Cystinuria in the Irish Terrier

Summary of the lecture on the current state of research on cystinuria.

Presented at the:

German Breeders Association and the Irish Terrier Association in Bad Sassendorf on 05.03.2023

by Dr. Ulrich Merschbrock

Dear Irish Terrier friends,

first of all, I have to thank the audience for their patience in listening to me for more than an hour as I tried to explain this very dry subject to them. However, there had been too much news in the last time, which I did not want to let fall under the table.

In the beginning, I mentioned the research findings on cystinuria in Irish Terriers that have been confirmed so far. It is important to note that neither an exact cause of the disease, nor the mode of inheritance and the method how to distinguish a healthy from a sick male has been found. Then it was also discussed that there are at least 6 reasons why a diseased male dog is not recognized clinically.

Prof. Leeb in Bern considers it most probable that at least two gene markers are defective, while I hold a model with the defect in only one gene, which I cannot and must not prove for animal ethical and laboratory reasons.

Because it has been found so far in genetic studies that the composition of the cystine transporter molecules responsible for the cause of the disease in the kidney is identical in both sick and healthy Irish Terrier males (i.e. the arrangement in the plane), I considered whether there might not be a disturbance in the spatial structure of these molecular chains rBAT and b0,+ -AT. One has to imagine these intact transporter molecules like spoons. And if these spoons are now completely deformed in shape, they can no longer function. The audience was therefore presented with some completely new scientific findings from the university in Beijing/China.

Based on my observations of the use of the hormone chip "Suprelorin" and the partial androgen receptor blocker "Ypozane", I found that the androgen receptor, which is activated by the male hormone testosterone, plays the decisive role in triggering the disease. I explained with the help of some diagrams how it can be possible that a genetic defect, starting

in the transmission of the activation of the androgen receptor and ending at the responsible protein (chaperone) for the formation of the spatial structure at the mainly acting cystine transporter amino acid chains, is crucial.

This thought approach is completely new and would also explain why clinically only the males, but not the females are affected, but the defects can possess both sexes! And because these affected genes exist in the chromosomes of males and females, I assume an autosomal recessive inheritance. In the opinion of Prof. Leeb this statement is still much too early and also uncertain, but he could not invalidate my opinion either. Nevertheless, I had shown the audience in a diagram the difficulty how clinically completely inconspicuous animals are seen despite massive genetic defects in cystinuria of IT.

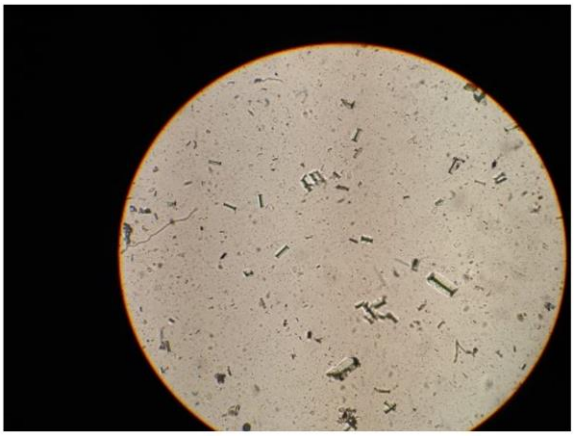
In the search for suitable males in the past, the diagnosis of the disease has always been based on the level of COLA in the urine (cystine, ornithine, lysine, arginine (amino acids)), which are jointly brought back from the primary urine to the kidney tissue by the above-mentioned amino acid transporters. Based on some observations made earlier, I can no longer share this I this line of thought. Recently, a second additional amino acid exchange model was found in addition to the return transport model with the above mentioned amino acid transporters, which may have genetic defects in some dog breeds and humans. It has been found that the second model has 15 percent and the first model 85 percent of the reabsorption capacities.

In search of a criterion for distinguishing between healthy and diseased males, I considered the following facts. If a sick male dog drinks a lot and is fed a low-protein diet, the possibility that the second, new cystine exchanger (in which no genetic damage has yet been proven) will catch the few cystine molecules formed is very high. Therefore, no cystine crystals should be visible in the urine. If the male dog is offered a lot of proteins and less drinking water, cystine crystals should form in the urine.

And with two examples I could show this with microscope pictures.

