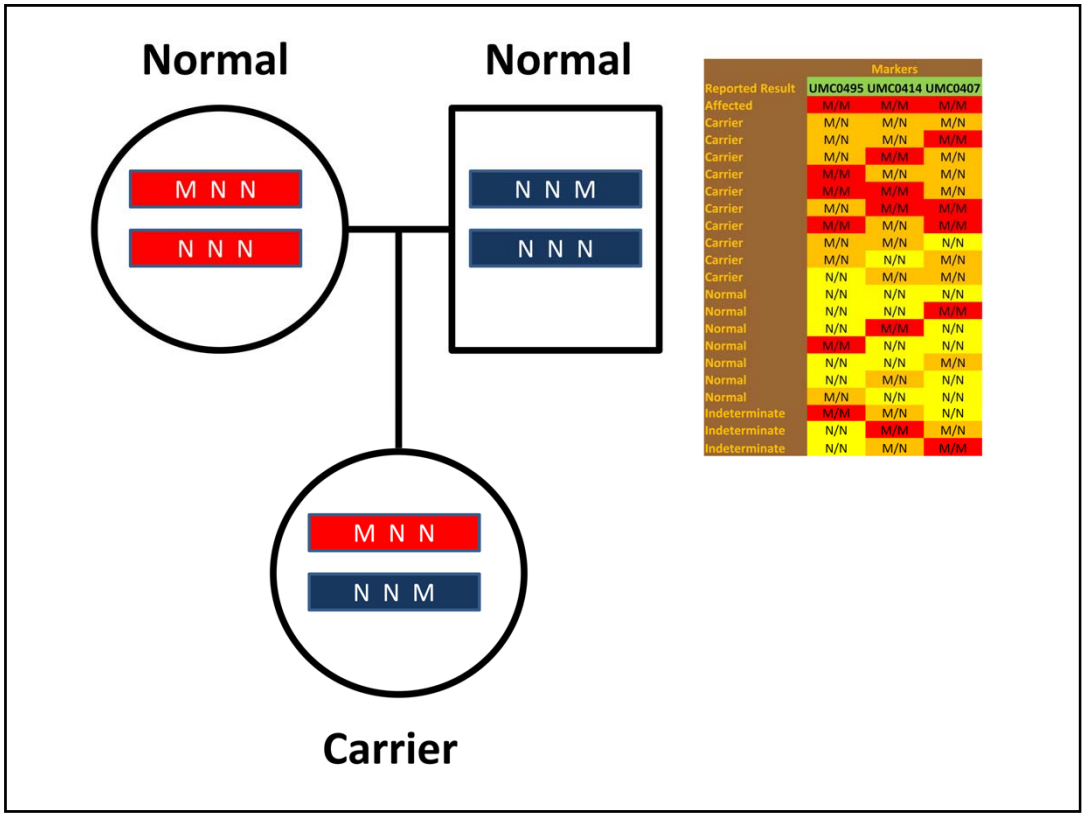
As of 29 August 2011 we replaced the linked marker test for Basenji Fanconi Syndrome with a direct test. We are not revealing the mutant gene until we are ready to publish.



The direct test should be a big improvement over the linked marker test which detects the genetic background, not the mutation itself. Linked marker tests have inherent flaws. For instance, if, as illustrated at the top of the slide, the chromosomal changes leading to the unique genetic background detected by the linked marker test take place before the disease-causing mutation, then some normal dogs may test positive. If, as illustrated at the bottom of the slide, the chromosomal changes leading to the unique genetic background detected by the linked marker test take place after the disease-causing mutation, then some affected dogs may test negative.



Another problem is that recombination can take place between the genetic background detected by the linked marker test. This can lead to both false positive and false negative test results.



