Genetics of Arrhythmogenic Right Ventricular Cardiomyopathy in Boxer dogs: a cautionary tale for molecular geneticists.

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In their paper published in this issue of the Veterinary Journal, Professor Bruce Cattenach and collaborators from four different institutions remind us of the importance of old fashioned pedigree analysis in moderating conclusions drawn from molecular genetic analysis (Cattenach et al., 2015). In this paper they use comprehensive pedigree analysis of a large proportion of the show section of the boxer breed to suggest that a mutation that has been thought to be causal of a monogenic disease and has been used in that way in two genetic testing laboratories is in fact not causal, but simply linked to the disease. What are the causes of and consequences of this conclusion?

We have become familiar with the idea that one approach to elimination of inherited diseases is to find the mutation, and to develop a DNA test. In the veterinary world, and in particular for dogs and other companion animals, this test is then offered to breeders to apply to individuals in the affected population: usually consisting of a single breed or a number of defined breeds. When combined with the correct advice, such testing can very quickly greatly reduce or even eliminate the disease. Over four hundred such tests (across all breeds) are now available worldwide. Testing laboratory data from both the UK and the USA suggest that there have been remarkable reductions in copper toxicosis in Bedlington terriers, canine leucocyte adhesion deficiency (CLAD) in Irish setters, a number of PRAs, primary lens luxation and several other diseases which can be credited in whole or part to the availability of these tests.

We have also become familiar with some limitations of the tests. Tests available at the moment are directed at diseases with monogenic inheritance, whilst the majority of inherited diseases problems are polygenic in nature, often with

34 additional substantial environmental components. But there are now multiple
35 tests for monogenic disorders, normally performed one by one. This has become
36 a large expense for conscientious breeders. (For example sixteen tests are
37 available for inherited disorders in the Labrador retriever.) In addition too many
38 tests may lead to narrowing of gene pools that are already restricted in pedigree
39 breeds, and this can itself lead to emergence of diseases that were previously at
40 low frequency, or simply to loss of fitness through inbreeding. Hence breeders
41 may be urged to retain carrier animals in order to maintain genetic diversity, and
42 to breed only to homozygous normal. This leads to further expense in
43 subsequent generations.

44
45 Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a well described
46 potentially fatal problem for boxer dogs (Harpster, 1983, 1991, Meurs, 2004)
47 characterized by progressive replacement of right ventricular myocardium with
48 fatty or fibrofatty tissue, with much less involvement of the left ventricle. In
49 gross respects boxer ARVC is similar to human ARVC, a major cause of cardiac
50 related sudden death in young adults. Similar diseases have been described as
51 isolated cases in other breeds including Labrador retriever, Bulldog, Dalmatian
52 dog, Shetland sheepdog and others (Nakao et al., 2011). The disease in the boxer
53 and in many human cases is familial and in humans has been associated with
54 mutations in genes encoding components of the desmosome, a plaque like
55 structure that binds adjacent muscle cells together.

56
57 The attempt to find the mutation responsible for boxer ARVC has been pursued
58 over several years in the laboratory of Prof Kate Meurs and her co-workers. They
59 have made searches for mutations in four candidate genes encoding desmosomal
60 proteins (Meurs et al., 2007), as well as in the cardiac ryanodine receptor gene
61 (Meurs et al., 2006), all of these being orthologous to the mutated genes in the
62 human disease. When these genes proved normal, the group and distinguished
63 collaborators from the canine genetics world used a genome wide association
64 study (GWAS) to search for single nucleotide polymorphisms closely linked to
65 the disease phenotype (Meurs et al., 2010). Two fairly closely linked loci were
66 identified on chromosome 17 that together occupy around three million bases

67 pairs. One of these loci is adjacent to the gene for Striatin (STRN), an attractive
68 candidate as it is also a desmosomal protein. Sequencing of parts of the two loci
69 encoding exons or well conserved non-coding regions showed few differences,
70 but crucially, an 8bp deletion in the 3' untranslated sequence of STRN. This
71 showed a close association between the number of ventricular premature
72 complexes and the number of copies of the mutant gene with essentially no
73 complexes in most control animals. Subsequently, the same group also showed
74 that right ventricular cardiomyopathy is associated with the striatin mutation,
75 completing the picture for STRN mutation as causing the ARVC syndrome.

