How Complex Can Resistance to Dieldrin, the Insect γ-Aminobutyric Acid Receptor, Get?

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ABSTRACT: Recently, Taylor-Wells et al published evidence that the γ-aminobutyric acid (GABA) receptor, resistance to dieldrin (RDL), from mosquitoes undergoes RNA A-to-I editing to generate an extraordinarily large range of isoforms. This editing was found to affect GABA receptor pharmacology, as it influenced the potency of GABA and ivermectin. This highlights RNA editing as a species-specific mechanism to fine-tune receptor function as well as possibly increase tolerance of mosquitoes to certain insecticides. This commentary also considers novel findings from analysis of *Rdl* transcripts from individual mosquitoes taken from different geographical areas.

KEYWORDS: *Anopheles*, GABA receptor, insecticide target, mosquito, RDL, RNA editing

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The insect γ-aminobutyric acid (GABA) receptor RDL (resistance to dieldrin) plays a central role in neuronal signalling and is the target of several insecticide classes.[1] It is a member of the Cys-loop ligand-gated ion channel (CysLGIC) gene superfamily, which consists of receptors comprising five subunits arranged around a central ion channel that opens when the receptor binds to a neurotransmitter such as GABA. The subunit composition determines the functional and pharmacological properties of the CysLGIC. It is possible that the receptor is made up of five different subunits, as is the case for a nicotinic acetylcholine receptor from the nematode, *Caenorhabditis elegans* (Figure 1A).[2] On the other hand, the simplest complement of subunits is possible where the CysLGIC consists of five identical subunits, which are referred to as a homomeric receptor. Usually, RDL is considered a homomeric receptor (Figure 1B), as only one subunit is required to generate functional GABA receptors in expression systems such as *Xenopus laevis* oocytes, although it remains to be clarified whether this is the case in vivo.[1,3]

The simple picture of five identical subunits forming RDL is made more complex, as the subunit undergoes alternative splicing at exons 3 and 6 to give four different variants.[4] In addition to this, differential use of splice acceptor sites generates RDL variants with differing lengths of an intracellular domain.[5,6] Furthermore, in some species, RDL diversity is expanded through A-to-I editing.[7,8] Here, certain adenosine residues in pre-mRNA are changed to inosine by adenosine deaminases that remove an amine group. Since inosine is interpreted by cellular machineries as guanosine, RNA editing can produce transcripts with a nucleotide composition that is different from the corresponding genomic DNA, potentially changing amino acid residues in protein products. Four amino acid residues in *Drosophila melanogaster* RDL were found to be altered through RNA A-to-I editing, giving rise to 8 different isoforms (including the unedited isoform).[9]

Recently, Taylor-Wells et al[10] pushed the complexity of the insect RDL even further with the identification of RNA A-to-I editing in RDL of the mosquitoes *Aedes aegypti*, *Anopheles gambiae*, and *Culex pipiens*. For the malaria mosquito, *A gambiae*, six amino acids can be altered (Figure 2) from which 24 isoforms were observed. Functional expression studies in *Xenopus* oocytes expressing individually 18 different isoforms showed that RNA editing of RDL effectively generates a spectrum of sensitivities to GABA. Editing decreased sensitivity of *A gambiae* RDL with the isoform having changes at all six editing sites being the least sensitive to GABA, as indicated by the highest EC_{50} value. RNA editing of the mosquito RDL was also shown to affect the potency of ivermectin, which is often used as an insecticide or antiparasitic drug.[11] Editing at two sites, N183G and I278V, were found to abolish the potentiating actions of ivermectin.[10] N183 is situated in the Cys-loop, a characteristic feature of CysLGICs, where two disulphide bond-forming cysteines separated by 13 amino acid residues are located in the N-terminal extracellular domain. I278 is located in the first transmembrane domain (TM1). The finding that changes at either of these sites abolishes potentiation by ivermectin highlights the Cys-loop and TM1 as being involved in the allosteric mechanism of CysLGICs.[12]

The combinations and permutations of alternative splicing and RNA editing of the mosquito RDL results in a staggering amount of potential isoforms from the single *Rdl* gene. Resistance to dieldrin from several insect species such as *Apis mellifera*[13] and *Triboilum castaneum*[14] do not undergo RNA editing, so why should the diversity of RDL from mosquitoes

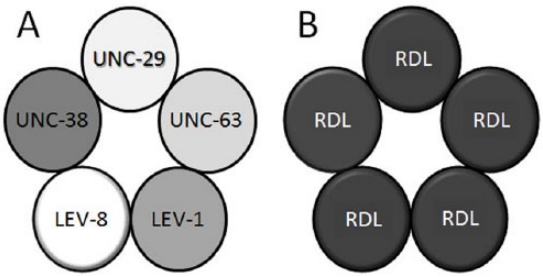

Figure 1. Cys-loop ligand-gated ion channels are pentameric proteins. They can consist of five different subunits, such as a nicotinic acetylcholine receptor from *Caenorhabditis elegans* (A), or just one subunit, an example being the insect GABA receptor RDL (B).

