The Search for Anticoagulant Resistance in Rats in New Zealand

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Resistance to anticoagulant toxins is a global and growing problem

Country	Norway	Ship	Mouse
Belgium			
Denmark			
Finland			
France			
Germany			
Great Britain			
Hungary			
Italy			
Netherlands			
Sweden			
Switzerland			
Canada			
USA			
Korea			
Japan			
China			
Indonesia			
Australia			

Pelz et al 2005; Rost et al 2009

Extent of existing anticoagulant resistance

	Anticoagulant	Known Resistance?
First generation	Warfarin	X
	Coumatetralyl	X
	Diphacinone	X
	Chlorophacinone	X
	Pindone	X
Second generation	Difenacoum	X
	Bromadialone	X
	Flocoumafen	??
	Brodifacoum	??*
	Difethialone	??

*low-level, inheritable

Blood Coagulation & Genetics 101

- Resistance to anticoagulant toxins associated with mutations in the VKORC1 gene
- VKORC1 = Enzyme - Vitamin K epoxide reductase complex sub-unit 1
- Vitamin K essential for activation of precursors to production of active blood clotting factors
- VKORC1 changes Vitamin K in to an activated form

- Anticoagulant toxins inhibit the action of VKORC1

- Mutations decrease sensitivity of VKORC1 to anticoagulants

- Mutations in VKORC1 are single nucleotide (ATCG) changes in DNA – called Single Nucleotide Polymorphisms (**SNPs**)
- SNPs **may** result in changes to Amino Acid (AA) composition of the enzyme coded by VKORC1 gene – less susceptible to ACs
- VKORC1 gene has three exons – SNPs found in each
- Most mutations involved with resistance in Exon 3

- Amino acids (AA) coded by sets of 3 nucleotides (codons)

- Example: Scottish Norway rats
  - VKORC1 – exon 3
  - Codon position 128
  - Codon wild-type CTG – AA Leucine
  - Codon mutation CAG – AA Glutamine

- Outcome – Resistance to Warfarin
  Resistance to Diphacinone
  Partial resistance to Coumatetralyl

