Comparative genetics of warfarin resistance

J. Oldenburg[1]; C. R. Müller[2]; S. Rost[2]; M. Watzka[1]; C. G. Bevans[3]

[1]Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Germany; [2]Institute of Human Genetics, University of Würzburg, Germany; [3]Frankfurt am Main, Germany

Keywords
Genetics, vitamin K 2,3-epoxide reductase complex subunit 1, VKORC1, warfarin resistance

Schlüsselwörter
Genetik, Vitamin-K-2,3-epoxid-Reductase-Komplex Untereinheit 1, VKORC1, Warfarinresistenz

Summary
Warfarin and other 4-hydroxycoumarin-based oral anticoagulants targeting vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1) are administered to humans, mice and rats with different purposes in mind – to act as pesticides in high-dosage baits for killing rodents, but also to save lives when administered in low dosages as antithrombotic drugs in humans. However, high-dosage warfarin used to control rodent populations has resulted in numerous mutations causing warfarin resistance. Currently, six single missense mutations in mice, 12 distinct missense mutations in rats, as well as compound heterozygous or homozygous mutations with up to six distinct missense mutations per *Vkorc1* allele have been described. Warfarin resistance missense mutations for human *VKORC1* have also been found world-wide, but differ characteristically from those in rodents. In humans, 26 distinct mutations have been characterized, but occur only rarely either in heterozygous or, even rarer, in homozygous form.
In this review, we summarize the known VKORC1 missense mutations causing warfarin and other 4-hydroxycoumarin drug resistance, identify genomics databases as new sources of data, explore possible underlying genetic mechanisms, and summarize similarities and differences between warfarin resistant VKORC1 variants in humans and rodents.

Zusammenfassung
Warfarin und andere 4-Hydroxycumarin-basierte orale Antikoagulanzien, die VKORC1 (Vitamin-K-2,3-epoxid-Reductase-Komplex Untereinheit 1) inhibieren, werden bei Menschen und den Nagern Maus und Ratte angewandt: als Rodentizid in hoher Dosierung und als antithrombotische Substanzen beim Menschen in niedriger Dosierung. Bald nach der Hochdosisanwendung bei Nagern kam es zu Resistenzen, die durch Mutationen in *VKORC1* hervorgerufen werden. Zurzeit sind 6 Missense-Mutationen bei Mäusen, 12 Missense-Mutationen bei Ratten, einschließlich compound heterozygoter oder homozygoter Mutationen mit bis zu 6 Missense-Mutationen pro *Vkorc1*-Allel bekannt. Ebenfalls wurden weltweit 26 Warfarin-resistente Missense-Mutationen beim Menschen im *VKORC1*-Gen in heterozygoter, selten in homozygoter Ausprägung beschrieben.
In dieser Übersicht fassen wir die *VKORC1*-Missense-Mutationen zusammen, die zur Resistenz gegen Warfarin und andere 4-Hydroxycumarine führen. Des Weiteren berichten wir über *VKORC1*-Varianten in genomischen Datenbanken, Mechanismen der Resistenzentwicklung und vergleichen die zu Warfarin-Resistenz führenden VKORC1-Varianten beim Menschen und bei Nagern.

Correspondence to:
Prof. Dr. Johannes Oldenburg
Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn
Sigmund Freud Str. 25, 53105 Bonn, Germany
E-mail: johannes.oldenburg@ukb.uni-bonn.de
Tel. +49/(0)228/287 51 75, Fax +49/(0)228/287 51 76

Vergleichende Genetik der Warfarinresistanz
Hämostaseologie 2014; 34: 143–159
http://dx.doi.org/10.5482/HAMO-13-09-0047
received: September 3, 2013
accepted in revised form: September 25, 2013
prepublished online: November 29, 2013

Vitamin K cycle, VKORC1 and its inhibition by 4HC

Since the middle of the 20th century, warfarin and similar synthetic derivative 4-hydroxycoumarins (4HC) have been used both as

- rodenticides, to control wild rat and mouse populations that cause extensive world-wide oeconomic loss (1, 2) and pose serious human health risks (2–4),
- therapeutic drugs for prophylaxis and treatment of human thrombotic diseases (5–7).

Design of these drugs was based on dicoumarol, a naturally occuring compound resulting from the microbial metabolism of coumarins that are abundant in sweet clover and grasses used as livestock feeds (8). Observations that spoiled feeds were responsible for dramatic haemorrhaging and death in livestock in the north-central USA and Canada in the early 20th century ultimately led to the discovery of dicoumarol and its application in high dosage as a rodenticide and, in low dosage, as an oral anticoagulant for human use (9).

For humans, attaining optimal therapeutic dosage for warfarin and other 4HC oral anticoagulant (OAC) drugs is complicated by both

- a narrow therapeutic window (too low dosage leading to ineffective antithrombotic therapy or too high dosage leading to potentially serious bleeding complications) and
- interpatient variabilities in pharmacodynamics (due to genetically defined expression levels and/or mutational polymorphism of *VKORC1*) and pharmacokinetics due to
  - genetically defined metabolic enzyme polymorphisms and
  - combination of comedications and
  - dietary vitamin K level.

Two enzymes essential for the vitamin K cycle

Initially, as vitamin K was already known to be essential for physiological blood coagulation (10), studies of laboratory rats by Matschiner, Bell and colleagues revealed that the vitamin undergoes oxidation (11). Several insightful studies by Bell & Matschiner in the early 1970s revealed that vitamin K is cycled between reduced and oxidized forms in vivo to enable coagulation and that warfarin blocks the conversion of oxidized vitamin K to the reduced form (12–14). Based on these cumulative results, Bell coined the term vitamin K-epoxide cycle (shortened by popular usage to vitamin K cycle) to summarize the cyclic reuse of vitamin K in carboxylation of thrombin, one of a number of vitamin K-dependent (VKD) clotting factors.(15)

In addition to recognizing that warfarin specifically inhibits reduction of vitamin K 2,3-epoxide – the oxidized form of the vitamin – Bell also hypothesized a general relationship between enzymatic oxidation of vitamin K to the 2,3-epoxide form and carboxylation of VKD clotting factors. Furthermore, it was found that the microsomal fraction (i.e., isolated intracellular membranes) prepared from liver tissue posesses all the distinct enzymatic activities that comprise the vitamin K cycle (17, 18) including

- γ-glutamyl carboxylation concomitant with epoxidation of vitamin K (16),
- reduction of vitamin K 2,3-epoxide to vitamin K quinone, and
- reduction of vitamin K quinone to vitamin K hydroquinone..

Suttie and coworkers provided evidence that γ-glutamyl carboxylation and vitamin K epoxidation were coupled, possibly catalyzed by a single enzyme (19). In 1991, a single enzyme catalyzing both reactions was isolated and its gene identified (*GGCX* encoding the human γ-glutamyl carboxylase) (20, 21). In 2004, an additional vitamin K cycle enzyme was discovered by identification of its respective gene (*VKORC1* encoding the human vitamin K 2,3-epoxide reductase complex subunit 1; NCBI Gene ID: 79001; located at chromosome 16: 31102175 – 31106276) (22, 23) that catalyzes both VKOR and VKR reactions and is inhibited by warfarin (24).

Independent genetic approaches were used by two research groups to identify VKORC1. One approach was based on positional cloning to identify candidate proteins that display warfarin-sensitive VKOR activity (22). The locus for combined vitamin K-dependent clotting factor deficiency type 2 (VKCFD2, OMIM #607473), was first mapped to a 20 Mb region of the 16p12-q21 locus (25). This region is orthologous to a locus for warfarin resistance on chromosome 1 in rats and on chromosome 7 in mice (26–28). The search was narrowed to a 4 Mb region on the short arm of human chromosome 16 by hypothesizing that allelic mutations in the orthologous genes could cause warfarin resistance in rodents and man (OMIM #122700). This region included 129 putative or annotated human genes. Using DNA of patients from two independently diagnosed families with VKCFD2 and from four warfarin resistant probands, genes were systematically screened and one gene of unknown function was found to be mutated. The gene extended over 5126 base pairs and comprised three exons and encoded a protein of 163 amino acids with a calculated relative molecular mass of about 18 kDa. Interestingly, the VKORC1 mutation (VKORC1:p.Arg98Trp) responsible for the life-threatening VKCFD2 bleeding pathology was identified in the same article. In homozygous individuals this results in lowered VKOR activity (22, 29). Without treatment, antigen levels and activities of all VKD clotting factors drop to hyperanticoagulated levels and result in bleeding. However, it was discovered that high, life-long supplementation with oral vitamin K completely restores VKD protein activities, apparently via a bypass pathway that directly reduces vitamin K quinone to the hydroquinone form (30).

The second approach to identify the *VKORC1* gene used siRNA gene knockdown to scan the region of chromosome 16 to which VKCFD2 and warfarin resistance had been mapped, but limited the candidate genes to just thirteen predicted to encode membrane-intrinsic proteins (23). Subsequently, each of the encoded candidate genes was knocked-down in cultured A549 cells that are known to constitutively express robust warfarin-sensitive VKOR activity. Just one candidate gene knockdown resulted in cells with substantially lowered VKOR activity, identifying the responsible gene. Ultimately, the putative gene from both approaches was confirmed by cloning and expression in a human cell line with low constitutive VKOR activity and, finally, assessing for warfarin-sensitive VKOR activity. Together, the enzymatic activities of *GGCX* and *VKORC1* are sufficient to drive the vitamin K cycle.

Substitutes for VKORC1 as vitamin K 2,3-epoxide and vitamin K reductases

Other enzymes including the flavoprotein NAD(P)H:quinone reductase (NQO1, formerly named DT-diaphorase) have been historically implicated in catalyzing the quinone to hydroquinone reduction step of the vitamin K cycle under certain non-physiological or disease conditions.(31) A VKORC1 paralog enzyme, VKORC1L1, was also identified and found to catalyse the same two enzymatic activities in vitro as VKORC1, but results from a study of VKORC1 knock-out mice showed that VKORC1L1 cannot substitute for the physiological function of VKORC1 with respect to blood coagulation in vivo (32, 33). Recently, investigations of cell biological function and in vitro enzymatic activity of VKORC1L1 strongly suggest that VKORC1L1 may be the hypothetical bypass enzyme responsible for vitamin K quinone reduction when VKORC1 activity is low due to low expression level or inhibition by warfarin and that it may provide essential VKOR activity in extrahepatic tissues and organs in the absence of VKORC1 function (34).

Variation of 4-hydoxycoumarin response

Warfarin binds to and directly inhibits the VKORC1 enzyme and, thus, interrupts the vitamin K cycle by preventing VKORC1 from catalyzing VKOR activity (22, 23). Surprisingly, little is understood about the molecular mechanism of wafarin action

and there is a complete lack of information about the location and nature of the warfarin binding site on VKORC1 (35, 36). However, informative reviews summarize various aspects of warfarin pharmacology for average patients successfully treated by oral anticoagulant therapy. Specific topics include

- pharmacogenetics (37, 38),
- phamacokinetics (39–41),
- pharmacodynamics (42, 43),
- loading and maintenance dosing algorithms (44–46),
- interactions with comedications and dietary supplements (47–50), and
- management of patients with warfarin hypersensitivity (51–56).

