Presence of the knockdown resistance mutation, Vgsc-1014F in Anopheles gambiae and An. arabiensis in western Kenya

Eric Ochomo1,2*, Krishanthi Subramaniam3, Brigid Kemei2, Emily Rippon3, Nabie M. Bayoh2, Luna Kamau4, Francis Atieli3, John M. Vulule2, Collins Ouma1,5, John Gimnig6, Martin J. Donnelly3,7 and Charles Mbogo8,9

Abstract

Introduction: The voltage gated sodium channel mutation Vgsc-1014S (kdr-east) was first reported in Kenya in 2000 and has since been observed to occur at high frequencies in the local Anopheles gambiae s.s. population. The mutation Vgsc-1014F has never been reported from An. gambiae Complex complex mosquitoes in Kenya.

Findings: Molecularly confirmed An. gambiae s.s. (hereafter An. gambiae) and An. arabiensis collected from 4 different parts of western Kenya were genotyped for kdr from 2011 to 2013. Vgsc-1014F was observed to have emerged, apparently, simultaneously in both An. gambiae and An. arabiensis in 2012. A portion of the samples were submitted for sequencing in order to confirm the Vgsc-1014F genotyping results. The resulting sequence data were deposited in GenBank (Accession numbers: KR867642-KR867651, KT758295-KT758303). A single Vgsc-1014F haplotype was observed suggesting, a common origin in both species.

Conclusion: This is the first report of Vgsc-1014F in Kenya. Based on our samples, the mutation is present in low frequencies in both An. gambiae and An. arabiensis. It is important that we start monitoring relative frequencies of the two kdr genes so that we can determine their relative importance in an area of high insecticide treated net ownership.

Keywords: Kdr, Insecticide resistance, Pyrethroids, Anopheles gambiae

Introduc­tion

The two most widely applied vector control tools, insecticide treated nets (ITNs) and indoor residual spraying (IRS) have contributed greatly to the decline in global malaria rates [1, 2]. Pyrethroids are the most commonly used insecticides in control programs due to their low human toxicity and high efficacy against vectors [3, 4]. Previously, DDT, an organochlorine, was the most widely used insecticide for vector control with its use spread out over multiple countries for malaria control [5, 6]. The widespread use of these insecticides has likely contributed to the selection of resistance across sub-Saharan Africa [7] (http://www.irmapper.com/).

Increased resistance to pyrethroids is particularly troubling since this is the only class of insecticides approved by WHO for use on ITNs [3]. If ITNs are rendered ineffective, a surge in malaria transmission could follow [8]. Resistance to pyrethroids has been reported from multiple sites in western Kenya [9, 10] with both target site and metabolic resistance mechanisms implicated [9–15]. DDT and pyrethroids function by binding to the voltage gated sodium channels (Vgsc) on the mosquito’s neurons delaying the closing of the sodium channel; prolonging the action potential and causing repetitive neuron firing, ultimately resulting in paralysis and death [8, 16].

In Anopheles gambiae s.l., knock down resistance (kdr) is commonly caused by mutations in the Vgsc- either from leucine (TTA) to phenylalanine (TTT) or leucine...
to serine (TCA) [11, 17] at codon 1014. Vgsc-1014S (formerly kdr-east) was first reported in Kenya in 2000 and has been observed to occur at high frequencies in the local An. gambiae populations [10, 11]. Thus far, there has been no report of the existence of Vgsc-1014F (formerly kdr-west) in Kenya but has been reported in Uganda and Tanzania in the recent past [18, 19]. Our work demonstrates the emergence of Vgsc-1014F in western Kenya in two principal malaria vectors, An. gambiae and An. arabiensis.

Findings

Material and methods

This study was conducted in four malaria endemic districts in western Kenya with two distinct Vector control interventions: Rachuonyo and Nyando where IRS (Deltamethrin in 2011 and lambdacyhalothrin in 2012) was combined with ITNs (treated with permethrin or deltamethrin); and in Bondo and Teso where only ITNs are deployed [9]. Mosquito collections were performed annually between June and September in 2011, 2012 and 2013. Mosquito sampling, rearing and bioassays of emergent adults were conducted as described in Ochomo et al. [9].

Species identification & Vgsc genotyping

DNA was extracted from whole specimens and a PCR assay [20] was used to distinguish between An. gambiae and An. arabiensis. DNA samples were genotyped to identify the kdr genotype at amino acid position 1014 of the Vgsc using a modification of the protocol by Bass et al., [21] as described in Mathias et al., [10].

Exon sequencing of Vgsc

Previous studies in western Kenya have only reported the presence of Vgsc-1014S mutation. Therefore, in order to confirm the presence of Vgsc-1014F and to determine if was a de novo origin, a subsample of the Vgsc-1014F carriers were Sanger sequenced. Prior to sequencing, conventional PCR was used to amplify the exon 20 [22] which contains the 1014 locus. Samples were sequenced at Centre for Genomic Research, University of Liverpool, UK and resulting sequences aligned using CodonCode aligner (http://www.codoncode.com/aligner/).

Analysis for the origin of Vgsc-1014F Mutation

Gene sequences obtained from the sequencing exons 20 and 27 were aligned using Codon Code aligner (http://www.codoncode.com/) and the contigs transferred to DnaSP (http://www.ub.edu/dnasp/) as FASTA files. The files were concatenated and then run using the PHASE algorithm in DnaSP [23]. The phased files were exported as a Phylip file to TCS, a statistical parsimony software for phylogenetic network estimation (http://darwin.uvigo.es/software/tcs.html).

