УДК 576.895.2 / 576.32/.36

EVOLUTION OF *Nf2e1/ dKeap 1* SIGNALING PATHWAY IN ACTIVATING *CYP6M2* GENE REGULATION: POTENTIAL ROLE IN RESISTANCE TO INSECTICIDES IN *ANOPHELES GAMBIAE* GILES, 1902 (DIPTERA: CULICIDAE) – A REVIEW

© 2020 B. R. Mohammed[a,b,*], M. K. Simon[a], A. M. Yayo[c]

[a] Department of Parasitology and Entomology, Faculty of Veterinary Medicine, University of Abuja, P.M.B 117, Abuja, Nigeria

[b] School of Science, Engineering and Technology, Abertay University, Dundee, DD1 1HG, United Kingdom (In collaboration with Liverpool School of Tropical Medicine, UK)

[c] Centre for Infectious Diseases Research, Bayero University, Kano Department of Medical Microbiology/Parasitology, Faculty of Medicine, Bayero University, Kano State, Nigeria

*e-mail: balarabemohammed161@yahoo.co.uk

Received 08.04.2020
Received in revised form 25.04.2020
Accepted 23.05.2020

Insecticide resistance is a worldwide menace in the control of vector borne diseases evolving in substantial vector control program. Cytochrome P450s including *CYP6M2* is known to be involved in the metabolism of insecticides preceding resistance. Information on regulatory mechanisms involved in the control of P450s in *Anopheles gambiae* Giles, 1902 is not yet clear. In this review, we analyze the potential function of Nuclear factor erythroid factor 2 (*Nf2e*1), which is an ortholog to Nuclear factor E2-related factor 2 (*Nrf2*) in vertebrates and Cap 'n' collar isoform C (*CnCC*) in *Drosophila melanogaster* in the countenance of the expression of *CYP6M2* gene encoding enzymes and conceivably to alienate insecticide resistance in the control of *Anopheles gambiae*. Under normal conditions, *Nf2e*1 aggregates in the cytoplasm where it synergizes with the actin binding protein, Kelch-like ECH associating protein 1 (*Keap*1) ortholog AGAP003645, and is instantaneously degenerated by the ubiquitin-proteasome pathway. This review article depicts contemporary knowledge of the *Nf2e*1/ AGAP003645 complex, consolidating chiefly on the molecular mechanism of *Nf2e*1 regulation and its potential implication in the control of mosquito borne diseases including malaria.

Keywords: *Anopheles gambiae*, *Drosophila melanogaster*, *Nf2e*1, d*Keap* 1, insecticide resistance

DOI: 10.31857/S1234567806030050

Nuclear factor-erythroid 2 (NF-E2)-related factor 2 (*Nrf2*) is a redox-sensitive basic leucine zipper transcription factor, which is implicated in the transcription of antioxidant defence enzymes including cytochrome P450s (Ramprasath et al., 2014; Swamy et al., 2016). The body defense system in vertebrates and insects is armed with the amplitude upregulating expression levels of these target genes including cytochrome P450s (Li et al., 2015). *Nrf2* (nuclear factor erythroid 2-related factor 2) is the dominant participant in the empirical expression insect cellular enzymes (Ben-Yehuda et al., 2016). These *Nrf2*-regulated enzymes are differentiated by the existence of a cis-acting element called antioxidant responsive element (ARE), which lies within the regulatory region (Ramprasath et al., 2012; Jiang et al., 2015). ARE-mediated response to oxidative stress pathway is conserved in all vertebrates and invertebrates including mosquitoes (Kumar et al., 2018). In sub-Saharan Africa, Anopheline mosquito vectors, including *Anopheles gambiae* s. s. is the target in most insecticide based malaria control activities, particularly those involving the use of residual pyrethroid insecticides (Ranson, Lissenden, 2016; Sinka et al., 2016). Resistance to pyrethroids is chiefly attributed to two principal mechanisms; increased detoxification by enzymes or reduced target site sensitivity (Horstmann, Sonneck, 2016). The detoxification enzymes typically linked with insecticide resistance in *Anopheles gambiae* include; Cytochrome p450s, carboxyl/ choline Esterases and glutathione S-transferase (GSTs) genes (Zhou et al., 2015; Chang et al., 2017). Additionally, p450s are the largest in comparison to all the other detoxification genes and are known to have synergistic effects with estrases and GSTs in insecticide resistance in *Anopheles* species (Chang et al., 2017). Of the p450s, CYP6 sub-family and especially *CYP6M2* is involved mainly in developmental process and is crucial for the metabolic detoxification of insecticides in *Anopheles gambiae* (Guo et al., 2013). *CYP6M2* is found exclusively in insects and repeatedly implicated in resistance to all three distinct classes of WHO recommended insecticides (Pyrethroids, carbamate, and organophosphates) that are important in mosquito vector control (Edi et al., 2014; Mohammed et al., 2017; Ibrahim et al., 2018). Erstwhile microarray investigations on insecticide-resistant mosquitoes, including *Anopheles gambiae* has established a comparatively modest number of up-regulated CYP genes consequent to exposures of the mosquitoes to various concentrations of insecticides (Liang et al., 2015). Up-regulation of these cytochrome oxidases (P450s) including *CYP6M2* has been associated with resistance and enzymatic metabolism of insecticides by *CYP6M2* has been demonstrated *in vitro* (Stevenson et al., 2011; Mitchell et al., 2012; Edi et al., 2014). The transcriptional up-regulation of *CYP6M2* in mosquitoes results in increased levels of protein production and enzymatic activities, which leads to the development of resistance (Liu, 2015). Contemporary investigations have established that the evolutionarily conserved *Nrf2 / Keap* 1 pathways orthologs perform a significant part in regulation of the correspondent transcriptional response to xenobiotic compounds in *D. melanogaster* (Misra et al., 2011; Jones et al., 2013; Guio et al., 2014; Kuzin et al., 2014).

