Final presentation at the breeders' meeting

Bad Sassendorf, 14.04.2024

Dr. Ulrich Merschbrock

Over 6 ½ years ago, during a conversation at the Dortmund autumn dog show, I learned that there were 3 cases of cystinuria (type III) in Irish Terriers, so I told some breeders that I would take on this problem because of my history. I had no idea what I was getting into. In the end, three professors have still not been able to unravel the mystery. In addition to my busy professional life, I had compiled all the scientific literature, correspondence and case reports with genealogical tables. Several people had already worked on this problem and had written down their data and hypotheses. Unfortunately, I was not always able to systematically record the blood samples and accurately categorise them as healthy or sick.

During this first intensive screening, I unfortunately had to realise that my work was not supported by all breeding kennels, so that some breeding lines were blocked to me for the future. Another difficulty was finding suitable male dog owners for collecting a pool of dogs that were certainly sick and healthy. Due to data protection regulations, in many cases I had to rely on the co-operation of breeding kennels, which provided me with the addresses after prior consultation. I also found it annoying when I either gave information by e-mail at the request of another person, but hardly ever received a response. Therefore, I cannot say anything about efforts from other scientific organisations that have also dealt with the problem of CU in Irish Terriers.

The basic maxim of all my work has always been on an extremely confidential basis coupled with respect for every owner, breeder and male dog. As I already did in my veterinary practice, no personal details or data were passed on to outsiders. I have always maintained anonymity with medical results. That's why I was able to acquire such a large number of test subjects.

As time went on, I had many contacts, some of which I still maintain today, with individual scientific institutes such as the leading genetic institute at the University of Bern, individual breeding centres, various contacts at different Kromfohrländer clubs

and suitable private individuals. I had originally hoped that an interdisciplinary research group, perhaps with financial support, would make progress in solving the CU problem and finding a genetic test. However, there was a lack of interested people, institutions and funds. So I actually had to continue as a lone fighter.

In the beginning, I had also thought that I had a tried and tested indicator with the categorisation of patients according to the level of COLA in their urine. However, this was not sustainable in the long term because

1. very many male dogs had high values, but demonstrably no cystine stone formation
2. the circumstances in which the COLA values were previously determined were not reproducible and comparable
3. due to protein feeding and drinking water intake, sometimes very different values were determined consciously and unconsciously

I had therefore studied the scientific publications on the physiological cause of all CU (i.e. type I, type IIa and IIb and type III) very intensively and was able to recognise that in all cases the reabsorption of cystine from the primary urine is characterised by two mechanisms. Firstly, by reabsorption into the blood via a COLA-binding amino acid chain, and secondly by a membrane exchange process in which cystine is reabsorbed from the urine into the blood. Due to known genetic defects in defective amino acid chain formation, a distinction is made between types I and II. However, no faulty structure has been seen in type III. And it was recognised almost 10 years ago that approx. 85 percent of cystine is reabsorbed via the first principle and approx. 15 percent via the second principle. And so far, genetic defects have only been described in the first resorption option.

I have already described the possible causes of elevated COLA levels in urine in one of my earlier statements and thus pointed out the inadequacy of this absolute statement. It has also not yet been scientifically clarified whether certain individuals have a defective glomerular filtering capacity in the kidney due to genetic influences, which is compensated for by intact reabsorption.

However, even when I have asked scientific institutions, they have not been able to tell me why crystals and stones do not form in the case of an elevated urine COLA value, despite some favourable boundary conditions such as a reduced fluid volume and low urine pH.

In my opinion, only the formation of crystals in the urinary tract is important for recognising pathological cystinuria (type I-III). This is the only way I can say with absolute certainty whether the dog is a cystine stone former or not. This is basically the

core of my PP test (by feeding a lot of protein, both reabsorption systems have to work, and if crystals form, the defect is in the first reabsorption principle; if only little protein arrives in the kidney, the "basic load disposal" is sufficient without recognising a pathological defect), which has proven to be excellent if carried out correctly and is also considerably cheaper than the COLA or amino acid test to be carried out in an external laboratory.

Based on this knowledge and the results so far, I can say that it is very likely that no cystine stone formation occurs with a low COLA value, but I cannot say that this affected male dog is a stone former with a high COLA value.

And as a former practitioner, I favour a safe and simple solution here. Even if it is considerably cheaper, the PP test should be prioritised over a COLA test and is recommended for young males after sexual maturity as a guide for the early detection of possible CU.

As the physiological cause of this metabolic disorder is still not clear, we can only speculate about the pathophysiology and the possible genetic defect(s) underlying it. Unfortunately, for various reasons, it is not possible to find a scientifically sound solution in the current situation. In any case, the presence of testosterone plays a decisive role in this form of cystinuria, and I have been able to prove on the basis of many urine samples from affected male dogs that it is not testosterone itself but the androgen receptor stimulated by this hormone that provides the impetus for the onset of this metabolic disorder.

When looking for test subjects for the study, I examined the urine of around 200 male dogs, analysed their pedigrees and suspected that we may have autosomal recessive inheritance in Irish Terrier CU because I was able to make a statistically accurate assessment of the puppies' disease status based on my knowledge of their parents' genetic make-up. Prof Leeb had advised me to be cautious with this assumption because, in his opinion, the number of animals examined was too small.

Unfortunately, in his genome-wide association study in Bern using next-generation sequencing, he did not succeed in recognising a significant difference when comparing the genomes of the two control groups (Prof. Giger's suggestion with a low COLA value and my suggestion with the non-formation of cystine crystals in the urine) with the genomes of the clearly diseased animals. Even in comparison with the reference genome of a German shepherd dog, no usable statements could be made. Prof Leeb was therefore forced to terminate the research project for the reasons mentioned in my last publication. As he kindly offered other interested scientists the opportunity to

continue using the data obtained to date and the blood samples, there is in principle the possibility of continuing the studies elsewhere with a different approach. However, further financial resources must be made available and breeding centres in Germany and abroad must also be interested in continuing the project. In the short term, the medical experts should meet again, if they have not already done so, and discuss the next steps. And it should be an absolute must for breeding centres never to use a sure cystine stone former as a stud dog in order to avoid unnecessarily introducing carriers or even stone formers into the population. Because we do not know the genetic status of the bitches.

In conclusion, despite the lack of results for a possible genetic defect, a reliable hereditary prediction and a genetic test, I would like to emphasise the following points:

1. we now have enough reasonably sorted test subjects and genetic material for further investigations
2. the PPT is a fairly simple procedure for the early detection of CU if carried out correctly
3. with a consistently low protein diet, it is not absolutely necessary to castrate a diseased male dog
4. the use of a partial androgen receptor blocker ("Ypozane") is suitable as a short-term and very fast-acting drug against the formation of new cystine crystals

As I cannot foresee at the present time whether this research endeavour will be continued in any form, this publication will probably be my last for the time being.

Heinsberg, 24.04.2024 Dr. Ulrich Merschbrock