Terminology

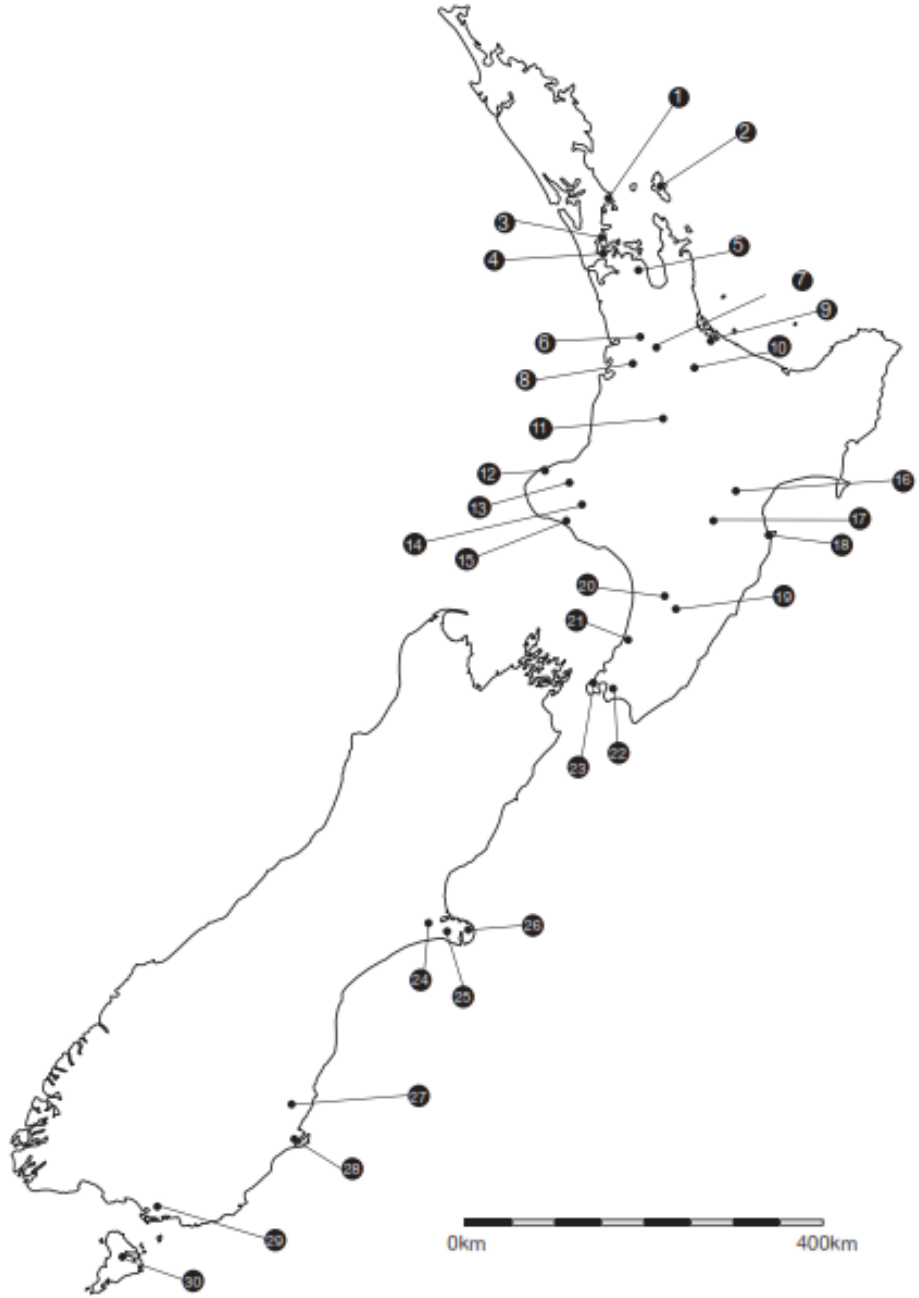
- “Wild type” VKORC1 = the commonly accepted Norway rat nucleotide/protein sequence
- Synonymous mutation - SNP that does NOT change Amino Acid
- Non-synonymous mutation - SNP that does change Amino Acid

Rats in NZ

- Ship rat (*Rattus rattus*)
- Norway rat (*Rattus norvegicus*)
- Polynesian rat (kiore) (*Rattus exulans*)

Screening for AC Resistance

- Sampled all three species of rats
- 30 sites around NZ with and without history of use of toxins
- Screened
  - 118 Norway rats (15/30 sites)
  - 482 ship rats (29/30 sites)
  - 31 kiore (2 sites)
- All 3 exons of VKORC1 gene sequenced