76
77 But Cattenach and co-workers show that in the UK, all boxers with ARVC
78 originate from a few imported ancestors derived from the same US lines
79 described in the original publications by Harpster. But the dogs have given rise
80 to three descendent lines of which two were available for analysis. One of these
81 showed the close association between STRN mutation and ARVC observed by
82 Prof Meurs, but the other, his line 2, showed no such association. Furthermore
83 STRN mutant dogs were found more widely distributed in the population, in
84 dogs with no sign of AFRC, and no US ancestry.

85
86 So what has gone wrong in the experiments leading to the introduction of this
87 genetic test? Nothing is wrong in the way the experiments were performed, but
88 there are intrinsic limitations to the GWAS technique which Prof Cattenach's
89 work has shone a bright light on. Many breeds have tracts of DNA which are
90 relatively homozygous, due to "selective sweeps", where selection for a given
91 trait and therefore a given allele has removed all chromosomes with other
92 variants in the selected gene and through the limitations of recombination,
93 therefore left no variation around the gene. In many breeds up to 20-25% of the
94 genome is inaccessible to GWAS mapping because these regions lack the variant
95 SNPs on which the technique relies.

96
97 In hindsight, the interpretation of the ARVC GWAS in the Meurs study may have
98 been influenced by this phenomenon. Two association signals (Regions 1 & 2 in
99 Figure 1) were seen on chromosome 17 at loci separated by only a short distance

in genetic terms. Both were analysed and a suggestive mutation was seen at STRN in Region 1, which was actually the less well supported of the two loci in terms of probability of association. These probabilities, although primary to the GWAS mapping technique, also depend on the level of variation at the site being measured, and the separation of two peaks by an area of homozygosity can occur because of a selective sweep for an allele close to a single mutant locus. Further investigation showed that although the STRN mutation was not expected to knock out the gene, it was associated with reduced STRN expression in cardiac myocytes, possibly through reduced RNA stability. Moreover, dogs homozygous for the mutation had more severe disease than heterozygotes. Four dogs in the study were originally diagnosed as having the STRN mutation without ARVC, but it was considered likely that these dogs, which were clinical patients, and had not had MRI to look for fatty degeneration of the ventricle, were incorrectly phenotyped. No other suggestive mutations were found in the region. Hence the STRN mutation was accepted as the likely cause of ARVC. With hindsight it is possible, or even likely, that the real mutation causing the disease is at a locus closely linked to STRN and the Region 2 peak, but under a selective sweep, and therefore not accessible by the GWAS technique.

Prof Cattenach's study also reminds us of the extra information that can be derived by looking at multiple populations. In the UK ARVC is a recent entry into the boxer population, and cases can all be traced back to the imported animals. But it now appears that the STRN mutation was already present in the UK population although undetected. Furthermore the phenomenon of line breeding has allowed a unique insight through the formation of a group of dogs (Line 2 in the Figure) in which the ARVC phenotype has become separated by recombination from the STRN mutation – the putative selective sweep has perhaps been broken, and in any case it clear that in this population the STRN mutation cannot be the primary cause of ARVC.

It is also possible that a new mutation causing ARVC has occurred in Line 2 in an entirely different part of the genome, whilst the old causative mutation has been lost and the descent of this line from the dogs seen by Harpster is coincidental.

The requirement for a phenocopy mutation in just this line descended from imported dogs perhaps makes this less likely. In any case there is still a role for striatin in the disease. Prof Cattenach documents that even in the UK dogs with mutant STRN genes develop a more severe phenotype than those without, suggesting that reduced striatin expression may still play a role in the disease.

And consequences? Well the data suggest that the mutation causing ARVC is closely linked to STRN, and occurred in the US on a chromosome already containing the unnoticed STRN mutation. Testing and selection against the STRN mutation may well have reduced both the frequency and severity of ARVC, particularly in the US where that linkage is still present in the majority of diseased dogs. So long as the proportion of ARVC dogs in the total population is low, it can be argued that removal of these dogs from breeding is valuable to the breed. Over the last five years the costs of DNA sequencing have decreased to a point where sequencing of the whole region surrounding the striatin gene and the second GWAS locus on chromosome seventeen is easily possible. It seems likely that DNA from ARVC dogs will be sequenced by the interested labs very soon, and that additional candidate mutations will be found and tested functionally, with the hope that the actual causative mutation for ARVC will soon be found.

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Figure Legend

Figure 1. A diagram showing in sketch form the chromosome 17 loci identified in their GWAS Meur et al., 2010, together with a hypothetical explanation of the results of Cattenach et al.. This figure is derived from the data presented by Meur et al., 2010 as their Figure 1b.

187 Figure 1.
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