In *Drosophila melanogaster*, some genes associated with metabolic activity are established to be upregulated by the transcription factors Cap ‘n’ collar isoform (*CnCC*) /

Drosophila Kelch-like-ECH-associated protein 1 (d*Keap* 1). These are orthologs to Nuclear factor erythroid -2 related factor-2 (*Nrf2*) / Kelch-like–ECH-associated protein 1 (*Keap* 1) signalling pathways in higher mammalians respectively (Cao et al., 2013; Misra et al., 2013; Das et al., 2014; Siller et al., 2014). No study appears to have been made in understanding these complex mechanisms regulating *CYP6M2* gene expression in insecticide resistance in *Anopheles gambiae*.

The recognition of these transcription factor binding sites (TFBSs) will improve knowledge of how wild populations of *Anopheles gambiae* become resistant to insecticide and are activated by different endogenous and exogenous xenobiotic challenges. One of such pathways is the *Nrf2*/*Keap* 1 signaling pathway, which was first shown to regulate P450s. The orthologs to this gene in *Drosophila melanogaster* an insect model are also known to be Cap 'n' collar isoform C (*CnCC*)/*Keap* 1) (Mohammed et al., 2014; Chatterjee et al., 2016). Significant explorations with regards to evolutionarily conserved signalling pathways have been accomplished employing *D*. *melanogaster* (Altintas et al., 2016).

Preliminary investigations employing insilico resources revealed the orthologs of *CnCC*/ d*Keap* 1 genes in *Anopheles gambiae* to be Nuclear factor erythroid 2 invertebrate (*Nf2e*1)/ AGAP003645. In contrast to the detailed studies in higher vertebrates and *D. melanogaster*, the complex regulatory mechanism regulating P450 gene including *CYP6M2* gene expression in *An. gambiae* is yet to be identified. Here we use bioinformatics and molecular biology technique to show that Nuclear factor erythroid 2, invertebrate) (*Nf2e*1) (*Nrf2*) / d*Keap*1 pathway play a key role in the regulation of xenobiotic responses in *An. gambiae*.

As a pre-requisite for understanding the molecular mechanism involved in the regulation of *CYP6M2*, searches for potential regulatory elements were made insilico using bioinformatic resources.

Insilico identification of *Nrf2 /* are orthologs

Searches were made insilico for the identification of the orthologs of *Nrf2* / ARE in *Anopheles gambiae*, Ensembl genome browser (http://www.ensembl.Org/Human/Search/ Results? q=%20*Nrf2*;m%20y=%203;site=ensemblallx=3;;page=1;facetspeciesHuman) was used to search for the *Nrf2* gene in *Homo sapiens*. Whilst Fly base (http://flybase.org/reports/ FBgn 0262975.html) data base was used to search for its orthologs in *Drosophila melanogaster* (Dm) and VectorBase data base (https://www.VectorBase.org/Anophelesgambiae/ Gene) was used to search for the orthologs in *Anopheles gambiae* (Ag).The insilico analysis results revealed that the orthologs to vertebrate *Nrf2* is identified as *CnCC* in *Drosophila melanogaster* and *Nf2e*1 in *Anopheles gambiae* gene promoters respectively (Mohammed et al., 2014) (Table 1).

While the orthologs to higher vertebrate *Keap* 1 in *Drosophila melanogaster* is d*Keap*1 and AGAP003645 in *Anopheles gambiae* respectively. In this review therefore, we have described the components mediating *Nrf2* signaling in *Anopheles gambiae* and their relationship to human and *Drosophila melanogaster* (Figure 1).

Table 1. The *CnCC* (ortholog to *Nrf2* /*Keap* 1) of vertebrates in *Drosophila melanogaster* and *Anopheles gambiae*

Vertebrates	*D. melanogaster*	*An. gambiae*	Source
Nrf2	*CnCC*	*Nf2e*1	Mohammed et al., 2014
Keap 1	d*Keap* 1	AGAP003645	Mohammed et al., 2014

K e y: *Nrf2* – Nuclear factor erythroid-2 related factor-2; *CnCC* – Cap 'n' collar isoform C; *Nf2e*1 – Nuclear factor erythroid-2, invertebrate.

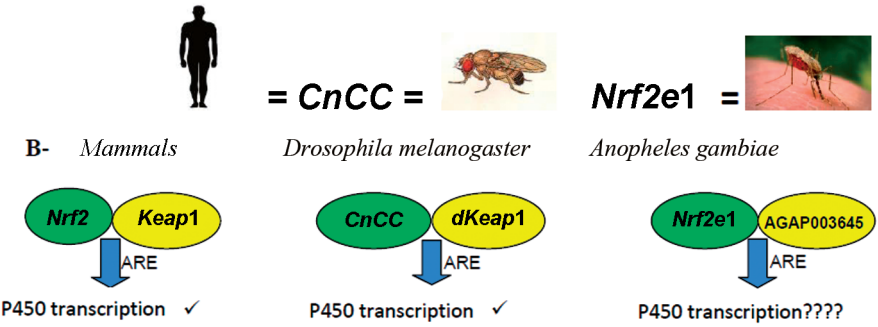

Figure 1. Graphic illustration of the vertebrate *Nrf2*- *Keap*1- Kelch-like ECH-Associated Protein *Keap*1 and its -Cap'n' Isoform C *CnCC* and Nuclear factor 2 invertebrate -*Nf2e*1-AGAP003645 orthologs of *Drosophila melanogaster* and *Anopheles gambiae* respectively and the and ARE-Antioxidant responsive element (Adopted and modified Mohammed, 2014).

