RNA-seq screening of the CPGs in *Culex pipiens pallens* among cypermethrin-resistant populations

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Abstract

Background Long-lasting overdependence on insecticides has led to the rapid spread of pyrethroid resistance in mosquito vectors, which is of great concern to the general public. There are many studies on metabolic resistance and target resistance, but fewer studies have been conducted on cuticle resistance and behaviour resistance. The cuticle of mosquitoes has been hypothesized to play a role in insecticide resistance by reducing penetration or sequestering insecticides.

Methods We used RNA sequencing (RNA-seq) to analyse the transcriptome of cypermethrin-resistant and cypermethrin-susceptible strains of *Culex pipiens pallens*. Sequenced 6 samples using an Illumina HiSeq platform, and generated approximately 6.66 Gb bases from each sample on average. Mapping the sequenced reads to a reference genome and reconstructing the transcripts, through gene expression analysis, we detected differentially expressed genes (DEGs) among the samples. Followed Gene Ontology (GO) classification and functional enrichment. Finally, we screened the genes of cuticle proteins associated with drug resistance throughout the genome, selected the significant DEGs with a log2 fold change > 3.0 and $P_{adj} < 0.05$, and applied real-time fluorescence quantitative PCR to verify the DEGs.

Results We obtained 13,517 novel transcripts, of which 8,653 were previously unknown splicing events for known genes, 665 were novel coding transcripts without any known features, and 4,199 were long noncoding RNA. A total of 1035, 944, and 657 genes were upregulated in comparisons between samples, and 2680, 1215, and 975 genes were downregulated in comparisons between samples. Finally, among all samples, 167 genes upregulated, and 145 genes downregulated. The GO classification and functional enrichment of DEGs as follows: molecular function, 224 genes; cellular component, 149 genes; and biological process, 272 genes. The expression of XM_001863852 and XM_001845881 in resistant strains of *Culex pipiens pallens* was lower than that in the laboratory sensitive strain, with fold changes in expression of 0.177 and 0.548, respectively; the expression of the XM_001845883.1 in the resistant strain was higher than that in the susceptible strain, and a 2.281-fold change in expression.

Conclusions The results provide a reference for resistance mechanisms through the mosquito cuticle, furthermore, could provide a new perspective for disease vector control.

Background

Culex pipiens pallens is the most common mosquito in northern urban areas and townships in China. In addition to stings and bites, it is also the main vector of several arboviruses, such as West Nile Virus (WNV), St. Louis encephalitis (SLE), Sindbis virus (SINV), Rift Valley fever (RVFV), Japanese encephalitis (JEV), and the main vector for lymphatic filariasis [1, 2]. Because most mosquito-borne diseases (except JEV) currently do not have effective vaccines or therapeutic drugs, effective control of vector mosquitoes is the main measure to prevent mosquito-borne diseases. Among the measures, chemical control is the primary method of vector management [3, 4]. Pyrethroid insecticides are synthetic analogs of naturally occurring pyrethrins from *Chrysanthemum* spp. [5]. Due to their low mammalian toxicity, high insecticidal

activity, fast action and ease of decomposition in the environment, pyrethroids are currently a dominant class of insecticides used globally against mosquitoes and other human disease vectors[6]. However, with the long-term use of insecticides, mosquito resistance is becoming increasingly more serious, which not only greatly reduces the control effect of existing insecticides but also makes the development of new drugs more difficult. The emergence of insecticide resistance has become a major obstacle for controlling mosquito-borne diseases [7].

Insecticides, pyrethroids in particular, remain a mainstay for the control of these important vectors. In this paper, we review what is known about the levels, mechanisms and fitness costs of pyrethroid resistance in *Cx. pipiens*. Pyrethroid resistance in *Cx. pipiens* is a global problem, and resistance ratios of up to 7000-fold have been found in larvae of field collected mosquitoes[8].

High levels of resistance to pyrethroids in *Culex* mosquitoes have been widely reported [9, 10]. Previous surveys have shown that *Culex pipiens pallens*/*Cx. quinquefasciatus* in southern China have different levels of resistance to pyrethroid insecticides. In Hainan and other provinces, the resistance level has reached several thousand fold[11]. The development of mosquito resistance has had an important impact on the control of *Culex pipiens pallens*.