Approximately 30 proteins known to be involved in regulating the vitamin K cycle and systemic availability of vitamin K, warfarin and other 4-hydroxycoumarin OAC drugs have been studied for linkage of polymorphisms with warfarin dosage requirement (57, 58). Of these, genome-wide association studies (GWAS) have reached sufficient statistical power to reveal that only three – VKORC1, CYP2C9 and CYP4F2 – are the major pharmacogenetic determinants of warfarin dosage (59–61).

Increased expression level for one additional protein – the intracellular chaperone calumenin (*CALU*) which modulates γ-glutamyl carboxylase and VKOR activity levels – was found to have an inhibitory effect on turnover of the vitamin K cycle. However, warfarin dosage-associated *CALU* polymorphisms appear to primarily affect ethnic blacks with associated minor allele frequency (MAF) of up to 14–15%, while Caucasian and Asian populations exhibit a MAF of <0.5% (62).

> Despite the best wafarin dosing algorithms explaining up to 70% of warfarin dosage variance in humans, a priori genetic testing has not yet become common practise.

However, *VKORC1* genotyping is increasingly used for patients who do not adequately respond to currently used warfarin loading algorithms and often reveals coding region polymorphisms that are known to be associated with human warfarin resistance phenotypes.

SNPs in *VKORC1*

The *VKORC1* gene has been the target of intense basic biomedical research to explain warfarin susceptibility and resistance since its discovery in 2004. Only a year later, non-coding region single nucleotide polymorphisms (SNPs) affecting VKORC1 expression levels were found to be associated with warfarin dosage requirement (42, 43). Subsequent studies investigated regulatory polymorphisms in the human *VKORC1* gene by in vitro expression and chromatin immunoprecipitation (ChIP) assays and established that, while transcription and mRNA processing were not affected, reduced mRNA expression was specifically associated with both *c.-1639G>A* (rs9923231) and *g.1173C>T* (rs9934438) allelic variants in liver, but not in heart or lymphocytes (63, 64).

Recently, a study of a South Indian cohort of 136 patients stably anticoagulated with warfarin was designed to assess contributions of *VKORC1* and *CYP2C9* SNPs to warfarin dosage variation (65). Unexpectedly, the authors found four patients (3%) with dosages consistent with warfarin resistance (warfarin dosages of 49–84 mg/week at stable, therapeutic INR values maintained over 2–12 months), but with *VKORC1:c-1639G>A* which typically reduces warfarin dosage requirement by ~25% for each allele present compared with patients with two G alleles (i.e., compared with wild-type *VKORC1 *1/*1* haplotype). Interestingly, the four warfarin resistant patients were found to carry a novel single nucleotide insertion at *VKORC1:c.-1586insG*. Furthermore, although not specifically stated in the preprint of the article, the authors have confirmed that each of the four warfarin resistant patients is heterozygous for the *VKORC1:c-1639G>A, c.-1586insG* haplotype and that no previously reported coding region missense mutations could be detected after sequencing all three exons and flanking regions (personal communication by Dr. Tanuj Shukla, 10 August 2013).

Using in silico transcription factor binding site analysis, the authors hypothesize that the insertion, which occurs just 53 bases downstream from the *VKORC1:c.-1639G>A* functional promoter region polymorphism, may possibly affect the binding of a transcription activator (or, we suggest, alternatively might prevent binding of a transcription inhibitor), but this point remains to be further clarified.

Although a number of seemingly non-genetic clinical factors including age, body weight, comedication and dietary vitamin K status account for up to 20% of inter-individual dosage variation among patients on stable oral anticoagulant therapy, one vary recent study of a Japanese patient cohort (n = 202) receiving stable anticoagulation therapy using warfarin has found a correlation between warfarin dosage requirement, the dietary level of vitamin K, and the occurrence of the 5'-UTR *VKORC1:c.-1639G* polymorphism which occurs in only a minority of Asian patients (the majority of Asian individuals have a homozygous *VKORC1:c.-1639A* genotype) (66). The authors of this study concluded that CYP2C9 genotype does not play a role in dietary vitamin K influence on therapeutic warfarin dosage requirement, but that the presence of a single *VKORC1:c.-1639G* allele is a statistically significant factor ($p < 0.028$) attenuated by low vitamin K intake compared with medium and high intake after adjustment for covariates (3.4 vs. 5.0 vs. 4.0 mg/d, respectively). This possible genetic linkage between *VKORC1:c.-1639* genotype and dietary vitamin K level requires further confirmation in world-wide OAC patient cohorts to determine if the association generally holds and if it can possibly explain a significantly greater fraction of interindividual OAC dosage variability among therapeutically anticoagulated patients.

In addition to *VKORC1* non-coding region polymorphisms, previously investigated polymorphisms in cytochrome P450 isoenzymes were also found to be responsible for interindividual differences in warfarin metabolism and systemic clearance (67–70). With regard to *CYP2C9*, homozygous wild-type (*1/*1) is the best warfarin metabolizer, while all other CYP2C9 haplotypes exhibit various degrees of

reduced warfarin metabolism (typically due to missense mutations that lower or even abolish CYP2C9 activity relative to that of the wild-type enzyme), meaning that lowered warfarin dosages are necessary for patients with these polymorphisms due to lower metabolic clearance of warfarin and, thus, in order to prevent elevated drug levels and potential bleeding risk. Independent of *CYP2C9* haplotype, the *VKORC1:c.-1639* haplotype alone is responsible for between ~2-fold (e.g., for *CYP2C9*1/*1or*2*) and ~8-fold interindividual differences in therapeutic warfarin dosage (e.g., for *CYP2C9*1/*3* or *CYP2C9*2/*2or*3*).(43, 71) Other non-coding region SNPs (intron 1, *VKORC1*:g.1173; 3'-UTR, *VKORC1*:g.3730; 7 SNPs significantly associated with warfarin dosage requirement) (42) are in linkage disequilibrium with c.-1639 (71, 72). Together, *VKORC1* and *CYP2C9* haplotypes have a major influence on determining therapeutic anticoagulation dosage and haplotyping have greatly improved theapeutic efficacy and safety for patients taking warfarin (71, 73–75).

Thus, combined SNP variations in both *CYP2C9* and *VKORC1* haplotypes are responsible for up to 14-fold difference in patient dosage requirement when all additional patient factors affecting warfarin dosage are considered (e.g., 0.5–2 mg/d for homozygous *CYP2C9*3* with any *VKORC1:c.-1639* haplotype compared to 5–7 mg/d for homozygous wild-type (*1) CYP2C9 haplotype with homozygous or heterozygous *VKORC1:c.-1639G* haplotype) (71).

Distribution of VKORC1 haplotypes

A recent study of the evolutionary development and relationships among human *VKORC1* haplotypes, based on analysis of seven non-coding region SNPs, provides insight into the main genetic determinant of 4HC anticoagulant response (76). In addition to six haplotype groups accounting for ~50% of the human population and closely related to the ancestral haplotype, the single variant haplotype that includes the *VKORC1:c.-1639G>A* SNP common among Asian populations was found to have arisen by strong positive selection of *VKORC1* and 24 nearby genes comprising a ~0.5 Mb locus on chromosome 16. Accordingly, the Asian haplotype and, specifically, the *VKORC1:c.-1639G>A* SNP in the 5' promotor region have been shown to be associated with reduced VKORC1 transcription and 4HC oral anticoagulant sensitivity which apparently spread through East Asian populations by a nearly complete selective sweep . However, due to strong linkage disequilibrium among *VKORC1* and three neighboring genes within a 45 kb region of the larger selected locus, the study could not conclude if *VKORC1* or one of the three other genes was the target of the strong positive selection (76). However, the authors were able to use maximum likelihood methods to estimate that the East Asian *VKORC1:c.-1639G>A* selective sweep began ~181 generations ago which is, assuming an average human generation time of 25 years, equivalent to ~4500 years ago.

VKORC1 missense mutations associated with OAC resistance

Since the 1960s, heritable autosomal dominant mutations of a warfarin resistance locus have been documented in wild rats and mice with initial reports coming from the UK and Denmark, respectively (77, 78). Similarly, patients from three families with heritable autosomal dominant warfarin resistance were firstly described between 1964 and 1985 (79–81). The three index patients required 6.5-fold, 11.2-fold and 7.0-fold increased warfarin dosage, relative to average warfarin-susceptible patients, to reach a stable therapeutic INR and had 18 relatives over two generations, 7 relatives over three generations, and one child (2nd generation), respectively, who had the same extended prothrombin time phenotype consistent with hereditary warfarin resistance (82). Since then, various operational definitions of warfarin resistance in humans have been suggested including

1. the inability of oral anticoagulant therapy to bring the prothrombin time (assessed as INR) to the adequate levels of anticoagulation when administered at or near equivalent to the normally recommended doses (83);
2. a more generalized, quantitative definition based on normalized dosage thresholds for each of the four oral anticoagulants currently used world-wide equal to two-fold the mean therapeutic dosages for patients with combined *non-VKORC1*2* (homozygous *VKORC1:c.-1639G*) and homozygous wild-type *CYP2C9* (*CYP2C9*1*1*) alleles, but without missense mutations in VKORC1, who achieved stable anticoagulation INR values (84); and, more recently,
3. a statistical definition based on therapeutic dosages greater than subpopulation mean dosages ±two-fold the population dosage standard deviation (i.e., mean dosage ±2) for each of 18 warfarin metabolism genotypes defined by all the possible combinations *of VKORC1:c.-1639(G or A)* and *CYP2C9:*X*Y(for X=1,2,3 and Y=1,2,3)* for large study populations (85).

The discovery that single nucleotide polymorphisms in the coding region of the *VKORC1* gene, responsible for single amino acid missense mutations in the VKORC1 enzyme, are associated with warfarin resistance in humans and rodents is intimately tied to the discovery of the *VKORC1* gene itself (22). Since then, warfarin resistance in wild rodent populations has been the thematic material of a distinctly separate literature from that reporting on and investigating human warfarin resistance. In the remainder of this article we summarize and compare what is currently understood about the similarities and differences in the genetics of warfarin resistance in humans and rodents.