*Nrf2 / Keap*1 signaling pathway in vertebrates

In unstressed conditions, *Nrf2* (Nuclear factor erythroid-2 related factor- 2) in vertebrates, and *CnCC* (Cap 'n' collar isoform C) in *Drosophila* are repressed by d*Keap*1 (*Drosophila* Kelch-like ECH-associated protein 1), which also functions as a sensor of oxidants and other electrophilic compounds (Mohammed et al., 2014; Loboda et al., 2016). In the absence of stress, *Nrf2* is maintained in the cytoplasm by the actin-binding protein *Keap*1, which also functions as an E3 ubiquitin ligase to stimulate *Nrf2* degradation by the 26S proteasome. Activation of this pathway through oxidative stress impedes the *Nrf2–Keap*1 synergy, allowing *Nrf2* to translocate to the nucleus, where it can heterodimerize with the small Maf (muscle aponeurosis fibromatosis) proteins and bind to antioxidant response elements (AREs) in the genome (Atia, Bin Abdullah, 2014). Overexpression of *Nrf2* and reduction of *Keap*1 in higher vertebrates switches on the transcription of numerous genes including *CYP6A2* that safeguards cells from xenobiotic compounds. *Nrf2*, *Maf* and *Keap*1 are all conserved in *D. melanogaster* and exert to maintain cognate regulatory synergy as described in vertebrates (Si, Liu, 2014; Dhanoa et al., 2013).

*CnCC / dKeap*1 signaling pathway in *Drosophila melanogaster*

Under oxidative stress situations, the repression of *CnCC* by d*Keap*1 are annihilated permitting these transcription factors to bind, together with other proteins, to ARE sequences upregulating downstream genes such as P450s. The *Drosophila* d*Keap*1 incorporates Kelch repeats homologous to those that intercedes d*Keap*1 synergy with *Nrf2* as well as a sequence motif that is mandatory for vertebrate *Keap*1 export from the nucleus (Deng, Kerppola, 2013). Overexpression of *CnCC* and reduction of d*Keap*1 in *Drosophila melanogaster* switches on the transcription of numerous genes including *CYP6G1* and *CYP6A2* that safeguard cells from xenobiotic compounds, albeit d*Keap*1 overexpression supresses their transcription, demonstrating that the activities of these protein families in the xenobiotic response are conserved amongst vertebrates and *Drosophila* (Deng, Kerppola, 2013; Misra et al., 2013).

Activation of this pathway through electrophilic xenobiotics / oxidative stress is necessary and sufficient for xenobiotic-induced transcription of a wide range of detoxification genes in *Drosophila* species (Misra et al., 2011; Deng, Kerppola, 2013).

Up-regulation of these cytochrome oxidases (P450s) including *CYP6M2* has been associated with resistance and enzymatic metabolism of insecticides by *CYP6M2* has been shown *in vitro* (Stevenson et al., 2011; Mitchell et al., 2012; Edi et al., 2014). In *Drosophila melanogaster*, some genes involved in metabolic activity are known to be upregulated by the transcription factors Cap 'n' collar isoform (*CnCC*) / *Drosophila* Kelch-like-ECH-associated protein 1 (d*Keap* 1). These are orthologs to Nuclear factor erythroid -2 related factor-2 (*Nrf2*) / Kelch-like–ECH-associated protein 1 (*Keap* 1) signalling pathway in higher mammalians respectively (Cao et al., 2013; Misra et al., 2013; Das et al., 2014; Siller et al., 2014).

The over-expression of *Nf2e*1 and repression of AGAP003645 (d*Keap* 1) in *Anopheles gambiae* potentially initiates the transcription of many P450 genes including *CYP6M2* and protect cells from xenobiotic compounds, whereas d*Keap*1 overexpression potentially represses their transcription, suggesting that the functions of these protein families in the xenobiotic response are conserved between vertebrates, *Drosophila melanogaster* and *Anopheles gambiae.* This has revealed therefore a connection between *Nf2e*1 / AGAP003645 signalling pathways and *CYP6M2* in insecticide resistance. A proposed scheme for the *Nf2e*1/ AGAP003645 pathway and that of its inhibition are described in Figures 2 and 3.

The potential nuclear factor erythroid 2 invertebrate (*Nf2e*1) / AGAP003645 signalling pathway in *Anopheles gambiae*

Activation of the *Nf2e*1/ AGAP003645 pathway in *Anopheles gambiae*

Under oxidative stress situations, the restriction of *Nf2e*1 by AGAP003645 is abolished permitting these transcription factors to bind together with other proteins, as maf (muscle aponeurosis fibromatosis) to ARE sequences up regulating downstream P450 genes such as *CYP6M2* responsible for detoxification of insecticides thereby conferring protection and possibly resistance to insecticides in *Anopheles gambiae* as seen in Figure 2.

