**Incidence of Cystinuria in North American Irish Terriers – ©ITCA 2016**

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**Cystine** [**sis**-teen, -tin]

Cystine, an amino acid (240.29 g/mol), is one of the building blocks of proteins. It is in the food we eat and makes up about 10% of skin and hair (Block, 1939). In blood it is found at a concentration of about 10 – 15 mg/L plasma (Hack, 1997). It is synthesized from two molecules of cysteine [**sis**-tee-een, -in] which is found in much lower (2-4 mg/L) concentrations. In the kidney, cystine is filtered into the urine but is almost completely reabsorbed in the proximal tubules (Fernandez, 2002 ). *(In the kidney, water, waste products and other small molecules are filtered from the blood and enter the renal tubules where water and specific molecules are reabsorbed or exchanged as necessary to produce the final urine.)*

**Cystinuria** [sis-tin-**yoo-**ree-*a*]

Cystinuria (**CU**) means that there is an abnormally high concentration of cystine in the dog's urine. This can result in the precipitation of solid cystine crystals and the formation of stones which can restrict the flow of urine from the bladder, and can cause bloody urine, urinary obstruction, renal failure and death (Brons, 2013).

**Cause**

Cystinuria is caused by the failure of reabsorption of cystine in the kidney. The reabsorption function of some other amino acids is also affected but cystine is less soluble and more likely to form stones. Throughout the dog (and other animal) populations there appear to be a number of genes involved in regulating the reabsorption process and a mutation in any of these genes can result in the disease. Defective genes have been identified in humans and some dog breeds (Brons, 2013). *(Genes are regions of the long strings of DNA that make up chromosomes. In each of these regions the DNA is coded to assemble a specific protein required for normal body structure or function. Dogs have 39 pairs of chromosomes in each cell with matching pairs of about 21,000 genes. Mutations occur when the code is changed so the protein is different. Many changes are harmless and make up the differences (e.g., hair color) between individuals, except for twins with identical genes. Other changes may cause a failure in metabolic function, resulting in a genetic disease.)* **Unfortunately, although we have known for a long time that there must be a gene mutation responsible for cystinuria in Irish Terriers, it is different from those which have been discovered for other breeds and has not yet been identified.** This could be because the mutation was introduced and spread from one dog as the lineage of the Irish Terrier (isolated for the most part in Ireland) developed separately from those other breeds.

Since most cells of the body carry two copies of each gene, if one copy of a gene has a recessive defect (doesn't work normally but doesn't produce anything additional that alters normal function) the other unaffected gene can maintain its role in organ function (reabsorption of cystine in the kidney). If both copies of the gene are defective (**CU** genes), the function fails. When the cell divides, both copies of the gene are duplicated so they can be passed to each daughter cell. Reproductive cells (egg or sperm), however, are produced by reduction division and only one of the copies is passed on. Dogs with one **CU** gene (carriers) are unaffected themselves but will pass on either the defective or normal gene to an offspring. Dogs with two **CU** genes always pass on a defective gene. During fertilization, the genes from the parents recombine so the offspring again has two copies in each cell. If there is no genetic test to detect the **CU** gene directly, only affected dogs with clinical signs of the disease can be identified as

having two defective genes. We know, however that, the parents and offspring of affected dogs must be carriers at least.

In many other breeds, both males and females can be affected (Giger, 2015) (Brons, 2013). In Irish Terriers suffer from a form of cystinuria known as Type III, where the effect is more complicated as it appears to be dependent on testosterone, a hormone normally present only in mature males. Thus, females and immature (or castrated) male dogs may have two defective genes but still have normal kidney function because testosterone levels are low. The exact genetic cause is as yet unknown but we do know that testosterone is involved and, because of the inheritance patterns, that it is due to a recessive genetic defect*. (In the classical testosterone signaling pathway, testosterone diffuses into the cell and binds with androgen receptors allowing them to move to the nucleus where they bind to specific DNA sequences* (Mooradian AD, 1987 )*. This could allow the recruitment of activator or repressor proteins that increase or decrease the expression of genes and thus alter cellular function. Thus, testosterone could turn off normal gene function due to altered gene regulation.)*

**Clinical signs in Irish Terriers**

Male and female carriers are unaffected. Females and puppies with two defective genes are unaffected. As testosterone levels rise when affected male puppies mature, cystine levels rise to detectable levels by about one year of age, although this may be variable. Even in adult males levels may be variable among dogs and fluctuate in any one dog. Below the solubility limit of about 250 mg/L (depending on pH etc. (D.S. Goldfarb, March 02 2006)) the cystine is passed in solution. At higher levels small hexagonal crystals precipitate out but are easily passed. When larger stones form, the symptoms occur with inflammation or concurrent infection in the urinary tract. Bloody urine, frequent urination, painful urination, and straining are common signs. When stones pass into the urethra, life threatening urinary blockage may occur (Giger, 2015).

