Genetic research in anticoagulant resistance

The world's rodenticide resistance experts recently met at a seminar in Lyon, France to review progress. Dr Alan Buckle, Visiting Research Fellow from the University of Reading and vice-Chair of the Rodenticide Resistance Action Committee (RRAC) filed this fascinating report.

Resistance in rats to anticoagulants has recently been much in the news. The media has picked-up on the reported westward spread of an advanced form of resistance that was once found only in Berkshire and Hampshire. Knowledge of this was made possible by dramatic advances in the study of resistance. This step change in the ability to identify and monitor resistance is a result of the development of new and sophisticated DNA-sequencing technology.

Significant advances in resistance research

Until recently, deciding whether a rat was resistant to anticoagulants or not depended on catching it alive and using one of several expensive and time-consuming laboratory tests. Needless to say, these tests were not much used as they were largely impractical for routine resistance monitoring. But recently researchers in Germany led by Dr Hans-Joachim Pelz made a critical break-though. They identified which part of the genetic code of rats and mice carried the DNA sequence, or gene, which alters when rodents become resistant to anticoagulants.

The gene they discovered produces the enzyme vitamin K1 epoxide reductase, a crucial enzyme in the vitamin K cycle and the one blocked by all anticoagulant rodenticides. The gene was given the name VKORC1 and the sequence of chemicals (amino-acids) used in its construction was decoded (Figure 1). Knowing the gene's DNA amino-acid sequence, it became possible for the first time to look for changes, or mutations, which resulted in anticoagulant resistance in rodents.

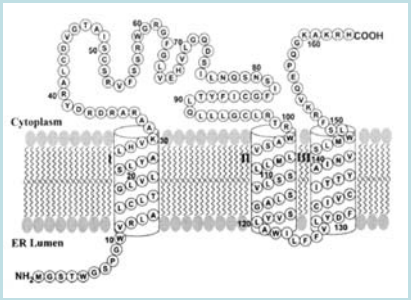
Pelz, and his many co-workers, went on to study the amino-acid sequence of the VKORC1 gene from Norway rat and House mouse resistance areas in Germany, France, Denmark and the UK. In a bench-mark paper published in 2005 in the journal *Genetics* they showed there were many different mutations of the gene.[1]

A fascinating pattern began to emerge. Anticoagulant resistance in Norway rats had evolved many times over the years, with different mutations in different places (Table 1). But occasionally the same mutation was found in rats from different countries indicating either that the same mutation emerged several times or that the rat populations developed from the same original stock. One such situation is the resistance found in Denmark and Germany is the same, and also the same as that found in north-west England.

Mutated genes are given names which describe the position of the mutated amino acid in the DNA sequence of the enzyme. In the case of the common French resistance mutation this is at position 139. The name of the original (wild-type) amino-acid is tyrosine and that of the mutant amino-acid is phenylalanine. These are put before and after the position number, hence tyrosine139phenylalanine. The names of the amino-acids are commonly abbreviated, i.e. tyr139phe. Other important mutations are shown in Table 1.

Figure 1 – The chemical structure of VKORC1

The enzyme is mainly found in liver cells. It is seen here as a chain of 163 amino-acids which passes several times through the membrane of the endoplasmic reticulum. The amino-acids are numbered in the chain and resistance mutations are most common at positions 120, 128 and 139. From Tie *et al*., 2005.[2]



"Soon all you will need to say if a rat is resistant or not is a tiny piece of tissue – a tip of the tail."

Dr Alan Buckle

Table 1 – Some of the VKORC1 mutations in Norway rats and House mice from different areas of resistance

Data provided by Dr Jo Pelz

Rodent species	Resistance area	Amino-acid position	Original amino-acid	Mutated amino-acid
Norway rat	Hampshire, UK	120	Leucine	Glutamine
	Berkshire, UK	120	Leucine	Glutamine
	Scotland, UK	128	Leucine	Glutamine
	Wales, UK	139	Tyrosine	Serine
	Yorkshire, UK	139	Tyrosine	Cysteine
	Yorkshire, UK	128	Leucine	Glutamine
	Nottinghamshire, UK	33	Arginine	Proline
	Denmark	139	Tyrosine	Cysteine
	Germany	139	Tyrosine	Cysteine
	Hungary	139	Tyrosine	Cysteine
	Belgium	139	Tyrosine	Phenylalanine
	France	139	Tyrosine	Phenylalanine
	USA	35	Arginine	Proline
House mouse	UK	139	Tyrosine	Cysteine
	UK	128	Leucine	Serine

[1]Pelz H-J, Rost S, H_nerberg M, Fregin A, Heiberg A-C, Baert K, MacNicoll AD, Prescott CV, Walker A-S, Oldenburg J, *et al.* 2005. The genetic basis of resistance to anticoagulants in rodents. *Genetics* **170**:1839–1847.