Human VKORC1 missense mutations

In humans, a large number of SNPs resulting in different spontaneous VKORC1 missense mutations have been discovered through genotyping patients with exceptional clinical resistance to OAC therapy (▶Tab. 1). Altogether, 209 cases of warfarin resistance have been published to date and include a total of 26 individual VKORC1 missense mutations associated with moderate to extreme or total warfarin resistance based on reported OAC drug

Tab. 1 Comprehensive data for human VKORC1 missense mutations associated with warfarin resistance including all literature reports as of 11 April 2013.

mutation	nucleotide	patient(s) ID (sex, age)	weekly dose*	INR	VKORC1	c.-1639	CYP2C9	ethnicity	reference
Ala26Pro	c.76G>C	patient 4 (f, 20)	700 F, 140 W	1.1, 1.2			*1*1	African	Bodin et al. 2008
Ala26Thr	c.76G>A	1 (m, 39)	42 P (aborted)	1,0		GG	*1*1	Morrocan	Watzka et al. 2011
Leu27Val	c.79C>G	(m, 41)	420 F (aborted), 49 W	3–4.5 st			*2*3	Bengali	Peoc'h et al. 2009
His28Gln	c.84C>T	2 (m, 79)	69 P, m	2.2 st	*3a*3a	GG	*1*1	German	Watzka et al. 2011
Val29Leu	c.85G>T	patient C (m, 57)	100 W	st		GG		Lithuanian	Rost et al. 2004
		(f, 24)	210 W	>2.0				Caucasian	Huff et al. 2005
		3 (m, 67)	180 P	2.0–3.0 st	*1*4a	GG	*1*1	Swiss	Watzka et al. 2011
Ala34Pro	c.100G>C	1 (m, 74)	189 W	2.2 st		GG			Harrington et al. 2011
Asp36Gly	c.107A>G	10 (f, ?)	140 W	2.5–3.0 st	*3b*3b	GG	*1*2	Russian	Watzka et al. 2011
Asp36Tyr	c.106G>T	(f, 41)	75 W	3,0			*1*11	Afro-Italian	D'Ambrosio et al. 2007
		(f, 42)	185 W	st	*1*3		*1*1	Israeli	Loebstein et al. 2007
		(m, 60)	140 W	st	*1*4		*1*1		
		(f, 84)	138 W	st	*1*4		*1*1		
		(f, 53)	135 W	st	*1*4		*1*2		
		(m, 53)	123 W	st	*1*2		*1*1		
		(f, 34)	123 W	st	*1*3		*1*1		
		(m, 55)	80 W	st	*1*2		*1*3		
		1 patient	>140 W		*1*2,3or 4			Sephardic Jewish	Scott et al. 2008
		49 patients	>140 W		*1*2,3or 4			Ashkinasic Jewish	
		1 patient	>140 W		*1*1				
		39 patients	19 *1*1, 13 *1*2, 7 *1*3	<----------	(CYP2C9 data to left)			non-Jewish Ethiopian	Aklillu et al. 2008
		3 patients			no *2,3,4		*1*1		
		patient 1 (f, 45)	315 F, 49 A, 98 W	1.2, 1.5, 2			*1*2	Ashkenasic Jewish	Bodin et al. 2008
		mother of patient 1 (f, 45)							
		father of patient 1 (f, 45)							
		subject 8 (m, 54)	175–245 W	st	*1/*1				Harrington et al. 2008
		4 (f, 39)	50 P	2.2 st	*1x*3a	GG	*1*1	German	Watzka et al. 2011
		5 (m, ?)	140 W	2.0–2.5 st	*1y*3b	GG	*1*1	Afro-American	
		6 (m, 56)	47 P	2.6 st	*1x*4a	GG	*1*1	Russian	
		7 (m, 71)	51 P, m	2.5 st	*1x*4a	GG	*1*1		
		8 (f, 66)	80 W (aborted)	1,2		GG	*1*1		
		9 (f, 47)	140 W	2.5 st	*1x*4a	GG	*1*1	Turkish	
		3 unrelated patients	49.7–84.7 (mean 70.0) W	st	*1*1	**G**A		Caucasian	Shuen et al. 2012

Tab. 1 Continued

mutation	nucleotide	patient(s) ID (sex, age)	weekly dose*	INR	VKORC1	c.-1639	CYP2C9	ethnicity	reference
Asp36Tyr	c.106G>T	1 paediatric patient (f, 2.2) 11kg, 0.50m^2	51 W (15.4 mg/wk is normal child dosage, based on 0.2 mg/kg/day)	3.3 st		GG	non-*2,*3	Moroccan	Moreau et al. 2013
		patient 1 (MAF 0.025)	98 W	st		GG	*1*1	Egyptian cohort	Shahin et al. 2013
		patient 2	87.5 W	st		GG	*1*1		
		patient 3	84 W	st		GA	*1*1		
		patient 4	77 W	st		GG	*1*1		
		patient 5	70 W	st		GA	*1*1		
		patient 6	45.5 W	st		GG	*1*1		
		patient 7	38.5 W	st		GA	*1*1		
		patient 8	24.5 W	st		GA	*1*1		
		patient 9	24.5 W	st		GA	*1*1		
		patient 10	21 W	st		GA	*3*3		
		1 patient (MAF 0.060)		st				Kenyan cohort	
		3 patients		st					
		5 patients (MAF 0.060)		st				Sudanese	
		5 patients (MAF 0.030)		st				Saudi Arabian	
		3 sequenced patients	36±3 A (pop. mean 14±6 A)	st				Spanish cohort (3949 total)	Anton et al. 2013
		11 genotyped patients	mean 22 A	st					
Ala41Ser	c.121G>T	(?, ?)	109 W	st				non-Asian/ non-African	Rieder et al. 2005
Val45Ala	c.134T>C	patient D (m, ?)	„complete resistance" W			GG		German	Rost et al. 2004
Ser52Leu	c.155C>T	patient 2	63 P (aborted)	<2.0	*1*1		*1*3	Dutch	Schmeits et al. 2010
Ser52Trp	c.155C>G	11 (f, 58)	69 P	2.0–2.2 st	*3a*4a	GG	*1*1	German	Watzka et al. 2011
Val54Leu	c.160G>C	patient 6 (m, 79)	420 F, 56 A, 70 W	1.3, 1.2, 1.2			*1*1	Caucasian	Bodin et al. 2008
		subject 9 (m, 81)	245–266 W	st	*3/*1				Harrington et al. 2008
		9ii-son of subject 9							
Ser56Phe	c.167C>T	12 (f, 55)	105 P (aborted)	1,2	*3a*3a	GG	*1*1	German	Watzka et al. 2011
Arg58Gly	c.172A>G	patient E (m, 80)	220–250 P	st		GG		Norwegian	Rost et al. 2004
		brother 1 of patient E (m, 82)	220–250 P	st					
		brother 2 of patient E (m, 87)	220–250 P	st					
		patient YH (complete diploid genome sequenced)	no thrombotic condition	-				Han Chinese	Wang et al. 2008
Trp59Arg	c.175T>C	(m, 79)	126–147 P	2.5–3.5 st			non-*2,*3	Caucasian	Wilms et al. 2008
		patient 1	84 A, 63 P (aborted)	1,4	*1*2		non-*2,*3	Dutch	Schmeitz et al. 2010
		patient 3	56 A, 63 P (aborted)	1,3	*1*2		non-*2,*3		

Tab. 1 Continued

mutation	nucleotide	patient(s) ID (sex, age)	weekly dose*	INR	VKORC1	c.-1639	CYP2C9	ethnicity	reference
Trp59Cys	c.177G>T	14 (m, 42)	74 P, s (aborted)	1,0	*3a*3a	GG	*1*1	German	Watzka et al. 2011
Trp59Leu	c.176G>T	13 (m, 55)	105 P (aborted)	1,2		GG	*1*2	Iranian	
Val66Gly	c.197T>G	18 (m, 51)	54 P	2.0 st	*3a*4a	GG	*1*2	German	
Val66Met	c.196G>A	(f, 64)	>175 W	1.7–2.2				Afro-Carribean	Harrington et al. 2005
		patient 3 (m, 69)	140 W	3, st			*1*1	Caucasian	Bodin et al. 2008
		patient 5 (f, 46)	245 W	3.4 st			*1*1	Caribbean	
		subject 2 (f, 68)	135–175 W	st	*1/*3				Harrington et al. 200
		subject 5 (m, 78)			*3/*4				
		subject 6 (f, 71)	168–245 W	st	*3/*3				
		subject 11 (m, 51)	210–294 W	st	*1/*1				
		15 (m, 46)	63 P (aborted)	1,2	*3a*4a	GG	*1*1	Austrian	Watzka et al. 2011
		16 (f, 51)	72 P, o, m	2.3 st	*1b*1b	GG	*1*1	Afro-Carribean	
		17 (f, 56)	294 W	2.5 st	(*1b*1b)	GG	*1*1		
		patient 1 (?, ?)						Afro-Brazilian	Orsi et al. 2010
		patient 2 (?, ?)							
		relative of subjects 2 & 3	224 W	st					Harrington et al. 2011
		1 patient (f, cohort 23–45) from 113 patient cohort	W	2.0–3.0				Black South-African	Mitchell et al. 2011
		1 individual (?, ?) from 100 person control group	no therapy	-					
Gly71Ala	c.212G>C	19 (m, 48)	42 P, r a f (aborted)	1,2	*3b*4a	GG	*1*1	German	Watzka et al. 2011
Asn77Ser	c.230A>G	20 (f, 28)	63 P (aborted)	1,6	*3a*4b	GG	*1*3		
Asn77Tyr	c.229A>T	21 (m, 50)	175 W; 73 P	3.0 st		GG	*1*3		
Ile123Asn	c.368T>A	22 (f, 68)	147 P (aborted)	1,4	*3b*4a	GG	*1*1		
Leu128Arg	c.383T>G	patient F (?, ?)	„complete resistance" W			GG		British	Rost et al. 2004
		(m, 63)	315 W, 210 P, 84 A, 560 F	1,0					Bodin et al. 2005
		(m, 77)	245 W	1,5				Irish	Áinle et al. 2008
		patient 2 (m, 75)	560 F, 70 A, 210 P, 350 W	1.2, -, 1, 1.2			*1*1	Caucasian	Bodin et al. 2008
		subject 4 (m, 80)	105–140 A	st	*3/*3				Harrington et al. 2008
		4ii-sister of subject 4							
		4iii-daughter of subject 4							
		subject 15 (m, 75)	280 W (aborted)		*2/*3				
		1 sequenced patient	36 A (pop. mean 14±6 A)	st				Spanish cohort	Anton et al. 2013
Tyr139His	c.415T>C	23 (m, 63)	63 P (aborted)	1	*2b*3b	GA	*1*1	German	Watzka et al. 2011

All mutations are heterozygous unless indicated with +/+ for homozygous. Patient IDs are indicated as in the original references. INR: International Normalized Ratio; st: stable therapeutic anticoagulation was achieved at the indicated INR value and dosage (mg/week). CYP2C9 and VKORC1 indicate respective haplotypes, c.-1639 indicates the determined *VKORC1:c.-1639G>A* SNP status where the A variant is associated with ~50% reduction of mRNA with respect to that of the G variant. OACs: A, acenocoumarol; F, fluindione; acenocoumarol; P, phenprocoumon; W, Warfarin.

Tab. 2 Additional human VKORC1 missense mutations from clinical laboratories, whole genome and exome sequencing projects

mutation	nucleotide	NCBI dbSNP identifier	clinical significance	reference, data source
Pro8Ser	c.22C>T	rs200328478	unknown	1000 Genomes
Gly19Ser	c.55G>A	rs151165999	unknown	NHLBI-ESP3
Ala32Glu	c.95C>A	rs111897490	unknown	HGSC – Baylor College of Medicine
Asp36Tyr^{V2}	c.106G>T^{V2}	rs61742245	unknown	
Pro60Leu^{V2}	c.179C>T^{V2}	not assigned	unknown	
Gly62Val	c.185G>T	rs200917074	unknown	NHGRI/NIH The ClinSeq Project
His68Arg	c.203A>G	rs201044348	unknown	1000 Genomes
His68Pro	c.203A>C	not assigned	unknown	
His68Tyr	c.202C>T	rs145273772	unknown	NHLBI-ESP3
Leu70Val	c.208C>G	rs202194968	unknown	1000 Genomes
Pro83Leu^{V2}	c.248C>T^{V2}	rs7200749	unknown	Tennessen et al. 2012 (NHLBI-NHGRI Exome Sequencing Project)
Leu111Pro	c.332T>C	rs201545927	unknown	1000 Genomes
Val112Met	c.334G>A	rs199740609	unknown	CSAgilent ClinSeq project1
Asp130Glu	c.390T>G	rs183024280	unknown	1000 Genomes
Val143Met	c.427G>A	not assigned	unknown	Tennessen et al. 2012 (NHLBI-NHGRI Exome Sequencing Project)
Arg151Gln	c.452G>A	rs199952041	unknown	Center for Statistical Genetics, University of Michigan

V2: VKORC1 mRNA splice isoform 2 variant; SNPs. 1population includes 662 participants of European descent from the ClinSeq project, all of whom have undergone whole-exome sequencing. 2AGI_ASP_population, PCR amplification of genomic DNA were analyzed by DNA sequencing. 3Data is derived from population cohorts participating in the NHLBI Exome Sequencing Project. For a list of participants see the program website (https://esp.gs.washington.edu/drupal/).

dosages phenotypes (22, 42, 84–94). Additionally, two naturally occurring VKORC1 missense mutations (VKORC1:p.Asp38Tyr, p.Arg151Gln) are associated with normal warfarin sensitivity (63), while 13 missense mutations have been confirmed through clinical reports or sequencing projects where there is currently no information concerning warfarin sensitivity or resistance phenotype (▶Tab. 2).