**Detection**

Urine test: The sodium cyanide–nitroprusside test (Brand's Test) is a qualitative determination (detects high levels but not the exact concentration) of cystine in the urine (Y. Nakagawa, 1999). Cyanide converts cystine to cysteine. Nitroprusside then binds, causing a purple hue in 2-10 minutes. The test detects cystine levels greater than about 75 mg/g of creatinine and is used for screening. Cystine is normally completely reabsorbed by the kidney and dogs with levels above 200 mg/g of creatinine are considered cystinuric. *(Creatinine is a waste product from muscle metabolism which is produced by the body at a relatively constant rate and is filtered into the urine. In dilute urine, due to a large water intake, the concentration of all solutes will be reduced so amino acid concentrations are often expressed as ratios to creatinine* (Hokamp, Feb 2016)*. This reduces the chance of false negative results in dilute urine specimens where concentrations are low just because there is a large volume of water. Concentrations can also be measured in weight per volume, e.g., mg/dL, or the molar concentration, e.g., µM.)* Cystine becomes less soluble in the urine if the pH decreases (more acidic) causing precipitation of crystals. More accurate measurement of cystine concentration can be obtained by chromatography (Wear, April 2005), but this is a much more expensive process and only used after a positive screening test if necessary.

Genetic test: The gene mutations responsible for cystinuria in some dog breeds have been identified and genetic tests have been developed so carriers and asymptomatic dogs can be identified. At present the mutation responsible for cystinuria in Irish Terriers has not been identified.

**Treatment**

All treatment should be done in consultation with a veterinarian who is familiar with Type III Cystinuria. When cystine stones block the urethra leading from the bladder, life threatening damage can occur to the renal system. (Giger, 2015) Catheterization can be used to help them pass or push them back into the bladder. They can be removed by surgery or broken up to allow them to pass. Treatment is recommended to prevent reoccurrence in these dogs and to prevent stone formation in all dogs with high cystine levels. Castration (surgical or with drugs) usually reduces cystine levels to normal. Drugs that combine with the cystine molecule to make it soluble (cystine-binding thiol drugs (D.S. Goldfarb, March 02 2006)) can help but can be expensive and have side effects. A high water intake will help keep the urine dilute and a reduced protein diet may keep levels low enough to prevent precipitation. Alkalinization, e.g., by adding potassium citrate to the diet, to keep the pH>7.5 will increase solubility, but going too high can increase the risk of calcium phosphate stones. In many affected dogs, the symptoms may not be severe but antibiotics may be required if inflammation occurs. In all cases, advice from an informed veterinarian is essential.

**How the genes are passed**

For the purposes of this discussion on cystinuria, the following definitions will be used:

**CU gene** – the recessive gene responsible for cystinuria in Irish Terriers.

**Unaffected** – No evidence of cystine in the urine.

**Affected** – A dog with high cystine levels in the urine as evidenced by a positive nitroprusside test, cystine crystals in the urine or cystine bladder stones (uroliths). These dogs will always have 2 **CU** genes.

**Pp** – the probability of passing on a **CU** gene. A dog will pass on one of its two genes. If it has 2 **CU** genes, Pp is 1.0. If it has 1 **CU** gene (carrier) Pp is 0.5.

**R2** – A dog with 2 recessive **CU** genes. In general only mature intact males will be affected, but even this may vary in severity. R2 males and females will always pass a CU gene to their offspring (Pp=1.0). Since they have to have received a **CU** gene from each of their parents, those parents both have to have at least one **CU** gene (R1).

**R1** – A dog with 1 recessive CU gene and one normal. These dogs are **carriers.** They are unaffected but there is a 50/50 chance the recessive gene will be passed to each of their offspring (Pp=0.5).

**R0 (or Clear)** – A dog with 2 normal genes. Unaffected. Always passes normal genes (Pp=0.0).

The parents of R2 dogs will always be R1 carriers at least, and offspring of R2 dogs, if bred to clear dogs will always be R1 carriers with a Pp of 0.5 (see Figure 1).

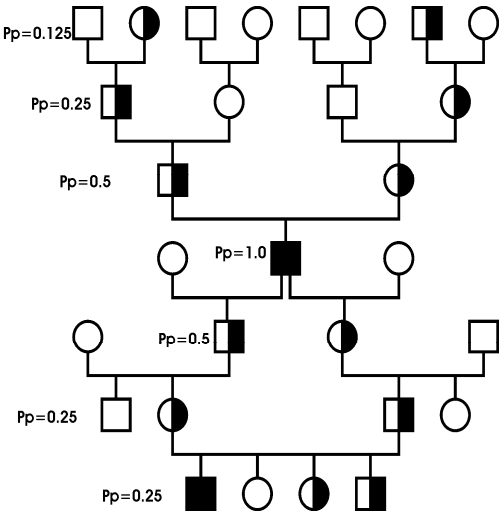



*Figure 1. Probability of passing on a recessive gene at each generation. Squares are male, circles are female. Open symbols are R0 dogs (PP=0.0) half-filled symbols are carriers (R1, Pp = 0.5) filled symbols are R2 dogs (Pp= 1.0). Working from the affected dog in the centre, both parents must be carriers, at least (Pp=0.5). At least one of each set of grandparents must be a carrier (average Pp=0.25. similar for the great grandparents (2/8 x 0.5 = 0.125). When bred to clear dogs, the offspring of the affected dog must be carriers. If these are bred to clear dogs, their offspring have a 50% chance of being carriers so the average Pp=0.25. The bottom line shows one example of a breeding between those offspring. The R1-R1 breeding (shown) gives an average Pp of 0.5, the R0- R0 a Pp of 0.0, and the two R1-R0 breedings a Pp of 0.25 giving an overall average Pp of 0.25. The R2 male in the bottom row should test positive for cystinuria, but if it were a female the CU genes would remain hidden. As the male pup is affected, its parents become known carriers (Pp=0.5) but the uncle and aunt remain Pp= 0.25.*

Since the parents are R1, each of them in turn has one parent that is R1 at least. Thus, the grandparents of an R2 dog have a 50% probability of being R1, and 50% of being R0 (overall Pp = 0.25).