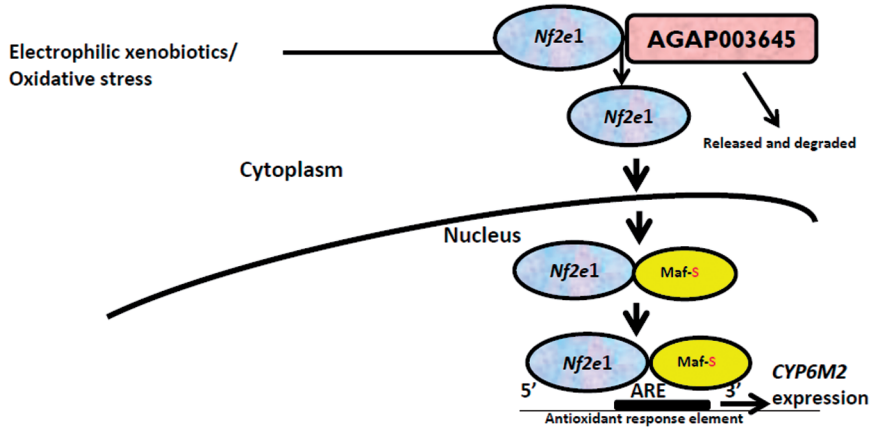

Figure 2. Schematic illustration for the induction of *Nf2e*1/ AGAP003645-(d*Keap*1) signalling pathway. The antioxidant response element (ARE) in the promoter region of select genes allows the coordinated up-regulation of antioxidant and detoxifying enzymes in response to oxidative/electrophilic stress. This up-regulation is mediated through Nuclear factor erythroid 2, invertebrate (*Nf2e*1) that may be activated by endogenous and exogenous molecules or stressful conditions. These agents disrupt the association between *Nf2e*1 and AGAP003645 with subsequent nuclear translocation of *Nf2e*1. In the cell nucleus, *Nf2e*1 interacts with small MAF-S (Muscle apoptosis fibromatosis) protein, forming a heterodimer that binds to the ARE (Antioxidant response element) sequence in the promoter region and up-regulates transcription of many genes encoding detoxifying enzymes such as P450s (*CYP6M2*) (Adopted and modified from Mohammed, 2014).

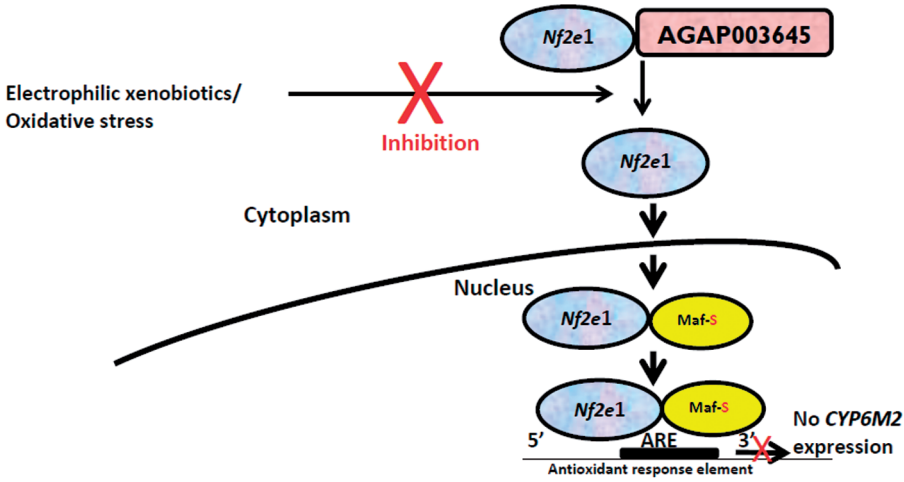

Figure 3. Schematic illustration general scheme for the inhibition of *NF2e*1/ AGAP003645 (d*Keap* 1) – signalling pathway. This up-regulation is mediated through Nuclear factor erythroid 2 invertebrate (*Nf2e*1) that may be activated by endogenous and exogenous molecules including xenobiotics (ligands) or stressful conditions. When these agents are inhibited, the association between *Nf2e*1 and AGAP003645 remains intact in the cytoplasm. This disrupts the up-regulation and transcription of many genes encoding detoxifying enzymes such as P450s (*CYP6M2*) (Adopted and modified from Mohammed, 2014).

Inhibition of the *Nf2e*1/ AGAP003645 pathway in *Anopheles gambiae*

Figure 3 is an Illustration of the hypothetical inhibition of the *Nf2e*1/ AGAP003645 signalling pathway which potentially hinders the up-regulation *CYP6M2* responsible for insecticide metabolism in *Anopheles gambiae*.

However, activation of this pathway is unlikely to be the only factor that contributes to insecticide resistance in *Anopheles gambiae*. Nevertheless, inhibition of the *Nf2e*1 / d*Keap* 1 (Figure 3) in particular response may potentially improve the efficacy of insecticides and development of methods to knock down or inhibit these pathways may prove fruitful. This work has increased our knowledge of the regulatory mechanisms involved in the control of *CYP6M2* in insecticide resistance in *Anopheles gambiae*. Even if the underlying mechanisms are still not very clear, the work shows the importance of these regulatory genes in the control of *CYP6M2* in response to insecticide selection.

The functionality of *Nf2e*1 can only be proven through wet-laboratory experiments with predetermined parameters, particularly since a potential binding site in a promoter can be functional in certain cells and non- functional under different conditions (Cartharius et al., 2005). These findings have implications in the ability to control the spread of malaria due to the reduction in insecticide resistance in *Anopheles gambiae*.

Activators and Inhibitors of *CnCC* signalling pathway

Activators of *CnCC* signalling pathway

Previous studies have identified Phenobarbital, Paraquat, Caffeine and GAL4UAS system among others as major activators of *CnCC*/Keap 1 signaling pathway (Misra et al., 2011; Deng, Kerppola, 2014) (Table 2).