The VKORC1:p.Asp36Tyr mutations that appear to be fixed in some geographically isolated (bottlenecked) populations including Israeli (Ashkenazim and Sephardim), Non-Jewish Ethiopian, European Ashkenazim Jewish, Russian, Egyptian, Kenyan, Sudanese and Spanish populations, have reached large enough minor allele frequencies to be considered standing variants in these populations and occur homozygously in some individuals (95, 96). However, several individual cases of isolated heterozygous VKORC1:p.Asp36Tyr individuals in the USA (one each Afro-American and Caucasian), Turkey and Germany also point to independent de novo events. Another warfarin resistance-associated mutation VKORC1:p.Val66Met, appears from literature reports to be fixed in Afro-Brasilian, Afro-Carribean and black South African populations. Some Caucasian, Irish and Spanish individuals with this mutation likely represent de novo events. With the exception of these two VKORC1 mutations, the remaining 24 warfarin resistance-associated mutations appear to be isolated reports, suggesting they are all de novo mutations.

Interestingly, three silent coding-region SNPs have been reported for human *VKORC1* that include amino acid residues Arg12, Cys43 and Leu120.(43, 97) Of these, the Arg12Arg and Leu120 Leu polymorphisms have recently been shown to result in small, but statistically significantly elevated therapeutic acenocoumarol dosages (85).

Vkorc1 missense mutations in mice

For the Western European *Mus musculus domesticus* mouse species, only six single Vkorc1 missense mutations have been found to be causative for warfarin and other 4HC rodenticide resistance (▶Tab. 3) (98–101). Two of these alter the native Trp59 to either Leu or Ser, while mutations in Glu37 and Arg58 lead to loss of formal charge – all three positions located in the large extramembraneous loop. Two further missense mutations, Leu128Ser and Tyr139Cys, appear to comprise the most often mutated residues and are located in two adjacent intermembrane α-helices near the vitamin K 2,3-epoxide binding site. Interestingly, since 2011, there have been reports of warfarin resistant mice with compound heterozygous missense mutations reported in Western Europe that include one Leu128Ser allele and either Leu124Gln or Tyr139Cys on the second *Vkcor1* allele (▶Tab. 4, bottom-most four entries representing 12 sequenced specimens) (101). Another unusual development involving multiple mutations in *Vkcor1* alleles of *Mus musculus domesticus* was first documented in 2009, whereby a combination of three missense mutations (Arg12Trp, Ala26Ser, Ala48Thr) together with a single coding region silent polymorphism (Glu37Glu), occur with other mutations including the formerly-reported single missense mutations Leu128Ser and/or Tyr139Cys, but also together with novel missense mutations including Arg58Gly, Arg61Leu, and also various combinations of these (▶Tab. 4, entries above the bottom-most four entries, except for the topmost entry).

For rodents, Vkorc1 missense mutation arise due to selective pressure by exposure to 4HC anticogulant rodenticides. Historically, rodent OAC resistance has been studied by field sampling, establishment of outcrossed and homozygous crossbred strains, and subsequent challenge with administered 4HC pesticides to provide complementary data on Vkorc1 function, genotypes, phenotypes and associated bio-

chemical appraisal of 4HC resistance (102–108). Warfarin resistance was assessed in animals by either survival of a no-choice feeding regimen with warfarin (and/or other 4HC based rodenticides) or by measurement of prothrombin time (PT) before and after administration of rodenticide.

Recently, acquired resistance to warfarin and other 4HC-based rodenticides was recently reported by Song et al. (2011), whereby an entire *VKORC1* allele was introduced as part of a large ~20 Mb chromosomal region into the European house mouse species (*Mus musculus domesticus*) by mating with the closely related Algerian species (*Mus spretus*) and natural backcrossing of interspecific hybrid offspring with the European species through a process called introgressive hybridization (100). This combination of mutations had been previously reported by Rost et al. for wild European mice collected from the Westphalia region in Germany prior to 2009, and futher reported by Pelz and colleagues from widely spread regions throughout Germany by 2012 (99, 101). Algerian mice exhibit a warfarin resistance phenotype even through they are not under 4HC rodenticide selection pressure. Transfer of the *M. spretus Vkorc1* allele confers strong resistance to warfarin and second generation superwarfarin rodenticides for European mice with the introgressed allele (100). *M. spretus* is known to be allopatric with *M. m. domesticus* throughout southern Spain where the two species hybridized under selection pressure from warfarin use.

As new missense mutations are generally believed to be coupled with fitness and viability costs, additional compensatory mutations may arise to offset the initial suvival deficits. It appears that mice have possibly three sources for acquiring compensatory genetic adaptation of initial warfarin resistance mutations – firstly, by acquiring novel, random missense mutations; secondly, by accumulation of missense mutations already present in the wild-type genetic background as standing variants with minor allele frequencies in populations of the original species; and thirdly, by adaptive introgression of the wild-type standing variation of multiple

Tab. 3 VKORC1 single missense mutations associated with warfarin resistance in *Mus musculus* sp.; see legend for Table 1

mutation	nucleotide	genotyped individuals	geographic region		reference
Glu37Gly	c.110A>G	12	Germany	Berlin	Rost et al. 2009
		1		Lower Saxony	
		12		Berlin	Pelz et al. 2012
Arg58Gly	c.172C>G	13		Westphalia	Rost et al. 2009
		13		Sassenberg	Pelz et al. 2012
		9	Germany		Song et al. 2011
Trp59Leu +/-	c.176G>T	5	Germany	Ahlen	Pelz et al. 2012
Trp59Leu +/+		7			
Trp59Leu		1	Germany		Song et al. 2011
Trp59Ser +/-	c.176G>C	1	Germany	Saarbrücken	Pelz et al. 2012
Trp59Ser +/+		1			
Leu128Ser +/+	c.383T>C	6	UK, York (CSL)		Pelz et al. 2005
Leu128Ser		17	Germany	Rhineland	Rost et al. 2009
		1		Bochum	Pelz et al. 2012
		2		Bonn	
		11		Dresden	
		5		Gelsenkirchen	
		6		Kalkar	
		27		Cologne	
		1		Lünen	
		64		Magdeburg	
		2		Neumünster	
		1		Oer-Erkenschwick	
		16		Saarbrücken	
		15		Stuttgart	
		11		Wesseling	
		1	Switzerland, Ecublens		
		1	Azores, Terceira		
		11	Germany		Song et al. 2011
		5	UK		
Tyr139Cys +/+	c.416A>G	4	UK, Reading (MHR)		Pelz et al. 2005
Tyr139Cys		1	Germany, Rhineland		Rost et al. 2009
		1	Azores, Terceira Island		
		8	Germany	Altenpleen	Pelz et al. 2012
		1		Bonn	
		12		Ennigerloh	
		27	Switzerland	Bern	
		1		Zürich	
		15	Azores	Terceira	
		5		São Miguel	

Tab. 4 VKORC1 compound missense mutations associated with warfarin resistance in *Mus musculus* sp.

mutations	nucleotides	genotyped individuals	geographic region		reference
Arg12Trp, Ala26Ser, (Glu37Glu), Ala48Thr	c.34C>T, c.76G>T, (c.111A>G), c.142G>A	wild-type *Mus spretus*	Algeria, Western Mediterranean		Song et al. 2011
Arg12Trp, Ala26Ser, Ala48Thr	c.34C>T, c.76G>T, c.142G>A	2	Switzerland, Zürich		Pelz et al. 2012
Arg12Trp, Ala26Ser, (Glu37Glu), Ala48Thr	c.34C>T, c.76G>T, (c.111A>G), c.142G>A	2	Spain		Song et al. 2011
Arg12Trp, Ala26Ser, (Glu37Glu), Ala48Thr, Arg61Leu	c.34C>T, c.76G>T, (c.111A>G), c.142G>A, c.182G>T	2	Germany, Westphalia		Rost et al. 2009
		1	Spain		Song et al. 2011
		7			
		4	Germany		
Arg12Trp, Ala26Ser, Ala48Thr, Arg61Leu	c.34C>T, c.76G>T, c.142G>A, c.182G>T	1	Germany	Datteln	Pelz et al. 2012
		13		Dortmund	
		1		Frankfurt/Main	
		6		Hamm	
		3		Lünen	
		3	Switzerland	Zürich	
Arg12Trp, Ala26Ser, Ala48Thr, Arg61Leu, Tyr139Cys	c.34C>T, c.76G>T, c.142G>A, c.182G>T, c.416A>G	1		Bern	
		1		Zürich	
Arg12Trp, Ala26Ser, Ala48Thr, Leu128Ser	c.34C>T, c.76G>T, c.142G>A, c.383T>C	4	Germany	Stuttgart	
Arg12Trp, Ala26Ser, Ala48Thr, Tyr139Cys	c.34C>T, c.76G>T, c.142G>A, c.416A>G	1			
		1	Switzerland	Bern	
		1		Zürich	
Arg12Trp, Ala26Ser, Ala48Thr, Arg61Leu, Leu128Ser	c.34C>T, c.76G>T, c.142G>A, c.182G>T, c.383T>C	3	Germany	Hamm	
		1		Lünen	
Arg12Trp, Ala26Ser, (Glu37Glu), Ala48Thr, Arg58Gly, Arg61Leu	c.34C>T, c.76G>T, (c.111A>G), c.142G>A, c.172C>G, c.182G>T	7		Westphalia	Rost et al. 2009
Arg12Trp, Ala26Ser, Ala48Thr, Arg58Gly, Arg61Leu	c.34C>T, c.76G>T, c.142G>A, c.172C>G, c.182G>T	1		Bochum	Pelz et al. 2012
		7		Sassenberg	
Arg12Trp, Ala26Ser, (Glu37Glu), Ala48Thr, Arg58Gly, Arg61Leu, Leu128Ser	c.34C>T, c.76G>T, c.142G>A, (c.147A>G), c.218G>T, c.383T>C	1		Achim	
		1		Berlin-Prenzlauer Berg	
Ala26Ser, (Glu37Glu), Ala48Thr, Arg61Leu	c.76G>T, (c.111A>G), c.142G>A, c.182G>T	1	Spain		Song et al. 2011
Leu124Gln, Leu128Ser	c.371T>A, c.383T>C	2	Germany	Kalkar	Pelz et al. 2012
Leu128Ser, Tyr139Cys	c.383T>C, c.416A>G	2		Bochum	
		4		Saarbrücken	
		4	Azores, Terceira		