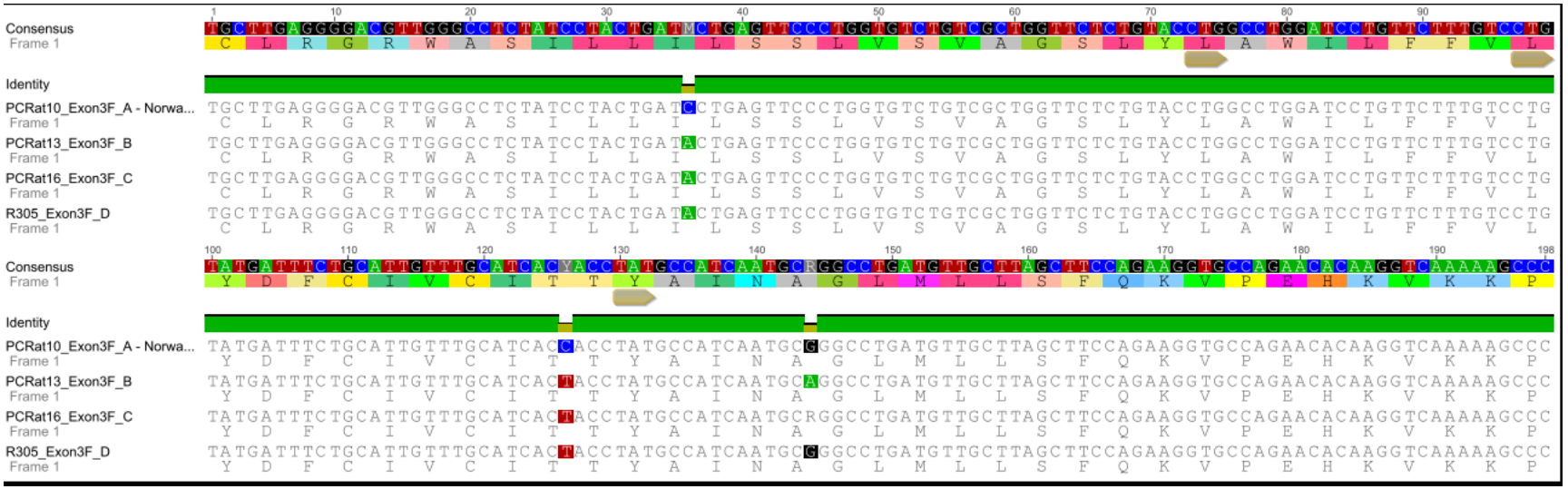


Map of rat sampling locations

Methods

- Begged, borrowed and bought bits of rat tail
- DNA extracted and sequenced by Ecogene®
- DNA sequences compared with those in Genebank & publications

EXON 3 – Nucleotide and Amino acid sequence



Genotyping Results

- SNPs found in all 3 exons

- 12 genotypes for Exon 1
- 7 genotypes for Exon 2
- 4 genotypes for Exon 3

- Multiple SNPs much more common in ship rats than Norway rats or kiore

Genotyping Results

No rats had the Tyr-139-Cys mutation

That is the most common mutation globally

Rattus norvegicus

75% of Norway rats matched the ‘wild type’ VKORC1 sequence

25% homo- or hetero- zygous for an Ile82Ile synonymous variant

No Norway rats found with mutations known to be associated with resistance

Rattus exulans (kiore)

97% of kiore rats matched the Norway rat ‘wild type’ VKORC1 sequence

One kiore heterozygous for wild type and an Ala14Val non-synonymous variant

No kiore found with mutations known to be associated with resistance

Rattus rattus

Found -

- 6 known synonymous variants
- 1 known non-synonymous variant
- 2 new variants
  - Synonymous SNP (Lys30Lys)
  - Non-synonymous SNP (Ala26Val)

- New variants only occurred in a few individuals

Rattus rattus

Known non-synonymous variant - Tyr25Phe
Spain and now NZ only records

Goulois J et al 2015
Evidence of a target resistance to antivitamin K rodenticides in the roof rat, *Rattus rattus*
Pest Management Science DOI 10.1002/ps 4020

Confers resistance to

- Warfarin
- Bromadialone
- Difenacoum

Rattus rattus – Tyr25Phe in NZ

- Found in 4 locations
  - Auckland city
  - Hamilton city
  - Wellington reserve
  - Invercargill reserve
- Uncommon
  - Only 8 (1.6%) ship rats
  - Only 1/8 rats homozygous

Rattus rattus –Tyr25Phe
What next?

- Trap more rats at those locations
- Test for resistance using blood clotting response test times

BUT

- Hamilton – no rats (council control)
- Invercargill – few rats (community control) – testing underway

Why is resistance not a problem in NZ?

1. Appropriate genetic variants missing from pool of founders
2. Insufficient selection pressure
3. Resistance to warfarin and other first generation anticoagulants could have been selected out when use stopped
4. Resistance to warfarin and other first generation anticoagulants could have been extinguished by use of more potent second generation anticoagulants and/or the combination of those with acute toxins

A Word of Caution

- We didn’t sample everywhere!
- Resistance needs to be confirmed by BCR/feeding trials or gene expression tests
- Need to assess the new Ala26Val variant
- Not all resistance is due to changes in VKORC1 - Warfarin-resistant ship rats in Japan have additional enhanced ability to metabolise warfarin
- Mice, in general, show more variable responses to anticoagulants

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