Mosquito resistance mechanisms include metabolic resistance, target resistance, cuticle resistance, and behavioural resistance [12]. Metabolic resistance refers to the degradation, isolation, or transportation/excretion of insecticides from cells prior to binding the target. Metabolic resistance results from increased detoxification caused by the overexpression of or conformational changes in the enzymes involved in chemical insecticide metabolism, sequestration, and excretion. P450-monooxygenases, glutathione S-transferases, and carboxy/cholinesterases are the main enzymes involved in this process [13–16]. Target-site resistance, or mutations in target binding sites for insecticides, is caused by a modification of the chemical insecticide site of action, reducing or preventing insecticide binding at that site. Mutations in the voltage sensitive sodium channel (*Vssc*) gene are the most common causes of target-site resistance [17]. Behavioural resistance results from selection pressure of mosquitoes under long-term exposure to pesticides, and mosquitoes thus show a series of behavioural changes to avoid pesticides. For example, long-term application of indoor residual spraying (IRS) and insecticide-treated nets (ITNS) caused mosquitoes to change from endophagy to exophagy and from endophily to exophily and caused peak bloodsucking to change from late night to dusk [18].

Cuticle thickening is implicated in insecticide resistance by reducing the uptake of the insecticide that reaches the target site in response to the modification of chemical composition of the cuticle[19]. However, the mechanism remains poorly understood, and its importance in *Aedes* species is yet to be confirmed[14, 20, 21]. A study revealed that this mechanism may play a major role in the development of resistance where it normally happens simultaneously with other mechanism(s) [22], causing resistance to single or multiple insecticides [23]. It has been reviewed elsewhere that cuticle thickening is associated with metabolic detoxification whereby thicker cuticle causes gradual insecticide absorption rate that will increase the effectiveness of metabolic detoxification in *Anopheles funestus* [24]. Moreover, it is crucial to

take note that insects with cuticular resistance will display resistance level of not more than 3-fold in comparison to susceptible insects, but the co-occurrence of other resistance mechanism will lead to a surge in insecticide resistance level markedly [25]. This is demonstrated by *Anopheles gambiae* in Benin [26] through which overexpression of cuticular genes and P450 genes gave rise to a relatively high resistance level.

High-throughput sequencing, i.e., RNA-seq, has become the main choice for measuring expression levels [27]. RNA-seq can be performed without prior knowledge of the reference or sequence of interest and allows a wide variety of applications such as the 'de novo' reconstruction of a transcriptome (without a reference genome), the evaluation of nucleotide variations, and the evaluation of methylation patterns [28]. RNA-seq technology has some advantages over the cDNA microarrays, such as a high level of data reproducibility, which reduces the number of technical replicates for experiments. In addition, RNA-seq allows identification and quantification of the expression of isoforms and unknown transcripts [29]. A transcriptome is a collection of complete transcripts within a cell at a specific developmental stage and physiological state. The key goals of transcriptomics are to list transcripts of all species, including mRNAs, noncoding RNAs (ncRNAs) and small RNAs (sRNAs); to determine the transcriptional structure of genes, i.e., the transcriptional starting position, 5' and 3' ends, splicing patterns and other post-transcriptional modifications; and to quantify changes in the expression levels of each transcript under different developmental and treatment conditions.

We used RNA-seq to analyse the transcriptome of cypermethrin-resistant and cypermethrin-susceptible strains of *Culex pipiens pallens* and obtained several cuticle protein genes that were differentially expressed in laboratory sensitive and resistant lines. However, these genes were unverified in the genome. We determined the sensitivity and resistance of mosquitoes to cypermethrin by real-time PCR and Centers for Disease Control and Prevention (CDC) Bottle Bioassay. In addition to the identification of the cypermethrin cuticle resistance genes, the relationship between the identified cuticle protein genes and mosquito resistance was verified to establish the specific mechanism of cypermethrin resistance in *Culex pipiens pallens* and provide new ideas for mosquito control and treatment.