All silent polymorphisms are indicated in parentheses; see legend for Table 1; * warfarin-resistant wild-type reference species *M. spretus* (first line entry); wild-type *M. m. musculus* and *M. m. domesticus* are both warfarin-senstive reference species.

coding and non-coding variants from a related, but distinctly unique, species (100, 109). One can consider introgression of a wild-type gene with multiple coding variants from one species to another as essentially passing a pre-optimized cassette of initial and compensatory mutations that confer adaptive advantage of immediate benefit. Such an advantage is proposed by Song et al. to have likely arisen from the development of mutations that increased affinity for dietary vitamin K as the original habitat of *M. spretus* is known to have only very low dietary sources of vitamin K. Thus, they hypothesize that, while *M. spretus Vkorc1* sequence differences arose due to selection for high vitamin K affinity due to low dietary vitamin K, the actual sur-

Tab. 5 VKORC1 single missense mutations associated with warfarin resistance in *Rattus* sp. (silent polymorphisms in parentheses); see legend for Table 1.

mutation	nucleotide	genotyped individuals	strain (reference)		geographic region	reference
(reference sequence)	reference SNPs: (c.36G, c.123G, c.268A, c.294G, c.321C, c.411C)	many	wild-type	*Rattus norvegicus*	Germany	Rost et al. 2004
(Arg12Arg), Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.268A>T, (c.321C>A, c.411C>T)			*Rattus rattus*	Japan	Tanaka et al. 2012
(Arg12Arg, Ala41Ala), Ile90Leu, (Arg98Arg, Ile107Ile, Thr137Thr)	(c.36G>A, c.123G>A), c.268A>T, (c.284G>A, c.321C>A, c.411C>T)			*Rattus losea*	China, Guangdong province	Wang et al. 2008
Arg33Pro	c.98G>C	2	wild, trapped in known or suspected resistance area		UK, Nottingham-shire	Rost et al. 2009
Arg33Pro	c.98G>C	7			Japan, Aomori	Tanaka et al. 2013
Arg35Pro	c.104G>C	6			USA, Chicago	Rost et al. 2009
Arg35Pro, (Ile82Ile)	c.104G>C, (?)	8				
Ser56Pro +/+	c.166T>C	1	wild resistant assessed by BCR		Germany, Münster-land/Emsland	Pelz et al. 2005
Ser56Pro +/-	c.166T>C	1				
Phe63Cys, (Ile82Ile)	c.188T>G	15	wild, trapped in known or suspected resistance area		UK, Cambidge/ Essex	Rost et al. 2009
Glu67Lys	c.199G>A	6			Japan	
Ile90Leu, (Ile107Ile, Thr137Thr)	c.268A>T, (c.321C>A, c.411C>T)	5			Indonesia	
(Arg12Arg), Ile90Leu, (Leu94Leu, Ile107Ile, Thr137Thr, Ala143Ala)	(c.36G>A), c.268A>T, (?, c.321C>A, c.411C>T, ?)	3			USA, Santa Cruz	
Ile90Leu, (Ile107Ile, Thr137Thr)	c.268A>T, (c.321C>A, c.411C>T)	1			Azores, Terceira	
Ile90Leu, (Leu94Leu, Ile107Ile, Thr137Thr, Ala143Ala)	c.268A>T, (?, c.321C>A, c.411C>T, ?)	1				
Leu120Gln	c.359T>A	2	HH, Hampshire resistant (Greaves & Cullen-Ayres 1988)		UK, Hampshire	Pelz et al. 2005
Leu120Gln +/-	c.359T>A	2	HB1, Berkshire resistant		UK, Berkshire	
Leu120Gln +/+	c.359T>A	2	HB2, Berkshire resistant (Hussain 1998)			
Leu120Gln	c.359T>A	26	wild, trapped in known or suspected resistance area		Belgium, Demer river catchment	Baert et al. 2012
Leu128Gln +/+	c.383T>A	2	HS, Scottish resistant (Greaves & Ayres 1973)		UK, Scottland	Pelz et al. 2005
Leu128Gln	c.383T>A	2	wild, trapped in known or suspected resistance area		UK, Lancashire	Rost et al. 2009
Tyr139Cys	c.416A>G	10	*Rw* (Kohn & Pelz 1999)		Gemany, Münsterland	Rost et al. 2004
Tyr139Cys	c.416A>G	1	wild susceptible (Kohn & Pelz 1999)		-	
Tyr139Cys +/+	c.416A>G	12	wild resistant (Thijssen &Pelz)		-	
Tyr139Cys +/-	c.416A>G	4			-	
Tyr139Cys +/-	c.416A>G	2	wild resistant assessed by BCR		UK, Yorkshire	Pelz et al. 2005
Tyr139Cys	c.416A>G	43			Denmark	
Tyr139Cys	c.416A>G	281			Germany, Münster-land/Emsland	
Tyr139Cys	c.416A>G	5	wild susceptible assessed by BCR			

Tab. 5 Continued

mutation	nucleotide	genotyped individuals	strain (reference)	geographic region	reference
Tyr139Cys	c.416A>G	1	wild, trapped in known or suspected resistance area	UK, Norfolk	Rost et al. 2009
Tyr139Cys	c.416A>G	1		UK, Gloucestershire	
Tyr139Cys	c.416A>G	9		Hungary, Maglód	
(Ile82Ile), Tyr139Cys	(?), c.416A>G	1		UK, Norfolk	
(Ile82Ile), Tyr139Cys	(?), c.416A>G	2		UK, Lincolnshire	
(Ile82Ile), Tyr139Cys	(?), c.416A>G	1		Hungary, Békés	
Tyr139Phe +/+	c.416A>T	1	wild resistant assessed by BCR	Belgium, Flanders	Pelz et al. 2005
Tyr139Phe +/-	c.416A>T	13			
Tyr139Phe +/+	c.416A>T	5		France, Burgundy	
Tyr139Phe	c.416A>T	6	wild, trapped in known or suspected resistance area	Korea, Seoul	Rost et al. 2009
Tyr139Phe	c.416A>T	81		Belgium, East & West Flanders	Baert et al. 2012
Tyr139Ser +/+	c.416A>C	2	HW, Welsh resistant (Greaves & Ayres 1969)	UK, Wales	Pelz et al. 2005
Tyr139Ser	c.416A>C	2	wild, trapped in known or suspected resistance area	UK, Shropshire	Rost et al. 2009
Ala143Val, (Ser103Ser, Ile107Ile, Thr137Thr)	c.428C>T, (?, c.321C>A, c.411C>T)	2		Thailand	

vival advantage for the *M. spretus Vkorc1* allele introgressed into *M. m. domesticus* is the pleiotropic effect of reduced affinity for warfarin binding that effectively confers warfarin resistance as a survival advantage in regions where 4HC pesticides are used (100).

Vkorc1 missense mutations in rats

In three *Rattus* species distributed worldwide, 12 distinct single missense mutations in Vkorc1 have been demonstrated to cause warfarin resistance (▶Tab. 5) (22, 98, 99, 110, 111). Interestingly, only one of these 12 missense mutations is found to cause warfarin resistance in mice – Vkorc1:p.Tyr139Cys (▶Tab. 1).

For these three species (*R. norvegicus* native to Europe, *R. rattus* native to Japan, *R. losea* native to Southeast Asia and China), compound Vkorc1 missense mutations have been reported (▶Tab. 6) (98, 99, 112, 113). In France and South Korea compound Vkorc1 missense mutations comprising Tyr139Phe together with Arg35Pro and Ala21Thr, respectively, have been reported. In the UK, compound Vkorc1 missense mutations comprising Phe63Cys together with either Ala26Thr or Tyr39Asn were found. Another compound heterozygous mutation involved the variants Leu128Gln and Tyr139Cys in another region of the UK (▶Tab. 6, bottom-most entry). As Arg35Pro, Phe63Cys, Leu128Gln, Tyr139Cys and Tyr139Phe are among the known rat single missense mutations responsible for warfarin resistance, we are temped to speculate that Ala21Thr, Ala26Thr and Tyr39Asn may be compensating mutations acquired to fine tune Vkorc1 enzymatics after the initial adaptive mutations arose (▶Tab. 6, entries 4–7 from top). Interestingly, comparison of wild-type Vkorc1 primary protein sequences for the three rat species shows that Leu90 is a wild-type residue common to *R. rattus* and *R. losea*, both species native to Asia, while Ile90 is the respective wild-type residue in *R. norvegicus* (▶Tab. 6, top three entries in grey-shaded box). By far the largest number of multiple mutations associated with warfarin resistance in wild rats includes the Vkorc1:p.Ile90Leu variant (▶Tab. 6, entries 8–20 from top). Also, wild-type warfarin-resistant *R. losea* from China were found to have an additional Arg58Gly mutation (▶Tab. 6, entries 8–10), while a resistant rat of unidentified species found in Argentina had both Trp59Arg and Ile90Leu (▶Tab. 6, entry 11). Japanese *R. rattus* specimens with warfarin resistance (▶Tab. 6, entries 12–16) were found to have Ala41Thr, Ala41Val, Arg61Trp, Leu76Pro or both Arg61Trp and Leu76Pro. Similarly, warfarin-resistant *R. losea* specimens found in Indonesia had either Ile141Val, Ala143Val or both of these mutations in addition to the native Leu90 (▶Tab. 6, entries 18–20). Finally, warfarin resistant rats found in the Azores, where the native species is *R. novegicus*, had both Ile90Leu and Val112Leu mutations (▶Tab. 6, entry 17). Interestingly, silent coding region polymorphisms were also detected at Leu94, Ile107, Thr137 and Ala143 suggesting two possible scenarios for acquisition of this combination of coding region and non-coding region variants. The first possibility is that specimens of *R. rattus* or *R. losea* may have invaded *R. norvegicus* natural habitats world-wide, establishing additional warfarin resistance mutations in new geographic areas. A recent study by Lack *et al.* supports this hypothesis, but also gives evidence that black rat taxa (*R. tanezumi*, *R. rattus I*, *R. rattus*

Tab. 6 VKORC1 compound missense mutations associated with warfarin resistance in *Rattus* sp.