[2]Tie, J.-K., C. Nicchitta, *et al.* (2005). Membrane topology mapping of vitamin K epoxide reductase by in vitro translation/cotranslocation. *Journal of Biological Chemistry* **280** (16):16410–16416.

The RRAC Seminar in Lyon

DNA anticoagulant resistance research is developing very rapidly and there are now research teams in several countries working on the genetics of rodent resistance. This March, the industry's Rodenticide Resistance Action Committee (RRAC) hosted a meeting in Lyon to allow these teams to come together to exchange techniques and ideas and to plan the future direction of this exciting area of research.

The Chairman of RRAC, Dr Stefan Endepols of Bayer CropScience, opened the meeting and described the objectives and work of the RRAC www.rrac.info. One objective, to support research into resistance and resistance management, was the aim of the seminar.

Dr Alan MacNicoll, from the Central Science Laboratory in York, summarised the status of genetic resistance research, highlighting areas of uncertainty and defining those requiring resolution so that results can be more easily interpreted for practical use. He posed a series of questions which speakers proceeded to address:

- why are there so many different resistance mutations;
- do all the mutations now found actually confer resistance;
- do they affect the biological fitness of rodents so that resistant individuals are less likely to survive than their susceptible counterparts;
- what do mutations of VKORC1 really tell us about practical resistance to the second generation anticoagulants, like bromadiolone and difenacoum?
- These questions, and many others, were answered during the seminar.

Professor Etienne Benoit of the National College of Veterinary Medicine, Lyon, France, said his research team had identified a single major resistance mutation, called tyr139phe (see box), found all over France and also in Belgium. However, the team had conducted a survey of resistance using samples sent by professional pest control technicians from all over France and had identified a total of nine different mutations in Norway rats. A resistance mutation was found in 50% of cases where professionals reported rat control problems. A remarkable hit rate!

Kristoff Baert of the Institute for Nature and Forestry in Belgium and Anne-Charlotte Heiberg from the Danish Pest Infestation Laboratory gave talks on the distribution of resistance in their respective countries. In Belgium, resistance is widespread in the west and the east of the country but, surprisingly, the central region remains clear. Resistance to bromadiolone, and to a lesser extent difenacoum, is also found in Belgium.

The speakers at the seminar. From left to right: back row, Alan MacNicoll, Anne-Charlotte Heiberg and Jo Pelz, Etienne Benoit. Front row, Michael Kohn, Kristoff Baert and Stefan Endepols.

Nowhere else in Europe is resistance more fully studied than in Denmark and the distribution and intensity of resistance is well known. Resistance to warfarin is widespread, and resistance in rats to bromadiolone and difenacoum is getting a strong hold. But the main thrust of Heiberg's presentation was that resistance is very complex and is not fully explained by single mutations of the VKORC1 gene.

The pioneer of this technique, Dr Jo Pelz from the German Federal Research Centre for Cultivated Plants gave a review of all of the currently-known VKORC1 mutations in rats, mice and humans – yes, some humans are resistant to warfarin! A total of about 30 mutations has now been identified, but not all mutations result in practical resistance. Finally, Dr Michael Kohn (Rice University, Houston, USA) detailed how rodents carrying one of the most common resistance mutations (tyr139cys) also had a high degree of hardening of the heart arteries. This is important as it may mean that these rats may be at an evolutionary disadvantage and die out naturally if we do not use ineffective anticoagulants against them.

For more information on the seminar visit the RRAC website at **www.rrac.info**

So how does this help me?

Often, much-heralded scientific advances offer little practical benefit to pest control technicians. But few have not found a troublesome rodent infestation and wondered whether anticoagulant resistance was the cause. In the past, wondering was usually as far as it got as resistance testing was so expensive and took far too long. Scientists in the UK are now putting in place the capacity to conduct routine DNA anticoagulant resistance assays. These include Dr Colin Prescott (University of Reading), Professor Robert Smith (University of Huddersfield) and Dr Alan MacNicoll (CSL).

To be able to say if the rodent is resistant or not, these tests require only a tiny piece of tissue, such as the tip of the tail, which can be sent in the post. Soon the common question: "have I got resistance?" will be answered at a cost of only a few pounds and the results would come 'by return of post'. We are not quite there yet but it will happen soon if the demand is there.

This new DNA technology holds great promise. Not only will it deliver more effective rodent control in problem areas. But it will allow us to make more effective use of existing anticoagulants and to prevent the 'doom scenario', in which there is practical resistance to all the anticoagulants currently in our tool-kit.