Methods

Materials and methods

Mosquito sample collection

We collected laboratory sensitive and resistant strains of *Culex pipiens pallens* in the following developmental stages: I, II, III, and IV instar larvae, pupa and female *Culex pipiens pallens* 3 days after hatching that had not fed on blood. A total of 200 mg of *Culex pipiens pallens* was collected at each developmental stage and placed into a 1.5 ml Eppendorf (EP) tube, to which 150 µl of TRIzol lysis buffer was added to soak the mosquitoes. The samples

were quickly stored in a -80 °C freezer, and RNA was extracted and sent to the BGI group for transcriptome sequencing. The resistant strain was screened from sensitive strains in our laboratory in accordance with the larvae dipping method recommended by the WHO.

RNA sequencing

Three RNA-seq libraries per population (3 biological replicates) were prepared using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.). The extracted RNA was treated with RNase-free DNase (Qiagen GmbH, Hilden, Germany) and purified using an RNeasy MinElute Cleanup Kit (Qiagen GmbH) to remove DNA. The amount of total RNA was measured in a NanoDrop 2000 (Thermo Fisher Scientific, Inc.). The quality of the extracted RNA was verified by agarose gel electrophoresis. Subsequently, the reaction systems to synthesize the first and second strands of cDNA were constructed, and after the second strand of cDNA was synthesized, the ends of the double-stranded cDNA were blunt ended with the EcoRI restriction sequence. After terminal phosphorylation and XhoI digestion, dscDNA was recovered using a recovery kit. An Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR System were then used for quality tests, and the Illumina HiSeq platform was used for RNA-seq after dscDNA quality was confirmed.

Sequenced reads were assigned to each sample (unplexing), and adaptors were removed. Read quality was assessed for each sample using FastQC. Reads were then filtered based on their length, pairing and quality using Trimmomatic [30] with the following parameters: Leading, 25; Trailing, 25; Minlen, 60; and Slidingwindow, 4-25. Only paired reads were kept. Reads were then mapped to the *Culex pipiens quinquefasciatus* genome using Tophat2 [31] with the following parameters: don't report discordant pair alignments; final read mismatches = 3; intron length = 45-300000; and use coverage search. Only read pairs mapping at a unique location (mapQ > 50) were retained. Quantification of the transcription levels was performed using the Cuffdiff2 module of Cufflinks implemented in Galaxy pipeline (<http://galaxyproject.org>) based on fragment per kilobase exon model (FPKM) values obtained for each gene across all samples.

Transcription ratios between each resistant and each susceptible strain were computed across all biological replicates using Cuff-diff. Genes showing an $FC \geq 3$ (in either direction) and a q value ≤ 0.001 between a given resistant population and all 3 susceptible strains were considered DEGs.

The DEseq2 and PossionDis algorithms were used to perform DEG detection. DEseq2 is differential analysis software based on the principle of negative binomial distribution, and the analysis was conducted according to the method used in Michael I et al. [32]. The PossionDis difference analysis algorithm is based on the Poisson distribution model, and the analysis was conducted according to the method described in Audic S et al. [33].

Screening and verification of DEGs between sensitive and resistant strains

Based on the results of the abovementioned transcriptome sequencing analysis, we analysed the genes related to cuticle proteins among the susceptible and resistant strain DEGs and identified 3 mRNAs with a fold change greater than 2 ($-\log_2\text{Ratio} \geq 1$) and $FDR \leq 0.001$ for subsequent real-time PCR verification.

Referring to the *Culex pipiens pallens* genome data in the gene library, real-time quantitative PCR primers were designed using Primer Premier 5 software and the nucleotide sequences of the selected mRNAs, and β -actin was used as the quantitative internal mRNA reference. The primers were synthesized by Shenzhen BGI. The base sequences of the specific primers are provided in Table 1. RNA was extracted using TRIzol reagent, and cDNA was synthesized. cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc.) using oligo(dT)18. qRT-PCR was performed using a CFX96 Touch (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). Twenty-five nanograms of cDNA and 500 nM of each forward and reverse primer were used in each reaction. The relative expression of each gene in resistant and susceptible mosquitoes was calculated by the $\Delta\Delta C_t$ method [34] using actin (ADIR001186-RA) as a control. Real-time PCR data were analysed using reliability simulation tool (REST) software and hypothesis testing; other data were expressed as the mean $\pm$ standard deviation ($X \pm S$) and analysed with STATA7.0 software.