Inhibitors of *Nrf2* signalling pathway

Inhibitors of *CnCC*, such as Drosophila Keap1 induce beneficial effects on survival and synaptic function in *Drosophila melanogaster* (Spiers et al., 2019). Furthermore, over expression of *Drosophila* Keap1 is also known to inhibit *CnCC* activity in vivo (Sykiotis, Bohmann, 2008). Other inhibitors such as Bromodomain and Extra-Terminal (BET) protein family in *Drosophila*, Fs (1) h, as an inhibitor of the stress responsive transcription factor *CnCC*, the fly ortholog of *Nrf2*. The mechanism by which Fs (1) h inhibits *CnCC* function is distinct from the canonical mechanism that stimulates *Nrf2* function by abrogating Keap1-dependent proteasomal degradation (Chatterjee et al., 2016). Finally, using an inducible *Drosophila* model, it was confirmed that Aβ42 inhibits activity of the fly homolog of *Nrf2* (cap-n-collar isoform C, *CnCC* (Sykiotis, Bohmann, 2008; Kerr et al., 2017). It is conceivable that the *CnCC* inhibition activity plays similar role in *Anopheles gambiae*.

Table 2. Typical activators of *CnCC* (*Nrf2* ortholog) signaling pathway in insects

S/ No	Insect	Activators	P450	References
1	*Drosophila melanogaster* (Fruit fly)	Phenobarbital (PB)	*CYP6D1, CYP6A2, CYP6A8* and *CYP12D1*	Misra et al., 2011; Deng, Kerppola, 2014
2	*Aphis gossypii* (Glover)	RNA interference (RNAi)	*CYP6A2*	Peng et al., 2016a
3	*Tribolium castaneum* (Red flour beetle)	Double stranded RNA (dsRNA)	*CYP6BQ*	Kalsi, Palli, 2015
4	*Aphis gossypii* (Glover)	Paraquat	*CYP6DA2*	Peng et al., 2016b
5	*Drosophila melanogaster* (Fruit fly)	Paraquat	*CYP6A2* and *CYP6A8*	Pitoniak, Bohmann, 2015
6	*Drosophila melanogaster* (Fruit fly)	GAL4/UAS system	*CYP6G1, CYP6A2*, *CYP6A8* and *CYP6A21*	Daborn et al., 2007; Misra et al., 2011
7	*Drosophila melanogaster* (Fruit fly)	chlorpromazine	*CYP6A2*	Misra et al., 2011
8	*Drosophila melanogaster* (Fruit fly)	caffeine	*CYP12D1*, *CYP6A8* and *CYP6D5*	Misra et al., 2011; Coelho et al., 2015
9	*Spodoptera litura* (Tobacco cutworm)	Piperonyl butoxide	*CYP6AB12*	Lu et el., 2020
10	*Bombyx mori* (Silk worm)	Curcumin High temperature	*CYP302A1, CYP306A1, CYP314A1*and*CYP315A1*	Li et al., 2019
11	*Aedes aegypti*	Fluoranthene	*CYP6M6*	Poupardin et al., 2008
12	*Aedes aegypti*	Copper	*CYP6M11*	Poupardin et al., 2008
13	*Tribolium castaneum* (Red flour beetle)	Latrophilin (Lph)	*CYP4BN6* and *CYP6BQ11*	Xiong et al., 2019
14	*Anopheles gambiae*	RNA interference (RNAi)	*CYP6M2, CYP6Z2, CYP6Z3* and *CYP6P4*	Ingham et al., 2017
15	*Leptinotarsa decemlineata* Colorado potato beetle	RRNA interference (RNAi)	*CYP6BJ, CYP6BJ1, CYP9Z25* and *CYP9Z29*	Kalsi, Palli, 2017a
16	*Tribolium castaneum* (Red flour beetle)	Double stranded RNA (dsRNA)	*CYP6BQ11*	Kalsi, Palli, 2015
17	*Leptinotarsa decemlineata* Colorado potato beetle	RNA interference (RNAi)	*CYP6BJ*	Kalsi, Palli, 2017b
18	*Drosophila melanogaster* (Fruit fly)	Tert-butylhydroquinone (tBHQ)	*CYP6BQ*	Deng, Kerppola, 2014

CONCLUSIONS

In this review, we have described findings regarding potential mechanisms by which *Nf2e*1 influences insecticide resistance in *Anopheles gambiae*. The roles of many *Nf2e*1 and its orthologs in xenobiotics are remarkably well conserved between higher mammals and other living organisms. The intervention of the *Nf2e*1 leads to an extended lifespan all living organisms. This suggests that the role of *Nf2e*1 xenobiotics is likely to be conserved across all living organisms. In addition, genetic variants of *Nf2e*1 components, including muscle apoptosis fibromatosis-S (MAF-S) and antioxidant response element (ARE), are associated with insecticide resistance. Thus, the evidence for the evolutionarily conserved nature of *Nf2e*1 and its orthologs-mediated longevity is extremely strong, ranging from invertebrates to humans. Further wet laboratory experiments such as qPCR (quantitative polymerase chain reaction) are required to establish the functionality of *Nf2e*1. We therefore conclude that inhibition of this *Nf2e*1 / d*Keap* 1 may potentially improve the efficacy of insecticides. Consistent with the previous studies on the *Drosophila* model pathway, these studies have established that the *Nrf2* / *Keap* 1(*Nf2e*1 / d*Keap* 1) pathway is differentially active as a key regulator of xenobiotic responses. These studies have implications for understanding the regulatory mechanisms of acquirement of insecticide resistance and its impact in the control of mosquito-borne diseases.

ACKNOWLEDGEMENT

This work was supported by Tertiary Education Trust Fund (TETFUND) and University of Abuja, Nigeria.

