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

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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.
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.
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.
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.
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.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Breed and Species</th>
<th>Gene</th>
<th>Type of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoporphyrja</td>
<td>Limosin cattle</td>
<td>FECH</td>
<td>Direct</td>
</tr>
<tr>
<td>Ceroid Lipofuscinosis</td>
<td>American Bulldog</td>
<td>CTSD</td>
<td>Direct</td>
</tr>
<tr>
<td>Ceroid Lipofuscinosis</td>
<td>Tibetan Terrier</td>
<td>ATP13A2</td>
<td>Direct</td>
</tr>
<tr>
<td>Fanconi Syndrome</td>
<td>Basenji dog</td>
<td>Unknown</td>
<td>Linked marker</td>
</tr>
<tr>
<td>Neonatal encephalopathy</td>
<td>Standard Poodle dog</td>
<td>ATF2</td>
<td>Direct</td>
</tr>
<tr>
<td>Degenerative myopathy</td>
<td>Several dog breeds</td>
<td>SOD1</td>
<td>Direct</td>
</tr>
<tr>
<td>Primary Lens Luxation</td>
<td>Several Terrier Breeds</td>
<td>ADAMTS17</td>
<td>Direct</td>
</tr>
<tr>
<td>Neonatal Cerebellar ataxia</td>
<td>Coton de Tulear</td>
<td>GRM1</td>
<td>Direct</td>
</tr>
</tbody>
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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.

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

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
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.
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.
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.'
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.
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.

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### 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
The sequences from an afflicted Basenji were aligned to a previously assembled Boxer sequence, producing approximately 8 million chromosomal positions where the sequences differ.
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.
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.
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.
This slide 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.
<table>
<thead>
<tr>
<th>Linked Marker Test Results:</th>
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<tbody>
<tr>
<td>Probably Normal</td>
</tr>
<tr>
<td>Probably Carrier</td>
</tr>
<tr>
<td>Probably Affected</td>
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<tr>
<td>Indeterminant</td>
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<table>
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<tr>
<th>New Fanconi Syndrome Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Carrier</td>
</tr>
<tr>
<td>Affected</td>
</tr>
</tbody>
</table>

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.
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.
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
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.
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.