Similarly, each of the great grandparents of an R2 dog has a Pp of 0.125 (there are 16 genes available to pass on and 2 are bad). Note that it cannot be determined which of the 8 great grandparents have the mutation, but at least 2 must (one on the maternal side, one on the paternal side).

Since the offspring of an R2 dog (when bred to a presumably clear mate) are all R1 (Pp = 0.5), the grandchildren have a 50% chance of receiving the recessive gene so they have a Pp of 0.25. Similarly the great grandchildren of an R2 dog have a Pp of 0.125 if the other parent is clear. This also applies to the g.g. grandparents and g.g. grandchildren which will have a Pp of 0.0625 etc.

In the case of parents and children of affected dogs, they must all be carriers (at least)**. I**n each of the proceeding generations there are a fixed number of dogs and the probabilities reflect that the exact location of the CU gene cannot be determined. In subsequent generations there can be any number of dogs where Pp only apply to overall numbers, and in any individual litter, there could be all carriers or none.

**Probability of passing on a mutation**

Since an R2 dog has only **CU** genes, the probability of passing one to any pup during mating on is 100% (Pp = 1.0). An R1 dog has one **CU** and one normal gene so there is a 50% chance of passing. If bred to a clear dog, the pup has a 50% chance of being R1 and 50% chance being R0 so its Pp = 0.25. This means that it has a 75% chance of passing on a normal gene (1 – Pp).

During a mating, Pp for the sire and dam can be different, so **Ps** and **Pd** are used to help keep thing straight.

As an example, what happens when 2 carriers mate? (Ps = Pd = 0.5) The offspring could receive one recessive gene from each parent (R2), a recessive gene from the sire or dam and a normal gene from the other (R1), or 2 normal genes (R0). The chances of each of these combinations is 25%, 50% and 25% respectively.

For any pup from this litter P2 is 0.25, P1 is 0.5 and P0 is 0.25.

**Calculations**

**P2** is the probability of being R2 (= Ps x Pd),

**P1** is the probability of being R1 (= Ps x (1-Pd) + Pd x (1-Ps)) and

**P0** is the probability of being R0 (= 1.0 – P2 – P1).

**Pp** - The probability of the pups passing on a recessive gene to the next generation is the probability of being R2 plus half the probability of being R1 so Pp = P2 + P1/2. Thus, the average Pp for the offspring of carriers is 0.25 + 0.5/2 = 0.5.

Again, the probabilities only apply to large numbers, and in any individual litter, there may be any combination of R2, R1 and R0 pups.

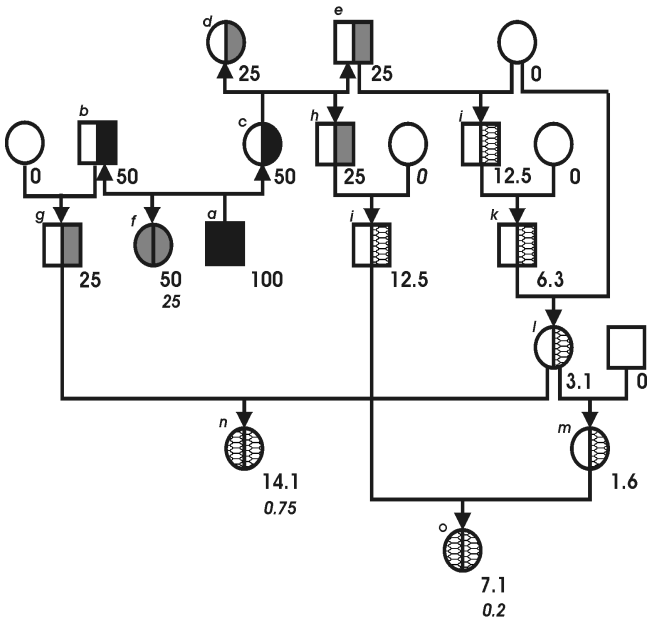
As another example, if the great grandfather of an affected dog (Ps = 0.125) had been bred to a carrier (Pd = 0.5), the offspring would have had a P2 of 0.0625 and a P1 of 0.5 and a Pp of 0.3125. This means that the pups would have about a 6% chance of being R2 (affected if male), a 50% chance of being a carrier and a 31% chance of passing on the recessive gene. Chances of being clear (P0) would be about 44%.

For any breeding the Pp of the litter will be the average of the Ps and Pd. For any breeding to a clear dog, the Pp will be reduced by one-half.