CONFLICT OF INTEREST

The authors declare that there are no competing interests regarding the publication of this paper.

REFERENCES

AltintasO., Park S., Lee S.J.V. 2016. The role of insulin/IGF-1 signaling in the longevity of model invertebrates, *C. elegans* and *D. melanogaster*. BMB reports 49 (2): 81–92.

Atia A.E., Bin Abdallah A. 2014. Modulation of *Nrf2* / *Keap1* pathway by dietary phytochemicals. International Journal of Research in Medical Sciences 2 (2): 375–381.

Ben-Yehuda G.M., Ben-Sasson S., Bianco-Peled H., Kohen R. 2016. Skin Redox Balance Maintenance: The Need for an *Nrf2*-Activator Delivery System. Cosmetics 3 (1): 1.

Cao C., Wang Z., Niu C., Desneux N., Gao X. 2013. Transcriptome Profiling of *Chironomus kiinensis* under Phenol Stress Using Solexa Sequencing Technology. PloS ONE 8 (3): e58914.

Cartharius K., Frech K., Grote K., Klocke B., Haltmeier M., Klingenhoff A., Werner T. 2005. MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics 21 (13): 2933–2942.

Chang K.S., Kim H.C., Klein T.A., Ju Y.R. 2017. Insecticide resistance and cytochrome-P450 activation in unfed and blood-fed laboratory and field populations of *Culex pipiens pallens*. Journal of Pest Science 90 (2): 759–771.

Chatterjee N., Tian M., Spirohn K., Boutros M., Bohmann D. 2016. Keap1-Independent Regulation of *Nrf2* Activity by Protein Acetylation and a BET Bromodomain Protein PLoS Genetics 12 (5): e1006072.

Coelho A., Fraichard S., Le Goff G., Faure P., Artur Y., Ferveur J.F., Heydel J.M. 2015. Cytochrome P450-dependent metabolism of caffeine in *Drosophila melanogaster*. PLoS One 10 (2): e0117328.

Daborn P.J., Lumb C., Boey A., Wong W., Ffrench-Constant R.H., Batterham P. 2007. Evaluating the insecticide resistance potential of eight *Drosophila melanogaster* cytochrome P450 genes by transgenic over-expression. Insect Biochemistry and Molecular Biology 37 (5): 512–519.

Das D.N., Panda P.K., Mukhopadhyay S., Sinha N., Mallick B., Behera B., Maiti T.K., Bhutia S.K. 2014. Prediction and validation of apoptosis through cytochrome P450 activation by benzo[a]pyrene. Chemico-Biological Interactions 208: 8–17.

Deng H., Kerppola T.K. 2013. Regulation of *Drosophila* Metamorphosis by Xenobiotic Response Regulators. PloS Genetics 9 (2): e1003263.

Dhanoa B.S., Cogliati T., Satish A.G., Friedman J.S. 2013. Update on the Kelch-like (KLHL) gene family. Human Genomics 7: 13.

Edi C.V., Djogbénou L., Jenkins A.M., Regna K., Muskavitch M.A.T., Poupardin R., Jones C.M., Essandoh J., Kétoh G.K., Paine M.J.I, Koudou B.G, Martin J., Donnelly M.J., Ranson H., Weetman D. 2014. CYP6 P450 Enzymes and ACE-1 Duplication Produce Extreme and Multiple Insecticide Resistance in the Malaria Mosquito *Anopheles gambiae*. PloS Genetics 10 (3): e1004236.

Guio L., Barron M.G., Gonzalez J. 2014. The transposable element *Bari-Jheh* mediates oxidative stress response in *Drosophila*. Molecular Ecology 23 (8): 2020–2030.

Guo H., Bao Z., Du H., Zhang L., Wang S., Sun L., Hu X. 2013. Identification of Cytochrome P450 (CYP) genes in *Zhikong scallop* (*Chlamys farreri*). Journal of Ocean University of China 12 (1): 97–102.

Horstmann S., Sonneck R., 2016. Contact Bioassays with Phenoxybenzyl and Tetrafluorobenzyl Pyrethroids against Target-Site and Metabolic Resistant Mosquitoes. PloS One 11 (3): e0149738.

Ibrahim S.S., Amvongo-Adjia N., Wondji M.J., Irving H., Riveron J.M., Wondji C.S. 2018. Pyrethroid resistance in the major malaria vector Anopheles funestus is exacerbated by overexpression and overactivity of the P450 CYP6AA1 across Africa. Genes 9 (3): 140.

Ingham V.A., Pignatelli P., Moore J.D., Wagstaff S., Ranson H. 2017. The transcription factor Maf-S regulates metabolic resistance to insecticides in the malaria vector *Anopheles gambiae*. BMC Genomics 18 (1): 669.

Jiang J., Shi D., Zhou X.Q., Yin L., Feng L., Liu Y., Zhao Y. 2015. Effects of glutamate on growth, antioxidant capacity, and antioxidant-related signaling molecule expression in primary cultures of fish enterocytes. Fish Physiology and Biochemistry 41 (5): 1143–1153.

Jones L.M., Rayson S.J., Flemming A.J., Urwin P.E. 2013. Adaptive and Specialised Transcriptional Responses to Xenobiotic Stress in *Caenorhabditis elegans* are regulated by Nuclear Hormone Receptors. PloS ONE 8 (7): e69956.

Kalsi M., Palli S.R. 2015. Transcription factors, *CnCC* and Maf, regulate expression of *CYP6BQ* genes responsible for deltamethrin resistance in *Tribolium castaneum*. Insect Biochemistry and Molecular Biology 65: 47–56.