Results

Frequency of Vgsc-1014S and Vgsc-1014F in the study sites from 2011 to 2013

We observed low frequencies of Vgsc-1014S in An. arabiensis, even though we had high frequencies of the same allele in An. gambiae, they were much lower than has been reported previously [10]. We saw a simultaneous appearance of Vgsc-1014F in both An. gambiae and An. arabiensis in 2012 in all four

| District  | Year | An. arabiensis | An. gambiae |
|-----------|------|----------------|-------------|
|           |      | Vgsc_1014S     | Vgsc_1014F  | Vgsc_1014S | Vgsc_1014F |
| Bondo     | 2011 | 105 0.052       | 0           | 0           |
|           | 2012 | 129 0.031       | 0.047       | 2           | 0           | 0           |
|           | 2013 | 236 0.008       | 0.125       | 2           | 0           | 0           |
| Nyando    | 2011 | 284 0.016       | 0           | 0           |
|           | 2012 | 82 0.012        | 0.024       | 1           | 0           | 0           |
|           | 2013 | 173 0.055       | 0.023       | 5           | 0           | 0           |
| Rachuonyo | 2011 | 20 0.05         | 0           | 0           |
|           | 2012 | 53 0           | 0.047       | 1           | 0           | 0           |
|           | 2013 | 136 0.018       | 0.011       | 5           | 0           | 0           |
| Teso      | 2011 | 7 0            | 0           | 211 0.94    | 0           |
|           | 2012 | 4 0            | 0           | 189 0.68    | 0.054       |
|           | 2013 | 60 0.019       | 0           | 317 0.85    | 0.025       |
study sites (Table 1) and thereafter compared the mean frequencies of the genes among the three years (Table 2). Of these, 19 samples (12 *An. gambiae* and 7 *An. arabiensis*) were sequenced. 3 *An. gambiae* and 3 *An. arabiensis* were confirmed to be homozygous for *Vgsc-1014F* with one *An. arabiensis* heterozygote detected. The sequences were deposited in GenBank (Accession numbers: KR867642- KR867651, KT758295-KT758303).

Only a single 1014F haplotype was observed (Fig. 1), suggesting a common origin in the species and subsequent interspecific transfer. However it should be noted that our ability to resolve different haplotypes was constrained by the low levels of diversity at this locus and our small amplicon length (478bp).

### Table 2 Comparison of mean frequencies of *Vgsc-1014F* and *Vgsc-1014S* mutations in *An. arabiensis* using ANOVA and Tukey’s test.

| Year   | Vgsc-1014F | Vgsc-1014S |
|--------|------------|------------|
|        | Difference | Lower limit | Upper limit | Adjusted P-value | Difference | Lower limit | Upper limit | Adjusted P-value |
| 2011–2012 | 0.043 | -0.019 | 0.105 | 0.186 | -0.084 | -0.877 | 0.709 | 0.953 |
| 2011–2013 | 0.046 | -0.016 | 0.108 | 0.152 | -0.032 | -0.824 | 0.761 | 0.993 |
| 2012–2013 | 0.003 | -0.059 | 0.065 | 0.99 | 0.052 | 0.741 | 0.845 | 0.982 |

**Discussion**

This is the first report of *Vgsc-1014F* in Kenya, which appears to have emerged in both *An. gambiae* and *An. arabiensis* around 2012 and is confirmed via DNA sequencing in multiple samples. The gene has previously been observed in Uganda [18], then much later in Tanzania [19] and now in Kenya. We have developed this report to alert researchers and programmatic staff to the presence of *Vgsc-1014F* mutation in these two important *Anopheles* vectors so that they can modify their resistance marker screening procedures. It is important therefore that we start monitoring allele and genotype frequencies so that we can assess their impact in an area of high bednet ownership.

**Fig. 1** A TCS plot of the three haplotypes present in the populations assayed. White colour represents *An. gambiae* while black colour represents *An. arabiensis*
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
EO, KS, NMB, LK, FA, JMV, CO, JG, MJD and CM designed and developed the study. EO, KS, BK, ER, NMB, LK, FA, JV, CO, KS, MID and CM contributed to development of the protocol. EO, KS, ER and MJD performed gene sequencing and data analysis. EO, KS and BK performed the laboratory analysis of the samples. All authors took part in manuscript preparation, read and approved the final manuscript.

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Author details
1 School of Public Health and Community Development, Maseno University, Maseno, Kenya. 2Centre for Global Health Research, Kenya Medical Research Institute, P. O. Box 1578, Kisumu 40100, Kenya. 3Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK. 4Centre for Biotechnology and Research Development, Kenya Medical Research Institute, Nairobi, Kenya. 5Health Challenges and Systems, African Population and Health Research Centre, Nairobi, Kenya. 6Centers of Disease Control and Prevention, Atlanta, USA. 7Malaria Programme, Wellcome Trust Sanger Institute, Cambridge, UK. 8Kenya Medical Research Institute, Centre for Geographic Medicine Research-Coast, Kilifi, Kenya. 9Malama Public Health Department, KEMRI-Wellcome Trust Research Program, Nairobi, Kenya.

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