mutations	nucleotides	genotyped individuals	strain (reference)		geographic region	reference
(reference sequence)	reference SNPs: (c.36G, c.123G, c.268A, c.294G, c.321C, c.411C)	many	wild-type	*Rattus norvegicus**	Germany	Rost et al. 2004
(Arg12Arg), Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.268A>T, (c.321C>A, c.411C>T)			*Rattus rattus**	Japan	Tanaka et al. 2012
(Arg12Arg, Ala41Ala), Ile90Leu, (Arg98Arg, Ile107Ile, Thr137Thr)	(c.36G>A, c.123G>A), c.268A>T, (c.284G>A, c.321C>A, c.411C>T)			*Rattus losea**	China, Guang-dong province	Wang et al. 2008
Ala21Thr, Tyr139Phe	c.61G>A, c.416A>T	1	wild, trapped in known or suspected resistance area		Korea, Seoul	Rost et al. 2009
Ala26Thr, Phe63Cys, (Ile82Ile)	c.76G>A, c.188T>G, (?)	1			UK, Cambridge/ Essex	
Arg35Pro +/-, Tyr139Phe -/+	c.104G>C, c.416A>T	1	wild resistant assessed by BCR		France, Burgundy	Pelz et al. 2005
Tyr39Asn, Phe63Cys, (Ile82Ile)	c.115T>A, c.188T>G, (?)	1	wild, trapped in known or suspected resistance area		UK, Cambridge/ Essex	Rost et al. 2009
(Arg12Arg, Ala41Ala), Arg58Gly, Ile90Leu, (Arg98Arg, Ile107Ile, Thr137Thr)	c.172C>G	29	wild warfarin-resistant *Rattus losea*		China, Guang-dong province	Wang et al. 2008
(Arg12Arg, Ala41Ala), Arg58Gly, Ile90Leu, (Arg98Arg, Ile107Ile, Thr137Thr)	c.172C>G	6				
(Arg12Arg, Ala41Ala), Arg58Gly, Ile90Leu, (Cys96Cys, Arg98Arg, Ile107Ile, Thr137Thr)	c.172C>G, (?)	1				
(Arg12Ag), Trp59Arg, Ile90Leu, (Leu94Leu, Ile107Ile, Thr137Thr, Ala143Ala)	(?), c.175T>A, c.268A>T, (?, ?, ?, ?)	7	wild, trapped in known or suspected resistance area		Argentina, Buenos Aires	Rost et al. 2009
(Arg12Arg), Ala41Thr, Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.121A>G, c.268A>T, (c.321C>A, c.411C>T)	16	warfarin-resistant by PT, APPT, HPT *Rattus rattus*		Japan, Osaka	Tanaka et al. 2012
(Arg12Arg), Ala41Val, Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.122T>C, c.268A>T, (c.321C>A, c.411C>T)	17			Japan, Niigata-Gunma-Fukuoka	
(Arg12Arg), Arg61Trp, Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.181T>C, c.268A>T, (c.321C>A, c.411C>T)	<38†			Japan, Tokyo area	
(Arg12Arg), Leu76Pro, Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.227C>T, c.268A>T, (c.321C>A, c.411C>T)	<44†				
(Arg12Arg), Arg61Trp, Leu76Pro, Ile90Leu, (Ile107Ile, Thr137Thr)	(c.36G>A), c.181T>C, c.227C>T, c.268A>T, (c.321C>A, c.411C>T)	2–37†				
Ile90Leu, (Leu94Leu, Ile107Ile), Val112Leu, (Thr137Thr, Ala143Ala)	c.268A>T, (?, ?), c.334G>T, (?, ?)	2	wild, trapped in known or suspected resistance area		Azores, Terceira	Rost et al. 2009
Ile90Leu, (Ile107Ile, Thr137Thr), Ile141Val	c.268A>T, (?, ?), c.421A>G	6			Indonesia	
Ile90Leu, (Ser103Ser, Ile107Ile, Thr137Thr), Ala143Val	c.268A>T, (?, ?, ?), c.421A>G	5				
Ile90Leu, (Ser103Ser, Ile107Ile, Thr137Thr), Ile141Val, Ala143Val	c.268A>T, (?, ?, ?), c.421A>G, c.421A>G	1				
Leu128Gln +/-, Tyr139Cys -/+	c.383T>A, c.416A>G	1	wild resistant assessed by BCR		Uk,Yorkshire	Pelz et al. 2005

Silent polymorphisms indicated in parentheses; see legend for Table 1; * warfarin-senstive wild-type reference species (entries 1–3); †Sequencing results reported 38 Arg61Trp- and 44 Leu76Pro-bearing indviduals, but some were compound heterozygotes.

IV) have already hybridized with other *Rattus* species on six continents (2). By analyzing gene sequences for mitochondrial cytochrome b, two nuclear genes and nine microsatellite loci, the authors suggest that significant hybridization has already occurred between *R. tanezumi* to *R. rattus I* in the USA and from *R. tanezumi*, to *R. rattus IV* in Asia. Additionally, there is microsatellite evidence for unidirectional introgression from both *R. rattus I* and *R. rattus IV* to *R. tanezumi* and that introgression has occurred to such an extent in the USA and parts of Asia that they were unable to detect any nuclear genetic signal for *R. tanezumi*. Thus, we venture to speculate that adaptive introgression among Asian and Western rat species may have played a role in the development in warfarin resistance in Western species. Further study and comparison of the fully sequenced Vkorc1 genes of Western and Asian species, including 5'-UTR, introns and 3'-UTR flanking regions will be required to test this hypothesis.

Comparision of VKORC1/Vkorc1 missense mutations

To summarize the distribution of warfarin resistance-associated VKORC1/Vkorc1 mutations of the 26 human, 22 rat and 13 mouse substitutions, there is a total of 37 positions in the protein primary sequences (nearly one-quarter of the amino acid residues) where naturally occuring warfarin resistance mutations have been found accross species. These positions are obseved to cluster in the predicted large ER lumenal loop (assuming the 4TMH topology) and topologically adjacent regions of predicted TM helices 1, 3 and 4 (Fig. 4 of Watzka et al. 2011) (84).

Compared to the 26 distinct VKORC1 single missense mutations associated with warfarin and other 4HC oral anticoagulant resistance in humans, there are relatively few rat and mouse 4HC rodenticide-associated Vkorc1 single missense mutations (ten and six, respectively). Thus, the vast majority of the single missense mutations appear to be species-specific in humans, mice and rats. Strikingly, of the OAC resistance missense mutations that are common among these mammalian species, four mutated residue positions are shared among all three species (Ala26, Trp59, Leu128 and Tyr139). Humans and mice share only one mutated residue (Arg58) associated with warfarin resistance. Similarly mice and rats share just one warfarin resistance mutation (Arg61). Humans and rats share three mutated positions (His28, Ala41, Ser56).

Selection pressure and pleiotropy in rodents

With respect to singly occurring rat Vkorc1 missense mutations, there is evidence that at least some warfarin resistance-associated mutations (*e.g.*, Leu128Gln and Tyr139Ser) actually confer an increased dietary vitamin K requirement and which, in turn, cause decreased fitness in the absense of 4HC rodenticides. Whether VKOR enzymatic efficiency (i.e., enzymatic „fitness") is improved by the warfarin-resistance mutations is a theme that had previously been examined by Lasseur and coworkers in a carefully performed series of Vkorc1 enzymatic studies of resistant rat and mouse strains (114–116). Interestingly, Czogalla et al. recently showed that most of the human warfarin-resistant mutations are associated with inceased VKOR enzymatic activity compared to that of the wild-type enzyme (117).

Sources of warfarin resistance genetics data

In collecting compehensive data for this review, we also included data from publicly available genomics databases and clinical repositories with entries for both noncoding polymophisms as well as coding region missense mutations. Specifically, we used the NCBI databases dbSNP, dbVar, OMIM, and ClinVar (▶www.ncbi.nlm.nih.gov/). While some databases indicate that some of the human polymorphisms have clinical significance, the overwhelming majority of the entries from either whole genome-sequencing or exomic sequencing projects reflect individuals who do not suffer clinical thrombotic symptoms and who have never been prescribed OAC therapy and for whom no VKORC1 phenotypes are known.

The NCBI Database for Short Genetic Variations (dbSNP) currently includes 161 human SNPs for *VKORC1*, although among these only five of 26 known warfarin resistance missense mutations are represented. Additionally, 34 *Rattus norvegicus* SNPs are included in dbSNP. Ten are noncoding region polymorphisms, five are silent coding region polymorphisms (Arg12Arg, Ile82Ile, Ser103Ser, Ala143Ala, Thr137Thr), and 19 are warfarin resistance-associated missense mutations (Ala21Pro, Ala26Pro, Arg33Pro, Arg35Pro, Tyr39Asn, Ser56Thr, Trp59Arg, Phe63Tyr, Glu67Gln, Ile90Leu, Leu94Ile, Ile107Met, Val112Met, Leu120Gln, Leu128Gln, Tyr139Phe, Ile141Leu, Pro154Leu, Ala143Glu). We could confirm, for example, that the Ile90Leu mutation, corresponding to the wild-type residue Leu90 in Asian rat species *R. losea* and *R. rattus*, has been found in *R. norvegicus* under the dbSNP entry rs66459383. For *Mus musculus* there are currently 139 SNPs and small insertions (<10 bp) listed in the dbSNP.

The NCBI database for genomic structural variation (dbVar) currently includes only a few entries including two insertions and one copy number variation in human *VKORC1*, and one mobile element insertion in mouse *Vkorc1*. Structural variants are reported in the chromosome 16 region including *VKORC1*of J. Craig Venter's diploid genome (esv1077864 and esv1138521, both are insertions ~3 kbp 3'-UTR to *VKORC1*) (118). Additional variants to the Venter genome set from Levy et al. 2007, including several additional completed human genomes, reported an additional single gene copy number gain of 629 bp occurring in the 3'-UTR flanking region of *VKORC1*.(119) The mobile element insertion esv628164 in the second *Vkorc1* intron of the species *M. m. domesticus* has not yet been functionally investigated.(120) Unexpectedly, we were able to link a genomics reference for the VKORC1:p.Arg58Gly warfarin resistance missense mutation to a literature reference of a study by Wang et al. 2008 (see their Supp. Table S11) in which the diploid genome sequence of one Asian individual was presented (121). This patient has no history of thrombotic disease or other known genetic diseases, has not ever been

treated with OACs, but carries the warfarin resistance mutation. To our best knowledge, this is the first documentation for an Asian individual with a warfarin resistance mutation (see Table 1; also indexed as HGMD entry: CM040501). Among medical practisioners and communities, it has been speculated that the rather obvious lack of Asian OAC patient reports of high warfarin dosage requirements probably due to the common knowledge that Asias require the lowest dosages world-wide and so physicians may be afraid to try substantially increasing dosage for fear of causing severe bleeding and possible death.

In the Online Mendelian Inheritance in Man (OMIM, ▶http://omim.org/), the entry #122700 for COUMARIN RESISTANCE surprisingly does not lead to any linked VKORC1 genotype/phenotype data. However, an alternative entry, *608547.VITAMIN K EPOXIDE REDUCTASE COMPLEX, SUBUNIT 1; VKORC1, does list warfarin-associated missense mutations, but is very outdated and includes only the original four human warfarin resistance missense mutations reported by Rost et al. in 2004 (22).

Finally, NCBI ClinVar is a new database launched in early 2013 that is intended to eventually supersede dbGAP, dbSNP and dbVar. Current entries for VKORC1 number only 14, among which are only five of the 26 missense mutations associated with human warfarin resistance.

Conclusion

In this review, we aimed to summarize the current knowledge of warfarin resistance mutations, especially addressing similarities and differences between warfarin and other OAC resistance-associated VKORC1 /Vkorc1 missense mutations in human, rat and mouse populations. With only two exceptions (VKORC1:p.Asp36Tyr, VKORC1:p.Val66Met), human OAC resistance-associated missense mutations, likely due to de novo mutations, represent extremely rare events.

Mouse and rat populations appear to acquire OAC resistance by 4HC pesticide selection pressure through either single or multiple Vkorc1 missense mutations on one or both *Vkorc1* alleles. Potential mechanisms include de novo mutations, selection of standing variants, and adaptive introgression of complete *Vkorc1* alleles from distinctly different species. We hope to have shed some light on the subject of comparative genetics of warfarin resistance among humans, mice and rats, and that awareness araised will lead to future studies of similarities and differences between human and rodent VKORC1 /Vkorc1 mutations and the mechanisms underlying them.

Conflict of interest

The authors declare, that they have no conflict of interest.