Kalsi M., Palli S.R. 2017a. Transcription factor cap n collar C regulates multiple cytochrome P450 genes conferring adaptation to potato plant allelochemicals and resistance to imidacloprid in *Leptinotarsa decemlineata* (Say). Insect Biochemistry and Molecular Biology 83: 1–12.

Kalsi M., Palli S.R. 2017b. Cap n collar transcription factor regulates multiple genes coding for proteins involved in insecticide detoxification in the red flour beetle, *Tribolium castaneum*. Insect Biochemistry and Molecular Biology 90: 43–52.

Kerr F., Sofola-Adesakin O., Ivanov D.K., Gatliff J., Perez-Nievas B.G., Bertrand H.C., Adcott J. 2017. Direct Keap1-*Nrf2* disruption as a potential therapeutic target for Alzheimer's disease. PLoS Genetics 13 (3): e1006593.

Kumar A., Srivastava P., Sirisena P.D.N.N., Dubey S., Kumar R., Shrinet J., Sunil S. 2018. Mosquito Innate Immunity. Insects: 9(3): 95.

Kuzin B.A., Nikitina E.A., Cherezov R.O., Vorontsova J.E., Slezinger M.S., Zatsepina O.G., Simonova O.B., Enikolopov G.N., Savvateeva-Popova E.V. 2014. Combination of Hypomorphic Mutations of the *Drosophila* Homologues of *Aryl Hydrocarbon Receptor* and *Nucleosome Assembly Protein* Family Genes Disrupts Morphogenesis, Memory and Detoxification. PloS One 9 (4): e94975.

Li J., Mao T., Wang H., Lu Z., Qu J., Fang Y., Gu Z. 2019. The *CnCC*/keap1 pathway is activated in high temperature-induced metamorphosis and mediates the expression of Cyp450 genes in silkworm, *Bombyx mori*. Biochemical and Biophysical Research Communications 514 (4): 1045–1050.

Li S., Tan H.Y., Wang N., Zhang Z.J., Lao L., Wong C.W., Feng Y. 2015. The role of oxidative stress and antioxidants in liver diseases. International Journal of Molecular Sciences 16 (11): 26087–26124.

Liang X., Xiao D., He Y., Yao J., Zhu G., Zhu K., 2015. Insecticide-mediated up-regulation of cytochrome P450 genes in the red flour beetle (*Tribolium castaneum*). International Journal of Molecular Sciences 16 (1): 2078–2098.

Liu N. 2015. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annual Review of Entomology 60: 537–559.

Loboda A., Damulewicz M., Pyza E., Jozkowicz A., Dulak J. 2016. Role of *Nrf2*/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cellular and Molecular Life Sciences 73: 3221–3247.

Lu K., Cheng Y., Li W., Li Y., Zeng R., Song Y. 2020. Activation of *CnCC* pathway by ROS burst regulates cytochrome P450 *CYP6AB12* responsible for λ-cyhalothrin tolerance in *Spodoptera litura*. Journal of Hazardous Materials 387: 121698.

Misra J.R., Horner M.A., Lam G., Thummel C.S., 2011. Transcriptional regulation of xenobiotic detoxification in *Drosophila*. Genes and Development 25: 1796–1806.

Misra J.R., Lam G., Thummel C.S. 2013. Constitutive activation of the *Nrf2* / *Keap1* pathway in insecticide-resistant strains of *Drosophila*. Insect Biochemistry and Molecular Biology, 43 (12): 1116–1124.

Mitchell S.N., Stevenson B.J., Müllera P., Wilding C.S., Egyir-Yawsond A., Field S.G., Hemingway J., Mark J. I.P., Ranson H., Donnelly M.J. 2012. Identification and validation of a gene causing cross-resistance between insecticide classes in *An. gambiae* from Ghana. PNAS 109 (16): 6147–6152.

Mohammed B.R. 2014. Regulatory mechanisms involved in the control of *CYP6M2* gene in insecticide resistant *Anopheles gambiae* (Diptera: Culicidae). PhD Thesis, Abertay University, Dundee, United Kingdom.

Mohammed B.R., Mailafia S., Kawe M.S., Agbede R.I.S., Finn R.D. 2017. Cytochrome P450s in *Anopheles gambiae* (Diptera: Culicidae) and Insecticide Resistance in Africa: A Mini Review. Entomology, Ornithology and Herpetology 6: 200.

Mohammed B.R., Wilding C.S., Collier P.J., Deeni Y.Y. 2014. Bioinformatic analysis of regulatory elements within the promoter region of the cytochrome P450 Gene, *CYP6M2* in *Anopheles gambiae*. European Journal of Biotechnology and Bioscience 2 (1): 24–31.

Peng T., Pan Y., Gao X., Xi J., Zhang L., Yang C., Bi R., Yang S., Xin X., Shang Q. 2016a. Cytochrome P450 *CYP6DA2* regulated by cap 'n'collar isoform C (*CnCC*) is associated with gossypol tolerance in *Aphis gossypii* Glover. Insect Molecular Biology 25 (4): 450–459.

Peng T., Pan Y., Yang C., Gao X., Xi J., Wu Y., Shang Q. 2016b. Over-expression of *CYP6A2* is associated with spirotetramat resistance and cross-resistance in the resistant strain of *Aphis gossypii* Glover. Pesticide Biochemistry and Physiology 126: 64–69.

Pitoniak A., Bohmann D. 2015. Mechanisms and functions of *Nrf2* signaling in Drosophila. Free Radical Biology and Medicine 88 (B): 302–313.