	Markers		
Reported Result	UMC0495	UMC0414	UMC0407
Affected	M/M	M/M	M/M
Carrier	M/N	M/N	M/N
Carrier	M/N	M/N	M/M
Carrier	M/N	M/M	M/N
Carrier	M/M	M/N	M/N
Carrier	M/M	M/M	M/N
Carrier	M/N	M/M	M/M
Carrier	M/M	M/N	M/M
Carrier	M/N	M/N	N/N
Carrier	M/N	N/N	M/N
Carrier	N/N	M/N	M/N
Normal	N/N	N/N	N/N
Normal	N/N	N/N	M/M
Normal	N/N	M/M	N/N
Normal	M/M	N/N	N/N
Normal	N/N	N/N	M/N
Normal	N/N	M/N	N/N
Normal	M/N	N/N	N/N
Indeterminate	M/M	M/N	N/N
Indeterminate	N/N	M/M	M/N
Indeterminate	N/N	M/N	M/M

This slides illustrates another feature of the previously used linked marker test. The reported results were based on three different markers located close to each other on chromosome 3. Usually all three markers were in agreement; however, sometimes these markers disagreed with each other. When two of the three markers were in agreement, our reported result was based on the two agreeing markers. When all three markers disagreed, we reported the test was indeterminate.

In the family shown here a sire and dam that were normal by our criteria produced an offspring that was a carrier by our criteria. This apparent discrepancy was not caused by laboratory error. It occurred because linked marker tests are not always accurate. If I were a Basenji breeder, I would want to retest my breeding stock with the new DNA test to ensure that I was not producing puppies destined to Fanconi Syndrome.

Linked Marker Test Results:

- **Probably Normal**
- **Probably Carrier**
- **Probably Affected**
- **Indeterminant**

New Fanconi Syndrome Test

- **Normal**
- **Carrier**
- **Affected**

The word "Probably" will not be used in the new test results and there will no longer be an "Indeterminate" result.

New test is currently available from The OFA

Linked marker test is no longer available

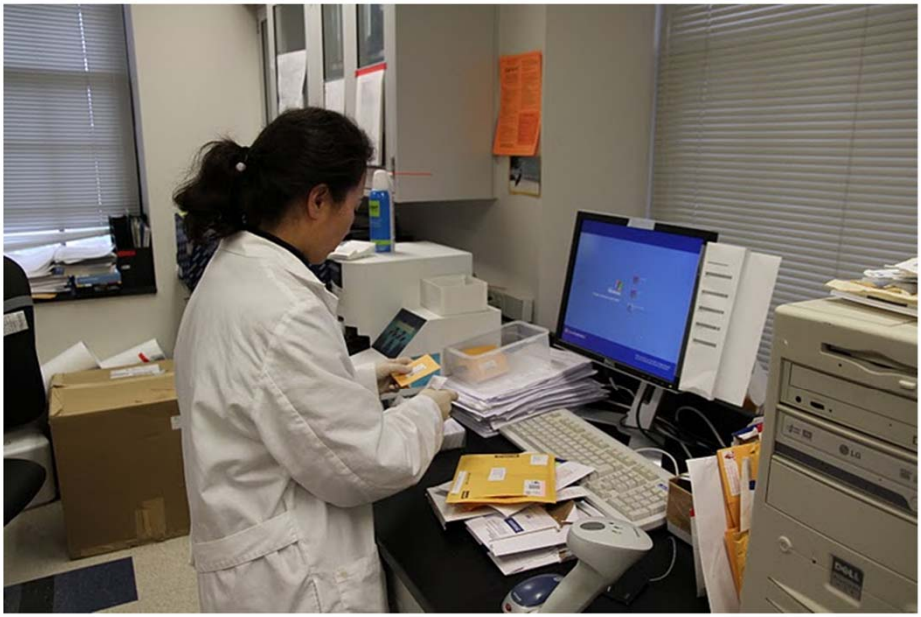
The Basenji Health Endowment will supplement the costs for retests

The new test will cost $65.00

The Basenji Health Endowment's supplement for retesting Basenjis previously tested by the linked marker test is $15.00