References

1. Gratz NG. Societal impact of rodents in rice agriculture. In: Quick GR, editor. odents and rice, report and proceedings of an expert panel meeting on rice rodent control. Manila, Philippines: Internaltional Rice Research Institute 1990, p. 17–26.
2. Lack JB, Greene DU, Conroy CJ et al. Invasion facilitates hybridization with introgression in the Rattus rattus species complex. Mol Ecol 2012; 21: 3545–3561.
3. Gratz NG. Rodents and human disease: a global appreciation. In: Prakash I, editor. Rodent pest management. Baton Rouge: CRC Press 1988, p. 101–71.
4. Gratz NG. Rodents as carriers of disease. In: Buckle AP, Smith RH, editors. Rodent pests and their control. Oxon, U.K.: CAB International 1994. p. 85–108.
5. Link KP. The discovery of dicumarol and its sequels. Circulation 1959; 19: 97–107.
6. Meehan AP. Rats and Mice: Their Biology and Control. East Grinstead, U.K.: Rentokill Ltd. 1984.
7. Tobin ME, Fall MW. Pest control: Rodents. Encyclopedia of Life Support Systems (EOLSS). Oxford, U.K.: Eolss Publishers (UNESCO) 2004; p. Paper 67.
8. Bellis DM, Spring MS, Stoker JR. The biosynthesis of dicoumarol. Biochem J 1967; 103: 202–206.
9. Campbell HA, Link KP. Studies on the hemorrhagic sweet clover disease. IV. The isolation and crystallization of the hemorrhagic agent. J Biol Chem 1941; 138: 21–33.
10. Dam H. The antihaemorrhagic vitamin of the chick. Biochem J 1935; 29: 1273–1285.
11. Matschiner JT, Bell RG, Amelotti JM, Knauer TE. Isolation and characterization of a new metabolite of phylloquinone in the rat. Biochim Biophys Acta 1970; 201: 309–315.
12. Bell RG, Matschiner JT. Vitamin K activity of phylloquinone oxide. Arch Biochem Biophys 1970; 141: 473–476.
13. Bell RG, Matschiner JT. Warfarin and the inhibition of vitamin K activity by an oxide metabolite. Nature 1972; 237: 32–33.
14. Bell RG, Sadowski JA, Matschiner JT. Mechanism of action of warfarin. Warfarin and metabolism of vitamin K 1. Biochemistry 1972; 11: 1959–1961.
15. Bell RG. Metabolism of vitamin K and prothrombin synthesis: anticoagulants and the vitamin K--epoxide cycle. Fed Proc Amer Soc Exp Biol 1978; 37: 2599–2604.
16. Willingham AK, Matschiner JT. Changes in phylloquinone epoxidase activity related to prothrombin synthesis and microsomal clotting activity in the rat. Biochem J 1974; 140: 435–441.
17. Fasco MJ, Principe LM. R- and S-warfarin inhibition of vitamin K and vitamin K 2,3-epoxide reductase activities in the rat. J Biol Chem 1982; 257: 4894–4901.
18. Enzyme Nomenclature: Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes by the reactions they catalyse. Queen May University of London 2013 [cited 1 August 2013]. www.chem.qmul.ac.uk/iubmb/enzyme/.
19. Sadowski JA, Schnoes HK, Suttie JW. Vitamin K epoxidase: properties and relationship to prothrombin synthesis. Biochemistry 1977; 16: 3856–3863.
20. Wu SM, Morris DP, Stafford DW. Identification and purification to near homogeneity of the vitamin K-dependent carboxylase. Proc Natl Acad Sci USA 1991; 88: 2236–2240.
21. Wu SM, Cheung WF, Frazier D, Stafford DW. Cloning and expression of the cDNA for human gamma-glutamyl carboxylase. Science 1991; 254: 1634–1636.
22. Rost S, Fregin A, Ivaskevicius V et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 2004; 427: 537–541.
23. Li T, Chang CY, Jin DY et al. Identification of the gene for vitamin K epoxide reductase. Nature 2004; 427: 541–544.
24. Jin DY, Tie JK, Stafford DW. The conversion of vitamin K epoxide to vitamin K quinone and vitamin K quinone to vitamin K hydroquinone uses the same active site cysteines. Biochemistry 2007; 46: 7279–7283.
25. Fregin A, Rost S, Wolz W et al. Homozygosity mapping of a second gene locus for hereditary combined deficiency of vitamin K-dependent clotting factors to the centromeric region of chromosome 16. Blood 2002; 100: 3229–3232.
26. Greaves J, Ayres P. Heritable resistance to warfarin in rats. Nature 1967; 215: 877–878.
27. Kohn MH, Pelz HJ. Genomic assignment of the warfarin resistance locus, Rw, in the rat. Mamm Genome 1999; 10: 696–698.
28. Wallace ME, MacSwiney FJ. A major gene controlling warfarin-resistance in the house mouse. J Hyg (Lond) 1976; 76: 173–181.
29. Oldenburg J, von Brederlow B, Fregin A et al. Congenital deficiency of vitamin K dependent coagulation factors in two families presents as a genetic defect of the vitamin K-epoxide-reductase-complex. Thromb Haemost 2000; 84: 937–941.
30. Marchetti G, Caruso P, Lunghi B et al. Vitamin K-induced modification of coagulation phenotype

in VKORC1 homozygous deficiency. J Thromb Haemost 2008; 6: 797–803.
31. Oldenburg J, Bevans CG, Muller CR, Watzka M. Vitamin K epoxide reductase complex subunit 1 (VKORC1): the key protein of the vitamin K cycle. Antioxid Redox Signal 2006; 8: 347–353.
32. Westhofen P, Watzka M, Marinova M et al. Human vitamin K 2,3-epoxide reductase complex subunit 1-like 1 (VKORC1L1) mediates vitamin K-dependent intracellular antioxidant function. J Biol Chem 2011; 286: 15085–15094.
33. Spohn G, Kleinridders A, Wunderlich FT et al. VKORC1 deficiency in mice causes early postnatal lethality due to severe bleeding. Thromb Haemost 2009; 101: 1044–1050.
34. Hammed A, Matagrin B, Spohn G et al. VKORC1L1, an enzyme rescuing the VKOR activity in some extrahepatic tissues during anticoagulation therapy. J Biol Chem 2013; DOI: 10.1074/jbc.M113.457119.
35. Bevans CG, Krettler C, Reinhart C et al. Determination of the warfarin inhibition constant Ki for vitamin K 2,3-epoxide reductase complex subunit-1 (VKORC1) using an in vitro DTT-driven assay. Biochim Biophys Acta – Gen Subj 2013; 1830: 4202–4210.
36. Van Horn WD. Structural and functional insights into human vitamin K epoxide reductase and vitamin K epoxide reductase-like1. Crit Rev Biochem Mol Biol 2013; 48: 357–372.
37. Johnson JA, Cavallari LH. Pharmacogenetics and cardiovascular disease--implications for personalized medicine. Pharmacol Rev 2013; 65: 987–1009.
38. Lee MT, Klein TE. Pharmacogenetics of warfarin: challenges and opportunities. J Hum Genet 2013; 58: 334–338.
39. Wittkowsky AK. Warfarin and other coumarin derivatives: pharmacokinetics, pharmacodynamics, and drug interactions. Semin Vasc Med 2003; 3: 221–230.
40. Ufer M. Comparative pharmacokinetics of vitamin K antagonists: warfarin, phenprocoumon and acenocoumarol. Clin Pharmacokinet 2005; 44: 1227–1246.
41. Lindh JD, Holm L, Andersson ML, Rane A. Influence of CYP2C9 genotype on warfarin dose requirements--a systematic review and meta-analysis. Eur J Clin Pharmacol 2009; 65: 365–375.
42. Rieder MJ, Reiner AP, Gage BF et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005; 352: 2285–2293.
43. Geisen C, Watzka M, Sittinger K et al. VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation. Thromb Haemost 2005; 94: 773–779.
44. Poller L, Wright D, Rowlands M. Prospective comparative study of computer programs used for management of warfarin. J Clin Pathol 1993; 46: 299–303.
45. Gage B, Eby C, Johnson J et al. Use of Pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther. 2008; 84: 430.
46. Finkelman BS, Gage BF, Johnson JA et qal. Genetic warfarin dosing: tables versus algorithms. J Am Coll Cardiol 2011; 57: 612–618.
47. Booth SL, Charnley JM, Sadowski JA et al. Dietary vitamin K1 and stability of oral anticoagulation: proposal of a diet with constant vitamin K1 content. Thromb Haemost 1997; 77: 504–509.
48. Nutescu EA, Shapiro NL, Ibrahim S, West P. Warfarin and its interactions with foods, herbs and other dietary supplements. Expert Opin Drug Saf 2006; 5: 433–451.
49. Anthony M, Romero K, Malone DC et al. Warfarin interactions with substances listed in drug information compendia and in the FDA-approved label for warfarin sodium. Clin Pharmacol Ther 2009; 86: 425–429.
50. Zhong SL, Yu XY, Liu Y et al. Integrating interacting drugs and genetic variations to improve the predictability of warfarin maintenance dose in Chinese patients. Pharmacogenet Genomics 2012; 22: 176–182.
51. Chu K, Wu SM, Stanley T et al. A mutation in the propeptide of Factor IX leads to warfarin sensitivity by a novel mechanism. J Clin Invest 1996; 98: 1619–1625.
52. Oldenburg J, Quenzel EM, Harbrecht U et al. Missense mutations at ALA-10 in the factor IX propeptide: an insignificant variant in normal life but a decisive cause of bleeding during oral anticoagulant therapy. Br J Haematol 1997; 98: 240–244.
53. Taube J, Halsall D, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. Blood 2000; 96: 1816–1819.
54. Linder MW. Genetic mechanisms for hypersensitivity and resistance to the anticoagulant Warfarin. Clin Chim Acta 2001; 308: 9–15.
55. Kristensen SR. Warfarin treatment of a patient with coagulation factor IX propeptide mutation causing warfarin hypersensitivity. Blood 2002; 100: 2676–2677.
56. Hermida J, Zarza J, Alberca I et al. Differential effects of 2C9*3 and 2C9*2 variants of cytochrome P-450 CYP2C9 on sensitivity to acenocoumarol. Blood 2002; 99: 4237–4239.
57. Wadelius M, Chen LY, Eriksson N et al. Association of warfarin dose with genes involved in its action and metabolism. Hum Genet 2007; 121: 23–34.
58. Wadelius M, Chen LY, Lindh JD et al. The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2009; 113: 784–792.
59. Takeuchi F, McGinnis R, Bourgeois S et al. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 2009; 5: e1000433.
60. Perera MA, Cavallari LH, Limdi NA et al. Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. Lancet 2013; 382: 790–796.
61. Daneshjou R, Tatonetti NP, Karczewski KJ et al. Pathway analysis of genome-wide data improves warfarin dose prediction. BMC Genomics 2013; 14 (Suppl 3): S11.
62. Voora D, Koboldt DC, King CR et al. A polymorphism in the VKORC1 regulator calumenin predicts higher warfarin dose requirements in African Americans. Clin Pharmacol Ther 2010; 87: 445–451.
63. D'Andrea G, D'Ambrosio RL, Di Perna P et al. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 2005; 105: 645–649.
64. Wang D, Chen H, Momary KM et al. Regulatory polymorphism in vitamin K epoxide reductase complex subunit 1 (VKORC1) affects gene expression and warfarin dose requirement. Blood 2008; 112: 1013–1021.
65. Shukla T, Reddy SC, Korrapatti S et al. A novel VKORC1 promoter mutation found causing warfarin resistance, along with –1639G>A promoter mutation – A pilot study on the genetic variation in patients on warfarin therapy in South India. Biomarkers Genomic Medicine 2013; http://dx.doi.org/10.1016/j.bgm.2013.05.001
66. Saito R, Takeda K, Yamamoto K et al. Nutri-pharmacogenomics of warfarin anticoagulation therapy: VKORC1 genotype-dependent influence of dietary vitamin K intake. J Thromb Thrombolysis 2013; DOI: 10.1007/s11239–013–0978–9.
67. Rettie AE, Korzekwa KR, Kunze KL et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S)-warfarin-drug interactions. Chem Res Toxicol 1992; 5: 54–59.
68. Zhang Z, Fasco MJ, Huang Z et al. Human cytochromes P4501A1 and P4501A2: R-warfarin metabolism as a probe. Drug Metab Dispos 1995; 23: 1339–1346.
69. Wienkers LC, Wurden CJ, Storch E et al. Formation of (R)-8-hydroxywarfarin in human liver microsomes. A new metabolic marker for the (S)-mephenytoin hydroxylase, P4502C19. Drug Metab Dispos 1996; 24: 610–614.
70. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1999; 353: 717–719.
71. Johnson JA, Gong L, Whirl-Carrillo M et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin Pharmacol Ther 2011; 90: 625–629.
72. Wells PS, Majeed H, Kassem S et al. A regression model to predict warfarin dose from clinical variables and polymorphisms in CYP2C9, CYP4F2, and VKORC1: Derivation in a sample with predominantly a history of venous thromboembolism. Thromb Res 2010; 125: e259–e264.
73. Oldenburg J, Bevans CG, Fregin A et al. Current pharmacogenetic developments in oral anticoagulation therapy: the influence of variant VKORC1 and CYP2C9 alleles. Thromb Haemost 2007; 98: 570–578.
74. Dzik WS. Reversal of drug-induced anticoagulation: old solutions and new problems. Transfusion 2012; 52 (Suppl 1): 45S-55S.
75. Frueh FW. On rat poison and human medicines: personalizing warfarin therapy. Trends Mol Med 2012; 18: 201–205.
76. Patillon B, Luisi P, Blanche H et al. Positive selection in the chromosome 16 VKORC1 genomic region has contributed to the variability of anticoagulant response in humans. PLoS One 2012; 7: e53049.
77. Boyle CM. Case of apparent resistance of Rattus norvegicus berkenhout to anticoagulant poisons. Nature 1960; 188: 517.