***The point is, that if Pp for each of the mating pair is known, the Pp of the pups can be calculated so the incidence throughout the population can be modeled.*** The ancestors of affected dogs can be assigned Pp values which can then be used to estimate Pp for any of their descendants. Again, the probabilities only apply to large numbers, and for any individual dog (apart from affected dogs, their parents or direct offspring) the presence of a recessive gene cannot be determined without a genetic test (see Figure 2).

*Figure 2. Example of calculation of Pp from a known affected dog (a). Numbers reflect the percent chance of passing on the recessive gene (Pp = 1.0 = 100%). The parents (b and c) must be carriers at least and one of the grandparents on each side (maternal shown) must also be carrier while the other can be clear. As we do not know which grandparent (d or e) is the carrier, each is given a Pp of 0.25. Preceding generations can also be calculated (not shown).*

*From these (a, b, c, d and e) the Pp for descendants can be calculated. In this case all outside mates are considered clear. The full sibling (f) of the affected dog could be R0, R1 of R2, with a Pp of 0.5 and a P2 of 0.25. The half sibling (g) has a Pp of 0.25. The uncle (h) has the average Pp of his parents but in this case only one is the carrier, so h cannot be R2. Successive breedings to clear dogs (j, k, l, m) decrease the Pp with no chance of producing an R2). Note that breedings between potential carriers could produce R2 dogs although in the case of n and o, the probability is very small (less than 1%)*



In Figure 2 all descendants have some probability of carrying the gene, but only the affected dog (*a*) and its parents (*b*, *c*) can be definitely identified. The sister (*f*) could also be R2, but with no symptoms she would appear perfectly normal.

Using a chart like the one shown in Figure 2, one can determine which dogs are more likely to pass on the gene (dark shading) and thus present a greater risk for breeding. A breeding between *l* and *g* is unlikely to produce an affected dog (0.75%), but if one did show up, the numbers would immediately change. Then, Pp for *n* = 0.5, *g* = 0.5, *f* = 0.5, *m* = 0.25, *k* = 0.125, etc. The grandmother of the affected dog (outsider at the top right) would also become Pp = 0.125 so that would affect any other siblings of *j* and so on. This complicates calculations as inbreeding means that the formula feeds back into itself (recursion) and a number of iterations (repeated calculations) are needed to approach the final answer so is best done with a computer program.

**How is this useful to breeders?**

For straightforward pedigrees and close relatives (as in figure 2), the calculations can be done by hand and breeders can use them to assess the risk of passing on the CU gene and balance them against the good features of a breeding. *For the future of the breed it would be unwise to eliminate good characteristics and diversity in the gene pool just because there is an outside chance of passing on the CU gene.* High risk dogs (eg. with a Pp of 0.25 or greater) should be avoided unless bred to a dog with a very low Pp. In that case carriers would be produced, but affected dogs unlikely. If one did show up, the calculations would have to be re-done to include the new information and other mates probably chosen.

**Incidence of the recessive gene responsible for cystinuria (CU gene) in North American Irish Terriers**

Recent studies on Irish Terriers from Australia and Europe have identified 51 affected males (Appendix I). Even if this represents, say, 1% of the breeding population, since there would be an equal number of unidentified R2 females it means that about 14% of the entire population would be carriers. With random breeding, the incidence of any variation in a particular gene (e.g., CU vs. normal gene) will remain constant in a population (Pp of offspring = average Pp of parents) provided the gene does not interfere with reproduction (a mutation that causes infertility will soon disappear!). With selected breeding, however, the incidence could increase, as in popular sire syndrome (Bell, August, 2004). By avoiding breeding dogs with a genetic defect, however, we can reduce the incidence of the gene in the population. The problem with a limited gene pool (as in Irish Terriers – almost all are related at some level) is to avoid limiting it further.

In recent years, there had been only one Irish Terrier in North America openly know to have cystinuria. As the dog had already sired numerous litters, the problem had to be dealt with by responsible breeders who owned the dogs. However, in the autumn of 2015 an additional Irish Terrier was reported with cystinuria. As this dog was related to two of thirteen entries at an Irish Terrier Specialty that was being held at that time, 3-generation pedigrees of the entries were examined and a total of 18 dogs were found with Pp values ranging from 0.13 to 1.0, indicating that this health issue was more widespread than formerly thought. Following the presentation of these findings at the 2015 Montgomery weekend AGM of the Irish Terrier Club of America, an additional affected dog was reported and the Board of Governors of the ITCA requested a more thorough examination of the potential problem with recommendations to club members concerning the disease.

Although only three confirmed cases of cystinuria were reported among North American Irish Terriers breeding population, these dogs and their parents, grandparents, offspring etc. have been used extensively so the gene mutation responsible for cystinuria may be wide spread through the population.

A few affected dogs that had not been involved in breeding programs were also identified and others were reported but not identified.

**Analysis**

A database was constructed of most dogs shown at major shows (Montgomery, Greyslake, Golden Gate, etc.) during the past 10 years, together with their sires and dams going back 4 generations or more if possible (about 1300 dogs total), based primarily on the Champion Report which is available to all club members. Web searches of affected dogs and their relatives revealed the pedigrees of additional offspring. Affected dogs were assigned a Pp of 1.0, parents 0.5, grandparents 0.25, great grandparents 0.125 and g. g. grandparents 0.0625. Some dogs in the data base did not descend from the dogs that were assigned Pp values so they and their ancestors were assigned a Pp of zero. Dogs for which neither parent was known were also assigned a Pp of zero for calculation of values for their progeny, but were not themselves included in overall counts of clear dogs.