Poupardin R., Reynaud S., Strode C., Ranson H., Vontas J., David J.P. 2008. Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: impact on larval tolerance to chemical insecticides. Insect Biochemistry and Molecular Biology 38 (5): 540–551.

Ramprasath T., Kumar P.H., Puhari S.S. M., Murugan P.S., Vasudevan V., Selvam G.S. 2012. L-Arginine ameliorates cardiac left ventricular oxidative stress by upregulating eNOS and *Nrf2* target genes in alloxan-induced hyperglycemic rats. Biochemical and Biophysical Research Communications 428 (3): 389–394.

Ramprasath T., Senthamizharasi M., Vasudevan V., Sasikumar S., Yuvaraj S., Selvam G.S. 2014. Naringenin confers protection against oxidative stress through upregulation of *Nrf2* target genes in cardiomyoblast cells. Journal of Physiology and Biochemistry 70(2), 407–415.

Ranson H., Lissenden N. 2016. Insecticide resistance in African anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends in Parasitology 32 (3): 187–196.

Si H., Liu D. 2014. Dietary antiaging phytochemicals and mechanisms associated with prolonged survival. The Journal of Nutritional Biochemistry 25 (6): 581–591.

Siller M., Goyal S., Yoshimoto F.K., Xiao Y., Wei S., Guengerich F.P. 2014. Oxidation of Endogenous N-Arachidonoylserotonin by Human Cytochrome P450 2U1. Journal of Biological and Chemistry 289: 10476–10487.

Sinka M.E., Golding N., Massey N.C., Wiebe A., Huang Z., Hay S.I., Moyes C.L. 2016. Modelling the relative abundance of the primary African vectors of malaria before and after the implementation of indoor, insecticide-based vector control. Malaria Journal 15 (1): 142.

Spiers J.G., Breda C., Robinson S., Giorgini F., Steinert J.R. 2019. *Drosophila Nrf2*/Keap1 Mediated Redox Signaling Supports Synaptic Function and Longevity and Impacts on Circadian Activity. Frontiers in Molecular Neuroscience 12: 86.

Stevenson B.J., Bibby J., Pignatelli P., Charoen M.S., O'Neill P.M., Lian L.-Y., Müller P., Nikou D., Steven A., Hemingway J., Sutcliffe M.J., Paine M.J.I. 2011. Cytochrome P450 6M2 from the malaria *vector Anopheles gambiae* metabolizes pyrethroids: sequential metabolism of deltamethrin revealed. Insect Biochemistry and Molecular Biology 41 (7): 492–502.

Swamy M.K., Akhtar M.S., Sinniah U.R. 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Complementary and Alternative Medicine 2016: 3012462.

Sykiotis G.P., Bohmann D. 2008. Keap1/*Nrf2* signaling regulates oxidative stress tolerance and lifespan in Drosophila. Developmental Cell 14 (1): 76–85.

Xiong W., Gao S., Mao J., Wei L., Xie J., Liu J., Li B. 2019. CYP4BN6 and CYP6BQ11 mediate insecticide susceptibility and their expression is regulated by Latrophilin *in Tribolium castaneum*. Pest Management Science 75 (10) 2744–2755.

Zhou D., Liu X., Sun Y., Ma L., Shen B., Zhu C. 2015. Genomic analysis of detoxification supergene families in the mosquito *Anopheles sinensis*. PloS One 10 (11): e0143387.

ЭВОЛЮЦИЯ СИГНАЛЬНОГО ПУТИ *Nf2e*1/ *dKeap*1 В ПРОЦЕССЕ РЕГУЛЯЦИИ ЭКСПРЕССИИ ГЕНА *CYP6M2*: ПОТЕНЦИАЛЬНАЯ РОЛЬ В РЕЗИСТЕНТНОСТИ К ИНСЕКТИЦИДАМ У *ANOPHELES GAMBIAE* GILES, 1902 (DIPTERA: CULICIDAE) – ОБЗОР

Б. Р. Мохаммед, М. К. Симон, А. М. Иайо

Ключевые слова: *Anopheles gambiae*, *Drosophila melanogaster*, *Nf2e*1, d*Keap* 1, резистентность к инсектицидам

РЕЗЮМЕ

Невосприимчивость (резистентность) к инсектицидам является угрозой мирового значения, т.к. препятствует борьбе с переносчиками возбудителей инфекций. Известно, что цитохром P450s, включая *CYP6M2*, вовлечен в метаболизм инсектицидов, и служит одной из причин возникновения резистентности. Работа регуляторных механизмов, участвующих в контроле цитохрома P450s у комара *Anopheles gambiae*, остается неясной. В настоящем обзоре авторы анализируют потенциальную роль ядерного эритроидного фактора 2 (*Nf2e*1), который является ортологом фактора 2 (*Nrf2*) у позвоночных животных и изоформой C белка Cap-n-Collar (*CnCC*) у *Drosophila melanogaster*, в экспрессии гена *CYP6M2*, раскодирующего энзимы и возможно препятствующего возникновению резистентности к инсектицидам у комара *Anopheles gambiae*. В нормальных условиях *Nf2e*1 накапливается в цитоплазме, где он объединяется со связывающим актин белком *Keap* 1 (Kelch-like ECH associating protein 1), ортологом AGAP003645, и мгновенно разрушается в убиквитин-протеасомной системе. В данном обзоре анализируются современные сведения о комплексе *Nf2e*1/ AGAP003645, причем особое внимание уделяется молекулярным механизмам регуляции и потенциальной возможности использования комплекса для борьбы с инфекционными болезнями, передаваемыми комарами, включая малярию.