be so greatly extended in comparison to these species? RNA editing of *Drosophila* and *Anopheles* RDLs modulates GABA potency and thus may fine-tune the sensitivity of the receptor to its neurotransmitter.[9,10] Does the mosquito RDL, therefore, need to be particularly flexible in how it responds to GABA? The finding that RNA editing affects the actions of ivermectin may suggest that it could be a mechanism to protect mosquitoes from compounds that have insecticidal effects by binding to the GABA receptor. This is relevant to the strategy of controlling disease vectors where mosquitoes biting humans or livestock treated with ivermectin to remove parasitic helminths have reduced survivorship.[15] It would be of interest to see whether altered levels of editing RDL is associated with any observed increased tolerance to ivermectin in mosquitoes. Recently, it was found that ivermectin was significantly less toxic to *Anopheles albimanus* than to *Anopheles stephensi*.[16] It was unclear what the basis of this difference in sensitivity is, although it was suggested that it may be due to ivermectin being less potent on the molecular target site in *A albimanus*. It would be of interest, therefore, to see whether the RDLs of both species have differential RNA editing, perhaps at different amino acid residues or distinct levels of editing.

Taylor-Wells et al[10] analysed cDNA from a group of ten *A gambiae* mosquitoes and observed that N183G editing occurred in 96% of the transcripts whilst the frequency of N289D editing was 38%. Is the mosquito RDL always edited at these levels or is the generation of different RDL isoforms a dynamic process depending on situations encountered by the mosquito? Studies on *Drosophila* have suggested that the latter can be the case as RNA editing levels were found to vary according to temperature.[17] Interestingly, our analysis of *Rdl* cDNA from *A hyrcanus* shows that A-to-I editing at certain sites can vary between individuals. As shown in Figure 2, a mosquito taken from the Bokeo province in Laos had no observable editing at I162, whereas 55% of transcripts were edited in a mosquito from Luang Prabang. It remains to be determined whether this difference is seen from one individual to another or perhaps reflects populations of mosquitoes in different geographical regions. No A-to-I editing was

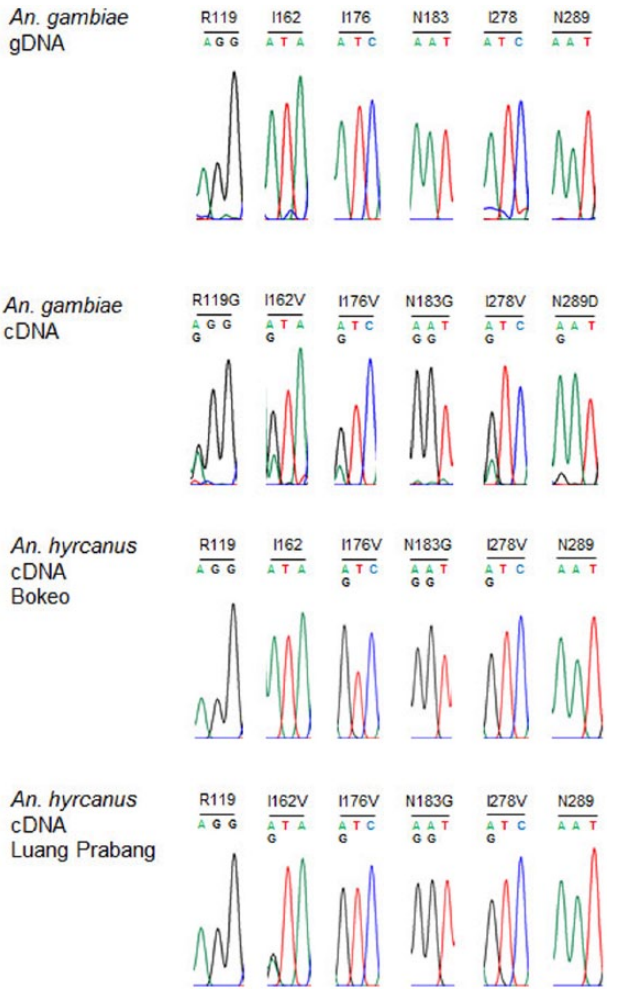

Figure 2. RNA A-to-I editing in RDL of *Anopheles gambiae* and *Anopheles hyrcanus* as shown by sequence chromatograms. Mixed adenosine/guanosine peaks or replacement of adenosine by guanosine in the cDNA sequences indicate RNA editing, and the resulting amino acid change is shown. The corresponding genomic DNA (gDNA) sequence of *A gambiae*, which lacks the G signal, is also included. Ten adult mosquitoes were used to generate the *A gambiae* sequences as previously described.[10] The *A hyrcanus* sequences, which have not been published, were obtained from individual adult females found in two provinces of Laos, Bokeo, and Luang Prabang, using the same methodology described in Taylor-Wells et al.[10]

observed at R119 or N289 for both *A hyrcanus* mosquitoes (Figure 2). It remains to be clarified whether editing changes four residues in *A hyrcanus* as opposed to six in *A gambiae*, which would show that the number of editing sites can vary amongst *Anopheles* species.

In conclusion, Taylor-Wells et al has highlighted that in some species RNA A-to-I editing can generate an extraordinarily high level of diversity in RDL. Even if insect GABA receptors do exist comprising only of RDL, are they truly homomeric? A major challenge for the future would be to determine how the different splice variants and editing isoforms assemble to make up native RDL receptors in vivo.

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Author Contributions

AKJ wrote the manuscript.

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