Based on the assigned Pp values for affected dogs, their progeny and ancestors, P0, P1, P2 and Pp values were modeled for the rest of the population using the formulas shown above (see Calculations). Because of the highly interconnected nature of the large database, up to 100 iterations were performed until a minimum change of 0.001 per iteration occurred for Pp values.

**Results**

Data shown in Figures 3-5 represent calculations on individual dogs in the database. However, apart from affected dogs which have two recessive genes and their parents and direct offspring which must be carriers, values shown here reflect only probabilities for the population as a whole and do not confirm values for any individual dogs.

Figure 3 shows the Pp values (= P2 + 0.5 x P1) for individual Irish Terriers born over the last half-century. Pp values of 1.0 represent affected dogs, while values of 0.5 represent parents or offspring which must be carriers. Siblings of affected dogs also have a Pp of 0.5 but may be affected (R2), carriers (R1) or clear (R0). The trend line indicates that there has been a gradual increase over the time period.

Details for the last 10 years of Figure 3 are shown in Figure 4, representing the current breeding population. Three affected dogs (Pp = 1.0) are shown and a number of carriers (Pp = 0.5). A few dogs have values between 0.5 and 1.0, probably the offspring of an affected dog and ones with a Pp closer to the average. Values for the last 4 years indicate that while the average Pp is only about 0.2 (80% chance of passing on a normal gene), no dog was *guaranteed* of being clear.

Figure 5 shows values for the probability of R2, R1 and R0 for each dog born since the beginning of 2006, with mean values of 0.043 ±0.044, 0.303 ±0.180 and 0.654 ±0.0204 (mean ±SD) respectively. Although no dogs had a P0 of 1.0 during the last four years, there did not appear to be a trend for change over the period.

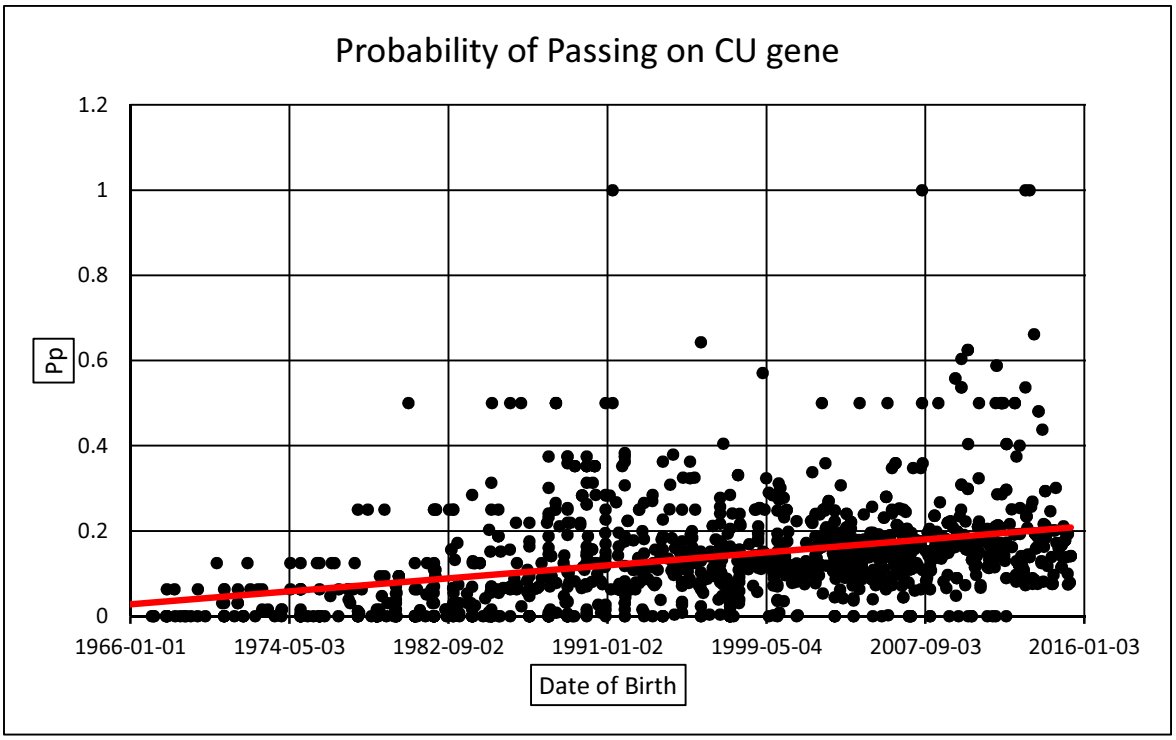



*Figure 3. Probability of passing on CU gene. Pp = P2+P1/2. Each dot represents the Pp for an individual dog shown on its date of birth (n=1297). This reflects changes in the known incidence of the gene in the Irish Terrier population since 1966. The trend line is a 2nd order polynomial fit showing a gradual increase over the time.*