Student's T test was performed for comparisons between groups, and $p<0.05$ was used as a basis for determining statistical significance.

Table 1 Nucleotide sequence of each gene primer and related information

Gene ID	Primer sequence (5'-3')	Primer length	Product size
XM_001845883.1-F	TGCCATCCGTTTCTTCCA	18	103
XM_001845883.1-R	GGGCTCAACCAGGGAGTAAG	20	
XM_001863852-F	ATGCCATCGTGAAGGGGTGT	19	93
XM_001863852 - R	GACTCTTGATGTCTCCGGTGTG	22	
actin-F	AGGACTCGTACGTCGGTGAC	20	
actin-R	TGGTGCCAGATCTTCTCTCCAT	22	-
XM_001845881-F	CACATTCGGATTACAAAATG	20	196
XM_001845881-R	GTGGTAGCTGTAGCTGTACTG	21	

Results

Overview of RNA-seq data

We selected 3 samples from different physiological stages of the sensitive and resistant strains, for a total of 6 samples, and each sample produced an average of 6.66 Gb of data. The sequenced clean reads were compared with the reference genome of *Culex pipiens pallens*, and the transcripts were reintegrated. A total of 13,517 new transcripts were detected, of which 8,653 were new alternative splicing isoforms of existing known protein-coding genes, 665 were transcripts of unknown protein-coding genes, and 4,199 were lncRNAs (see Tables 2, 3).

Table 2 Summary of differentially expressed genes

VS	Upregulated	Downregulated
Cx_S_strain-VS-Cx_R_strain.DEseq2	167	145
Cx_S_strain-VS-Cx_R_strain.DEseq3	1035	2680
Cx_S_strain-VS-Cx_R_strain.DEseq4	944	1215
Cx_S_strain-VS-Cx_R_strain.DEseq5	657	975

Table 3 Summary of whole genome expression

Sample name	Total gene number	Number of known genes	Number of novel genes	Total transcript number	Known transcript number	Novel transcript number
R_strain_1	14597	14036	561	19094	11413	7681
R_strain_2	14507	13921	586	19460	11415	8045
R_strain_3	14592	14016	576	19733	11533	8200
S_strain_1	14603	14040	563	19381	11440	7941
S_strain_2	14551	13980	571	19671	11562	8109
S_strain_3	14568	13999	569	19679	11501	8178

Prediction of new transcripts

After comparing the clean reads to the *Culex pipiens pallens* genome, we used StringTie [35] software to perform transcript reintegration for each sample and then used cuffmerge and cuffcompare software (both are packages in Cufflinks [36]) to compare the reintegrated transcripts with the annotation information for the *Culex pipiens* genome. We selected transcripts with a class code type of u, i, o, and j as candidates for novel transcripts. A total of 13,517 new transcripts were detected. Detailed statistical information is provided in Table 4.

[Due to technical limitations, Table 4 could not be displayed here. Please see the supplementary files section to access the table.]

Detection of SNPs and INDELs

After comparing clean reads to the *Culex pipiens pallens* genome, we used Genome Analysis Toolkit (GATK) [37] software to call each chromosome, identify single nucleotide polymorphisms (SNPs) and insertion and deletion (INDEL) sites for each sample, and store the final results in variant call format (VCF). The SNP statistical information for all samples is provided in Table 5. We then analysed the site information for each SNP and INDEL, as shown in Figure 1 and Figure 2.

[See supplementary files for Table 5.]

Numbers, functional categorization and pathway analysis of DEGs

DEGs were obtained by comparing the expression levels of differential genes between the sample groups. The results are shown in Figure 3.

DEGs were compared with the GO library for functional classification. GO included 3 major categories: molecular function, cellular composition and biological process. Subsequently, the 3 categories were separately classified. The results are provided in Figure 4.