The cost for a retest is $50.00

When possible, retests will be done on previously submitted samples



Tests are usually done by Dr. Ginny Guo

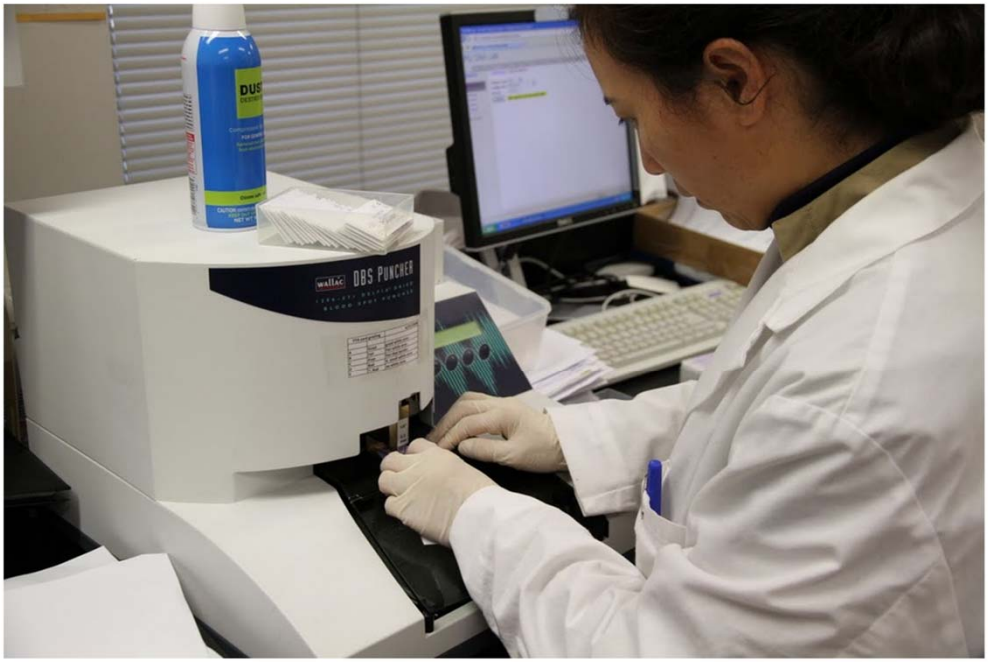
The Fanconi Syndrome tests are usually done by Dr. Jenny Guo who is shown here logging in FTA cards with the aid of a barcode reader.

Jenny stores the logged in cards in a bin until she is ready to test them. She usually does Fanconi Syndrome tests once a week.

When she is ready to perform the test, she scans the cards again so that the computer knows which dogs are being tested.

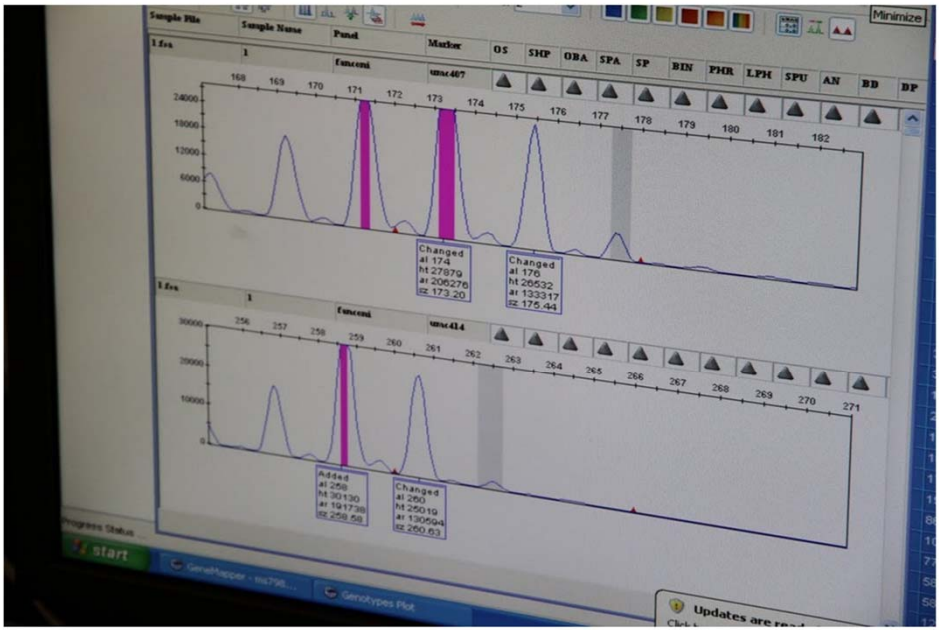

FTA card grading
Good
Fair
Poor
Bad
V. Bad
A
B
C
D
E
good white area
Fair white area
Fair-Bad border
V. small white area
no white area
1/8"
3.2
mm

Here Jenny has an FTA card and is ready to start the analysis.