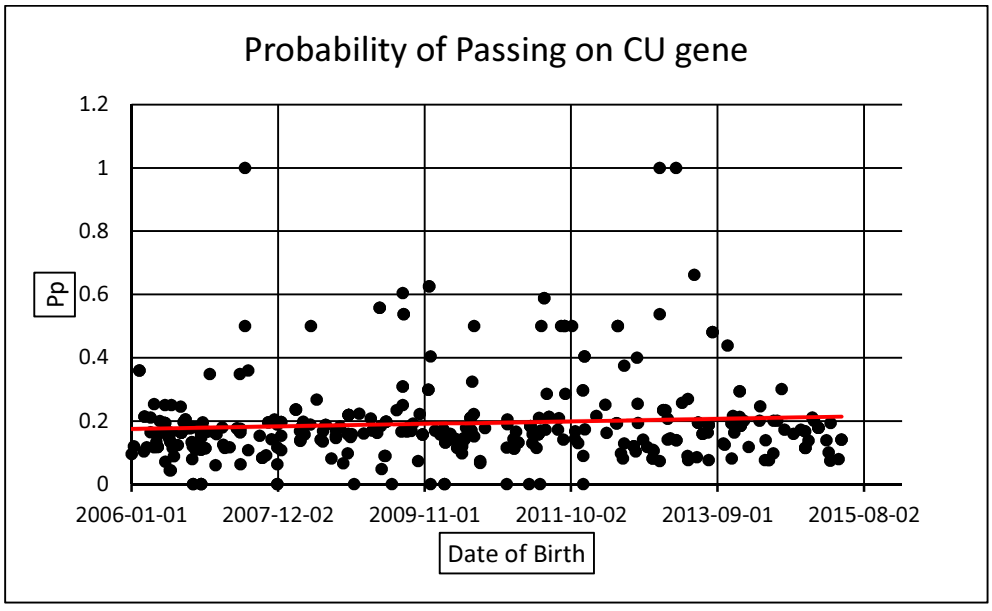



*Figure 4. Detail from Figure 3. Showing dogs born during the past 10 years (n=365). Note that the data predict no dogs guaranteed to be free of the CU gene since 2012. The trend line shows that the average Pp is about 0.2 however, indicating that most dogs have an 80% chance of passing on a normal clear.*

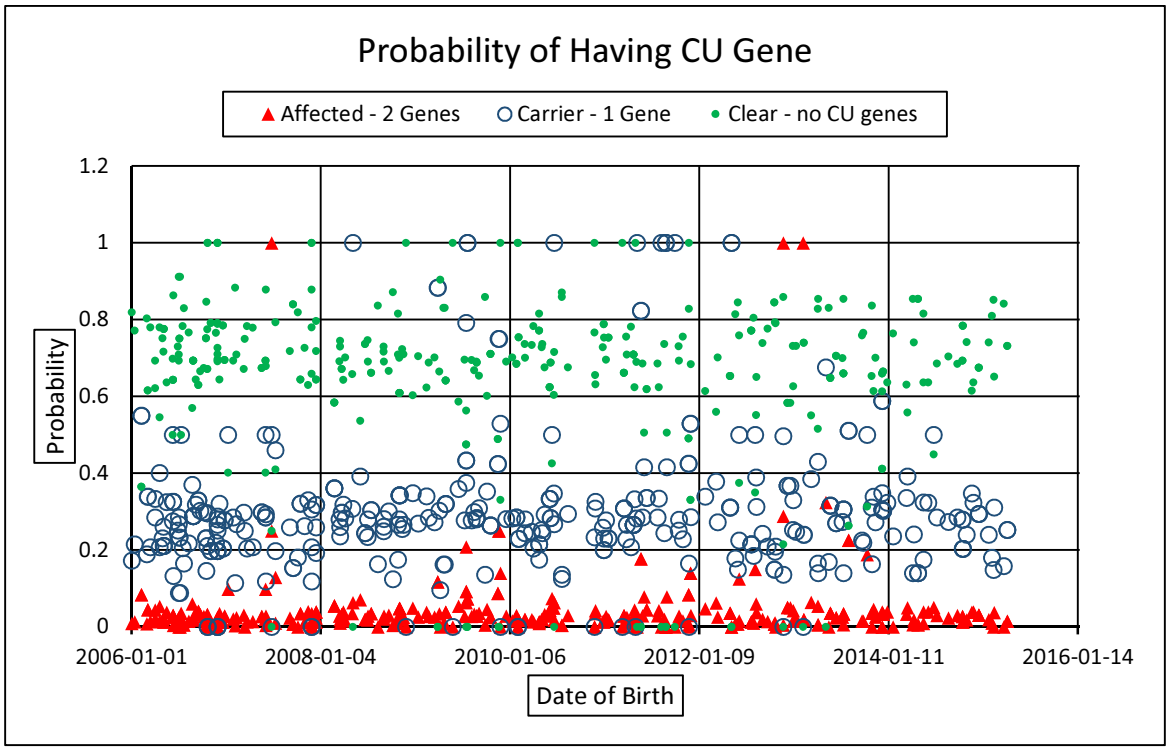



*Figure5. Probability of individual dogs (n = 256) being affected (triangles), carriers (open circles) or clear (dots). Most dogs have a good probability of being clear (>60%) and a low probability (<5%) of being affected. Although no dogs were shown to be clear for the past four years, no overall change was evident.*