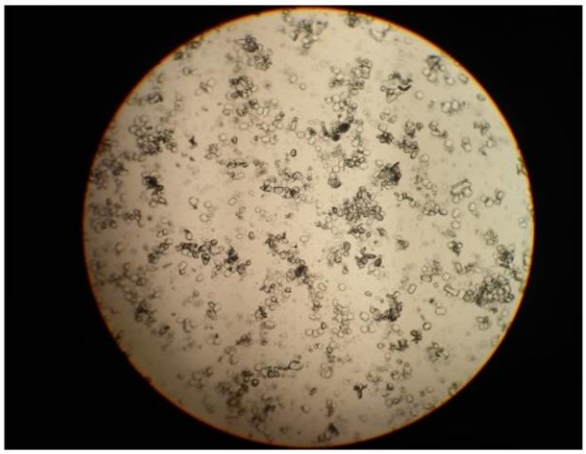
Urin 2 vor

- **Proteinarme Fütterung mit Trockenfutter und 20 %**
- **Urin pH 7**
- **Spez. Gewicht 1028**
- **Steinbildnerstatus nicht bekannt**
- **Unkastriert, 2 Jahre alt**



Urin 2 nach

- **Hochproteinreiche Trockenfütterung mit 30 % über 6 Tage**
- **Ca. 20 % Verminderung der Wasseraufnahme**
- **Spez. Gewicht 1046**
- **Urin pH 6,5**
- **sehr viele Cystin- und Struvitkristalle**



Based on this knowledge, new healthy test persons were acquired for the genome study at the University of Bern, which was continued last year. Furthermore, I was also asked to examine clinically completely inconspicuous male dogs by means of this procedure, which I call the protein provocation test. Thereby, I was able to find 5 samples out of a total of 30 urine samples as containing cystine crystals. The test has thus proven itself.

Protein provocation test (PPT)

You are the owner of an un-neutered and unmedicated male Irish Terrier that is at least 12 months old. This easy to perform test will help you determine if your pet has hereditary cystinuria.
Please feed your male dog for a period of 3-5 days only with a feed that has a very high protein content:

1. dry food with approx. 30% crude protein.
2. wet food with approx. 15% crude protein
3. BARF food with a very high meat or lean curd content
and also reduce the intake of drinking water during this time so that the urine is thickened somewhat.

Afterwards, collect the urine and have your veterinarian

1. with a urine multistick for various parameters
2. for specific gravity
3. the composition of the urine sediment incl. crystals

examined. Ideally, the specific gravity values should be well above 1.040 and the pH value should be around 5.5-6.5. With this result a very safe statement can be made whether your male dog will form cystine crystals and thus also later cystine stones. If the thorough microscopic examination for cystine crystals is negative, the probability of disease in the future is extremely low.

Unfortunately, at the end of January 2023, the review of all samples in the biodatabase of the genetic institute of the University of Bern revealed that no significant differences could be found between the previously cataloged stone- and crystal-forming carriers and the newly procured stone- and crystal-forming free carriers. This news was devastating to me. But after reviewing the criteria for the earlier sample classification, I had to realize that here, among other things, the old (for me unsuitable COLA criteria) standards were used for evaluation and thus at least 75 percent of all samples must not be considered. Therefore, the result of such a comparison between "certainly non-stone formers" as "possible" and "proven stone formers" can only result in nonsense!
I have suggested a procedure that, based on my new findings with the PPT for finding stone-forming males as early as 1.5 years of age, we reform the previous biodatabase and find new test persons. This is because, according to the genetics institute, it is necessary to have 100 samples from each cohort "healthy" and "sick" in order to obtain reliable statistical conclusions. This would mean that all test persons would have undergone the same suitability procedure, and after so many years of searching around, we would finally have the reliable

possibility of a genetic comparison of healthy to sick dogs. Prof. Leeb was impressed by my proposal and accepted me as an external collaborator in his research group at the University of Bern with effect from 08.03.2023 [this communication could not yet be refereed]. I will now, with the help of the contact data from the biodatabase, ask the owners of the males listed as unsafe in my opinion to perform the above PPT. And here one can sort these animals then surely into one of the two groups, because the blood samples are already available.
We will proceed in the same way with the help of as many breeders and private dog owners as possible. I gladly offered to send me the urine for examination, because I perform this free of charge and send the result with a valid certificate and a photo of the microscope image by e-mail on the same day of receipt of the sample to the dog owner. After the PPT has been performed and the sorting has been done, blood would be taken and this would be sent to Switzerland with the documents required by the University of Bern. A detailed instruction will be created by me and will be published on this page shortly.
This examination should be actually obligation for each dog owner, if we do not have yet a safe gene test for this illness.
Furthermore, they have the certainty very early, if their Irish Terrier will form cystine stones later on. If the test is positive, the male dog does not necessarily have to be neutered or treated with a hormone chip according to the current state of knowledge. A low-protein diet combined with an occasional administration of the preparation "Ypozane" and plenty of drinking water would be sufficient for a good life without surgery. Only in the case of clinical complaints would it be essential to remove the urinary bladder stones.
With a positive outlook on the further CU research direction and the realization that we now have a reasonable diagnosis and also an acceptable therapy at hand, I ended my lecture.

If you have any further questions, please do not hesitate to contact me. I would be pleased if I can recruit as many younger Irish Terrier males as possible for the genome study and my protein provocation test PPT).

Dr. Ulrich Merschbrock
Schafhausener Str. 40
DE - 52525 Heinsberg
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Phone ++2452.180448
ulrich.merschbrock@t-online.de