Next she places the cards in a Wallac puncher which cuts disks in the FTA card. The disks drop into a plastic plate with 96 wells. The computer keeps track of which dog's samples are in each well.

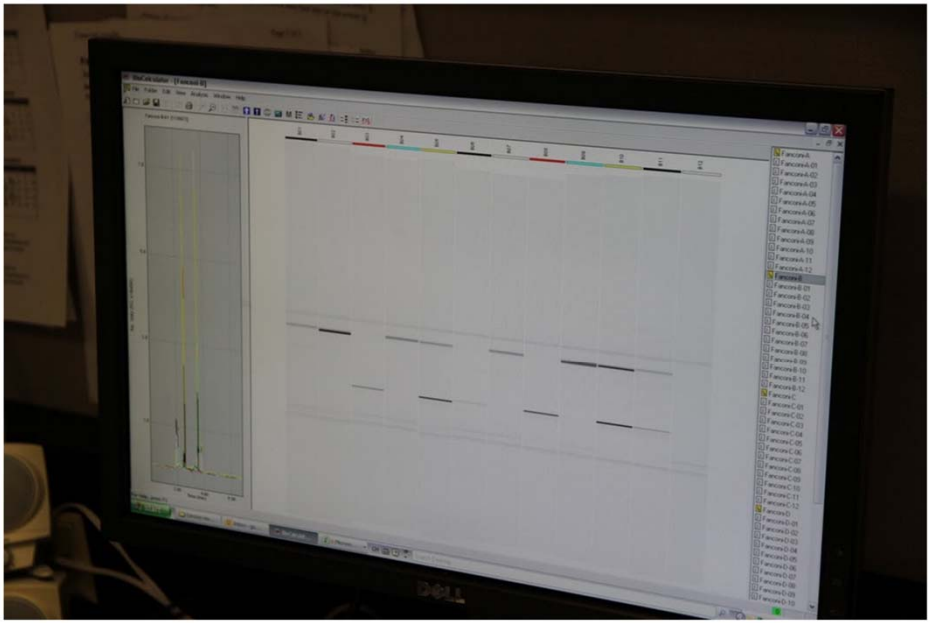
Here is what a card looks like after four disks have been punched out.



Each of the three markers in the linked marker test produced a complex pattern of peaks that were compared and interpreted.

With the new test the samples, still in a 96 well plate, are amplified with an instrument called a thermocycler.

Next the same 96 well plate is placed into another instrument where the amplified DNA is separated by size.



The results for the new direct test are much easier to interpret. Normal dogs are represented by a single band. Affected dogs also produce a single band that is lower on the computer screen. Both bands appear in tests of Carriers with one normal gene and one affected gene.

The OFA will issue certificates reflecting the new test results.

After the tests are completed, the FTA cards are stored in binders and can be efficiently recovered. In most cases we expect to be able to do retesting with the cards already in our possession. Nonetheless, in some cases there will be insufficient DNA on the stored cards and we will have to request new samples.

Liz Hansen

This is Liz Hansen. Many of you know her from the dog show circuit. She is the one most likely to take your call if you phone our laboratory.

Future Studies

Investigation of the mechanisms by which the newly found mutation causes kidney disease

Investigate the molecular genetic causes of progressive retinal atrophy (PRA) in Basenjis

Generate a *de novo* assembly of the Basenji Genome

Fabiana H. G. Farias

Most of the laboratory experiments on Basenji Fanconi Syndrome were conducted by Ms. Fabiana Farias. Fabiana is a graduate student from Brazil. She will graduate this winter and the Basenji work will be one of three discoveries of disease-causing mutations described in her PhD thesis.

Special Thanks to Jon Curby

Throughout the 15 years we have worked on Basenji Fanconi Syndrome, Jon Curby has played an essential role in all aspects of the research. We have never before had such excellent cooperation from a breed enthusiast and we are extremely grateful for the hundreds of hours he devoted to this effort.