**Discussion**

Cystinuria has the potential to be fatal to Irish Terriers. Cystine stones formed in the bladder can block the urethra causing pressure build-up and irreparable damage to the kidneys. Fortunately, symptoms in Irish Terriers are often less severe than in some other breeds and because the problem is androgen dependant, females and immature males are not affected. If the problem is detected early enough neutering or even chemical castration are reported to reduce cystine levels in the urine and some owners indicate that the disease can be managed by diet and drugs to control infection. Managing the problem, however is no substitute for breeding to avoid or eliminate the incidence of cystinuria in the population.

In some breeds a defective gene has been identified, and those dogs can be tested to determine if they are affected or carriers. This means that any of those dogs can be bred to an appropriate mate with no risk of producing affected pups and carriers can be dropped from the gene pool when appropriate. Unfortunately no such gene has been identified for Irish Terriers, so breeders must depend on the identification of affected dogs and the probability that the defective gene is passed through the pedigree. Figure 1 is an example of related dogs where over half are at least carriers but less than 7% show the disease.

Figure 2 provides an example of how a breeder can use the calculations to assess the probability that a breeding pair will pass on a defective gene. Although blood relatives of the affected dog have some probability of passing on the gene defect those with a high Pp should in general be avoided and thus reduce the average Pp in the population. The calculations, however, do not include “negative”

information from highly used dogs. For instance, if a dog with a high Pp has been used extensively (especially with other dogs with a high Pp) and has not produced any affected pups, it may be less likely that he is a carrier. The pups should be screened (nitroprusside test) periodically during maturation, none the less, to ensure that the defective gene has not slipped through from both parents.

Looking at the Pp (probability of passing on the CU gene by carriers or affected dogs) of dogs over the past 50 years (Figure 3), the values are seen to be increasing. This however may be due to the fact that there are no identified cases in the earlier years. One or two cases in the 60's would probably level out that increase with time, but the overall incidence would be increased. Over the past 10 years (Figure 4) PP values appear to be fairly steady. Because of the close relationships within Irish Terriers, however, there are no dogs born in the past four years in the population analyzed that can be guaranteed free of the CU gene. It must be repeated that that probabilities are low and reflect the situation for the population as a whole. An individual dog would have to be either clear, a carrier or affected with a Pp of 0%, 50% or 100% respectively. P0, P1 and P2 give the probability of being each of these based on the Pp of the parents.

Figure 5 shows the values of P0, P1and P2 for dogs during the past 10 years. The mean P0 value of 0.654 indicates that about 65% of the dogs are clear, while 30 % might be expected to be carriers and 4.3% affected. The clear and carriers which make up about 96% of the 256 dogs would be unaffected and one might expect about 4 of the 177 males to be affected, which isn't far off the 3 reported. However, as there have been a number of additional reported but unidentified dogs this number might be low.

An additional factor which has not been included in the analysis, and which should be taken into consideration with individual dogs is the fact that there are some dogs with a fairly high Pp that have had a lot of offspring but no reported affected pups. This could mean that the dog may be less likely to have a high Pp, (e.g., if dog ***e*** in figure 2 had a lot of litters with other mates and there were no pups with evidence of the disease, it could be more likely that dog ***d*** carried the CU gene).

It is expected that a number of affected dogs will be added to the database in the future, which would increase the average Pp for the population. However, if they are primarily related to those already with a high Pp, the overall population would not be affected proportionally.

**Take Home Message**

There is a small but finite probability that the gene responsible for cystinuria in Irish Terriers could be carried by any dog in the population studied. Apart from identified affected dogs, their parents and immediate offspring, no individual dog can be positively identified as a carrier, and about 65% are expected to be completely clear. Whenever possible, by breeding dogs with an average Pp less than the population average Pp, the incidence of the CU gene in the population will decrease.

**Recommendations**

1. Work out the Pp of your dog. Go back 4 generations and see if any of those dogs appear in the pedigree of a known affected dog. For every generation your dog is separated from the affected dog (Pp=1.0), divide by 2. This should serve as a guideline although any "inbreeding" will complicate the calculations. The breeding objective should be to reduce the incidence of the CU gene in the population and to avoid affected pups by keeping the average Pp of the breeding pair low (e.g., less than 0.15) and the product of the Pps lower (e.g., less than 0.02).

2. Do nitroprusside screening tests on all males used for breeding (and perhaps on the brothers of females used for breeding). Do nitroprusside tests on all male pups (when old enough and repeat a couple of times at intervals) where there is a probability of being affected (e.g., P2 greater than 0.05). A positive result will immediately change the Pps of related dogs so you can revise your breeding plans. Keep a number of dogs available for breeding (don't put all your eggs in one basket). Because the disease may not show up for several years, you should keep a backup, even if other traits are not ideal.

3. Treat dogs immediately if there is urinary distress or signs of blockage and surgery is required. Use preventive treatment and/or castration for dogs with a positive screening test. Recommend that pups from suspect litters be screened at appropriate times with reports to the breeder. Ideally pups should be monitored while maturing to reveal the disease if present before neutering or serious symptoms occur.