78. Lund M. The toxicity of chlorophacinone and warfarin to house mice (Mus musculus). J Hyg (Lond) 1971; 69: 69–72.
79. O'Reilly RA, Aggeler PM, Hoag MS et al. Hereditary Transmission of Exceptional Resistance to Coumarin Anticoagulant Drugs. the First Reported Kindred. N Engl J Med 1964; 271: 809–815.
80. O'Reilly RA. The second reported kindred with hereditary resistance to oral anticoagulant drugs. N Engl J Med 1970; 282: 1448–1451.
81. Alving BM, Strickler MP, Knight RD, Barr CF, Berenberg JL, Peck CC. Hereditary warfarin resistance. Investigation of a rare phenomenon. Arch Intern Med 1985; 145: 499–501.
82. Hulse ML. Warfarin resistance: diagnosis and therapeutic alternatives. Pharmacotherapy 1996; 16: 1009–1017.
83. Lefrere JJ, Horellou MH, Conard J, Samama M. Proposed classification of resistances to oral anticoagulant therapy. J Clin Pathol 1987; 40: 242.
84. Watzka M, Geisen C, Bevans CG et al. Thirteen novel VKORC1 mutations associated with oral anticoagulant resistance: insights into improved patient diagnosis and treatment. J Thromb Haemost 2011; 9: 109–118.
85. Anton AI, Cerezo-Manchado JJ, Padilla J et al. Novel associations of VKORC1 variants with higher acenocoumarol requirements. PLoS One 2013; 8: e64469.
86. Harrington DJ, Underwood S, Morse C et al. Pharmacodynamic resistance to warfarin associated with a Val66Met substitution in vitamin K epoxide reductase complex subunit 1. Thromb Haemost 2005; 93: 23–26.
87. D'Ambrosio RL, D'Andrea G, Cafolla A et al. A new vitamin K epoxide reductase complex subunit-1 (VKORC1) mutation in a patient with decreased stability of CYP2C9 enzyme. J Thromb Haemost 2007; 5: 191–193.
88. Bodin L, Perdu J, Diry M et al. Multiple genetic alterations in vitamin K epoxide reductase complex subunit 1 gene (VKORC1) can explain the high dose requirement during oral anticoagulation in humans. J Thromb Haemost 2008; 6: 1436–1439.
89. Wilms EB, Touw DJ, Conemans JM et al. A new VKORC1 allelic variant – p.Trp59Arg – in a patient with partial resistance to acenocoumarol and phenprocoumon. J Thromb Haemost 2008; 6: 1224–1226.
90. Peoc'h K, Pruvot S, Gourmel C et al. A new VKORC1 mutation leading to an isolated resistance to fluindione. Br J Haematol 2009; 30: 30.
91. Schmeits PC, Hermans MH, van Geest-Daalderop JH et al. VKORC1 mutations in patients with partial resistance to phenprocoumon. Br J Haematol 2010; 148: 955–957.
92. Harrington DJ, Siddiq S, Allford SL et al. More on: endoplasmic reticulum loop VKORC1 substitutions cause warfarin resistance but do not diminish gamma-carboxylation of the vitamin K-dependent coagulation factors. J Thromb Haemost 2011; 9: 109–118.
93. Mitchell C, Gregersen N, Krause A. Novel CYP2C9 and VKORC1 gene variants associated with warfarin dosage variability in the South African black population. Pharmacogenomics 2011; 12: 953–963.
94. Huff JD, Lawson HL, Harish VC et al. Warfarin Resistance Due to a Val29Leu Mutation in the VKORC1 Protein. Blood 2005; 106: A4146.
95. Scott SA, Edelmann L, Kornreich R, Desnick RJ. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. Am J Hum Genet 2008; 82: 495–500.
96. Shahin MH, Cavallari LH, Perera MA et al. VKORC1 Asp36Tyr geographic distribution and its impact on warfarin dose requirements in Egyptians. Thromb Haemost 2013; 109: ?? AUTOR BITTE ERGÄNZEN: SEITEN??)).
97. Osman A, Enstrom C, Arbring K et al. Main haplotypes and mutational analysis of vitamin K epoxide reductase (VKORC1) in a Swedish population: a retrospective analysis of case records. J Thromb Haemost 2006; 4: 1723–1729.
98. Pelz HJ, Rost S, Hunerberg M et al. The genetic basis of resistance to anticoagulants in rodents. Genetics 2005; 170: 1839–1847.
99. Rost S, Pelz HJ, Menzel S et al. Novel mutations in the VKORC1 gene of wild rats and mice--a response to 50 years of selection pressure by warfarin? BMC Genet 2009; 10: 4.
100. Song Y, Endepols S, Klemann N et al. Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. Curr Biol 2011; 21: 1296–1301.
101. Pelz HJ, Rost S, Muller E et al. Distribution and frequency of VKORC1 sequence variants conferring resistance to anticoagulants in Mus musculus. Pest Manag Sci 2012; 68: 254–259.
102. Lorusso DJ, Suttie JW. Warfarin binding to microsomes isolated from normal and warfarin-resistant rat liver. Mol Pharmacol 1972; 8: 197–203.
103. Shah DV, Suttie JW. The vitamin K dependent, in vitro production of prothrombin. Biochem Biophys Res Commun 1974; 60: 1397–1402.
104. MacNicoll AD. A comparison of warfarin resistance and liver microsomal vitamin K epoxide reductase activity in rats. Biochim Biophys Acta 1985; 840: 13–20.
105. Thijssen HH. Warfarin resistance. Vitamin K epoxide reductase of Scottish resistance genes is not irreversibly blocked by warfarin. Biochem Pharmacol 1987; 36: 2753–2757.
106. Thijssen HH, Janssen CA, Mosterd JJ. Warfarin resistance: biochemical evaluation of a warfarin-resistant wild brown rat. Biochem Pharmacol 1989; 38: 3129–3132.
107. Grandemange A, Lasseur R, Longin-Sauvageon C et al. Distribution of VKORC1 single nucleotide polymorphism in wild Rattus norvegicus in France. Pest Manag Sci 2010; 66: 270–276.
108. Endepols S, Klemann N, Song Y, Kohn MH. Vkorc1 variation in house mice during warfarin and difenacoum field trials. Pest Manag Sci 2013; 69: 409–413.
109. Staubach F, Lorenc A, Messer PW et al. Genome patterns of selection and introgression of haplotypes in natural populations of the house mouse (Mus musculus). PLoS Genet 2012; 8: e1002891.
110. Baert K, Stuyck J, Breyne P et al. Distribution of anticoagulant resistance in the brown rat in Belgium. Belg J Zool 2012; 142: 39–48.
111. Tanaka KD, Kawai YK, Ikenaka Y et al. A novel mutation in VKORC1 and its effect on enzymatic activity in Japanese warfarin-resistant rats. J Vet Med Sci 2013; 75: 135–139.
112. Wang J, Feng Z, Yao D et al. Warfarin resistance in Rattus losea in Guangdong Province, China. Pesticide Biochem Physiol 2008; 91: 90–95.
113. Tanaka KD, Kawai YK, Ikenaka Y et al. The genetic mechanisms of warfarin resistance in Rattus rattus found in the wild in Japan. Pest Biochem Physiol 2012; 103: 144–151.
114. Lasseur R, Longin-Sauvageon C, Videmann B et al. Warfarin resistance in a French strain of rats. J Biochem Mol Toxicol 2005; 19: 379–385.
115. Lasseur R, Grandemange A, Longin-Sauvageon C et al. Heterogeneity of the coumarin anticoagulant targeted vitamin K epoxide reduction system. Study of kinetic parameters in susceptible and resistant mice (Mus musculus domesticus). J Biochem Mol Toxicol 2006; 20: 221–229.
116. Lasseur R, Longin-Sauvageon C, Berny P, Benoit E. Biochemistry of resistance to warfarin in a French strain of the Norway rat (Rattus norvegicus). Int J Pest Management 2007; 53: 273–280.
117. Czogalla KJ, Biswas A, Wendeln A-C et al. Human VKORC1 mutations cause variable degrees of 4-hydroxycoumarin resistance and affect putative warfarin binding interfaces. Blood 2013; doi: 10.1182/blood-2013–05–501692.
118. Levy S, Sutton G, Ng PC et al. The diploid genome sequence of an individual human. PLoS Biol 2007; 5: e254.
119. Pang AW, MacDonald JR, Pinto D et al. Towards a comprehensive structural variation map of an individual human genome. Genome Biol 2010; 11: R52.
120. Keane TM, Goodstadt L, Danecek P et al. Mouse genomic variation and its effect on phenotypes and gene regulation. Nature 2011; 477: 289–294.
121. Wang J, Wang W, Li R et al. The diploid genome sequence of an Asian individual. Nature 2008; 456: 60–65.