4. Provide blood samples for DNA research from any dog with a positive screening test. Once a DNA test is available, we can select a breeding for any dog with no risk of producing affected pups while maintaining the genetic diversity in the breed and gradually eliminating the defective gene from the population.

5. At the club level, collection of urine samples from all male Irish Terriers should be organized at shows. By supporting the collection and shipping of samples it will make it easier for owners to make better breeding decisions. In addition, participation in the CHIC DNA repository program should be encouraged and perhaps financially supported (http://www.caninehealthinfo.org/dnabankfaq.html) at show events. This could be particularly important for older dogs before the information is lost forever. The bank will provide material for researchers and if DNA tests for Irish Terriers are developed in the future, owners can go back to the bank for appropriate testing.

## Glossary

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|---|---|
| CU | Cystinuria. Abnormally high concentrations of cystine in the urine. |
| CU gene | Recessive gene mutation responsible for CU in Irish Terriers |
| **Unaffected** | No evidence of cystine in the urine |
| **Affected** | A dog with high cystine levels in the urine as evidenced by a positive nitroprusside test, cystine crystals in the urine or cystine bladder stones (uroliths). These dogs will always have 2 **CU** genes. |
| **Pp** | The probability of passing on a **CU** gene. A dog will pass on one of its two genes. If it has 2 **CU** genes, Pp is 1.0. If it has 1 **CU** gene (carrier) Pp is 0.5. |
| Ps | Pp of the sire |
| Pd | Pp of the dam |
| **R2** | A dog with 2 recessive **CU** genes. In general only mature intact males will be affected, but even this may vary in severity. R2 males and females will always pass a CU gene to their offspring (Pp=1.0). Since they have to have received a **CU** gene from each of their parents, those parents each have to have at least one **CU** gene (R1). |
| **R1** | A dog with 1 recessive CU gene and one normal. These dogs are **carriers.** They are unaffected but there is a 50/50 chance the recessive gene will be passed to each of their offspring (Pp=0.5). |
| **R0** | **(or Clear)** – A dog with 2 normal genes. Unaffected. Always passes normal genes (Pp=0.0). |
| **P2** | The probability of being R2 (= Ps x Pd) |
| **P1** | The probability of being R1 (= Ps x (1-Pd) + Pd x (1-Ps)) |
| **P0** | The probability of being R0 (= 1.0 – P2 – P1) |
| **Pp calculation** | The probability of a dog passing on a recessive gene to the next generation is the probability of its being R2 plus half the probability of being R1 so Pp = P2 + P1/2. Thus, the average Pp for the offspring of carriers is 0.25 + 0.5/2 = 0.5. |

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**Appendix I**

CHARACTERIZATION OF NON-TYPE I CYSTINURIA IN IRISH TERRIERS Urs Giger1, Jessica W. Lee1, Cait Fitzgerald1, Junlong Liu1, Angela Erat1, Adrian C. Sewell2, Paula S. Henthorn1.

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Cystinuria is a hereditary renal tubular reabsorption defect of cystine, ornithine, lysine and arginine (collectively, COLA). The low solubility of cystine in acidic urine predisposes to the formation of uroliths. Type I cystinuria in Newfoundland and Labrador Retriever dogs is an autosomal recessive trait caused by mutations in the SLC3A1 gene, whereas in other breeds, the cause of cystinuria has not yet been determined. We report here on the clinical, biochemical and molecular features of cystinuria in Irish Terriers.

Urine and EDTA blood were collected from 222 Irish Terriers from Europe and Australia. A nitroprusside screening test was used to identify increased cystine in urine. Urinary amino acid concentrations were determined by high-pressure liquid chromatography. Cystinuric dogs were defined as having cystine calculi, a positive nitroprusside result, urinary cystine (>179 µmol/g creatinine) and/or a COLA concentration of >700 µmol/g creatinine.

All 83 females tested nitroprusside negative and had normal urinary cystine (<150 µmol/g creatinine) and COLA (<500 µmol/g creatinine) concentrations. The 10 intact males that formed calculi as adults exhibited cystine concentrations ranging from 323-1580 and COLA from 1029- 4302 µmol/g creatinine. An additional 41 males had similarly high COLA values with cystine levels from 0-1580 µmol/g creatinine. Among the affecteds tested, 75% were nitroprusside positive. The negative nitroprusside results and/or low urinary cystine levels of affecteds may be due to precipitation of cystine in acidic urine.

Sequencing the coding regions of the SLC3A1 and SLC7A9 genes from EDTA blood identified no mutations. The mode of inheritance remains undetermined. However, castration appears to lower the urinary cystine and COLA concentrations and to prevent cystine calculi formation, while diet changes have lesser effects.

In conclusion, non-type I cystinuria in Irish Terriers (and several other breeds like Mastiffs and Scottish Deerhounds) is a unique form characterized by increased aminoaciduria only in males, with lower cystine and COLA excretion and fewer and later urolith formation compared to type I cystinuria. Castrating cystinuric Irish Terriers lowers their cystine and COLA excretion and thus their risk for calculi formation.

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