The obtained DEGs were compared with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and the related metabolic pathways were identified. The results are provided in Figure 5.

Validation of the 3 target cuticle protein genes

To ensure amplification of the target genes and the housekeeping gene, we performed primer verification. The results showed that the amplification curve for the primers was good and that the melting curve was monomodal, and the electrophoresis results revealed specific target fragments. The average Ct value for the actin gene in the sensitive group was 25.905; the average Ct value of the actin gene in the resistant group was 26.227; the average Ct value of the XM_001863852 gene in the sensitive group was

26.813; the average Ct value of the XM_001863852 gene in the resistant group was 29.633; the average Ct value of the XM_001845883.1 gene in the sensitive group was 36.797; the average Ct value of the XM_001845883.1 gene in the resistant group was 35.93; the average Ct value of the XM_001845881 gene in the sensitive group was 32.647; and the average Ct value of the XM_001845881 gene in the resistant group was 33.837 (see Tables 6 and 7 for details).

Subsequently, we used the $2^{-\Delta\Delta Ct}$ method to analyse the expression of target gene mRNA in the extracted RNA and normalized the result based on the housekeeping gene.

The specific calculation method is as follows:

$$\Delta\Delta Ct = (Ct \text{ target gene} - Ct \text{ housekeeping gene}) \text{ experimental group} - (Ct \text{ target gene} - Ct \text{ housekeeping gene}) \text{ control group}$$

The relative expression level of the target gene = $2^{-\Delta\Delta Ct}$, which indicates the fold change in expression for the target gene in the experimental group relative to the control group.

The calculation results are provided in Tables 6 and 7.

Table 6 Quantitative PCR results

Target ID	Target Name	Sample ID	Sample Name	Ct Avg (SDM)	Rel. Qty (SDM)
T001	Actin	S001	S (sensitive) 1	25.475	1.00E+00
T001	Actin	S002	S 2	26.205	1.00E+00
T001	Actin	S003	S 3	26.035	1.00E+00
T001	Actin	S004	R (resistance) 1	25.37	1.00E+00
T001	Actin	S005	R 2	26.19	1.00E+00
T001	Actin	S006	R 3	27.12	1.00E+00
T002	XM_001863852	S001	S 1	31.79	1.00E+00
T002	XM_001863852	S002	S 2	24.00	3.67E+02
T002	XM_001863852	S003	S 3	24.65	2.08E+02
T002	XM_001863852	S004	R 1	30.235	2.73E+00
T002	XM_001863852	S005	R 2	30.61	3.72E+00
T002	XM_001863852	S006	R 3	28.055	4.16E+01
T003	XM_001845883.1	S001	S 1	36.19	1.00E+00
T003	XM_001845883.1	S002	S 2	37.07	9.01E-01
T003	XM_001845883.1	S003	S 3	37.13	7.68E-01
T003	XM_001845883.1	S004	R 1	35.43	1.58E+00
T003	XM_001845883.1	S005	R 2	35.94	1.95E+00
T003	XM_001845883.1	S006	R 3	36.42	2.67E+00
T004	XM_001845881	S001	S 1	36.48	1.00E+00
T004	XM_001845881	S002	S 2	29.33	2.36E+02
T004	XM_001845881	S003	S 3	32.13	3.01E+01
T004	XM_001845881	S004	R 1	33.04	1.01E+01
T004	XM_001845881	S005	R 2	35.15	4.13E+00
T004	XM_001845881	S006	R3	33.32	2.80E+01

Table 7 Calculation of the relative quantitative Ct values for target genes and the internal reference gene

	β -actin	XM_001863852	XM_001845883.1	XM_001845881
Average Ct value in the sensitive group	25.905	26.813	36.797	32.647
Average Ct value in the resistant group	26.227	29.633	35.93	33.837
Δ Ct value in the sensitive group	-----	0.908	10.892	6.742
Δ Ct value in the resistant group	-----	3.406	9.703	7.61
$\Delta\Delta$ Ct value	-----	2.498	-1.189	0.868
$2^{-\Delta\Delta}$ Ct value	-----	0.177	2.281	0.548

It can be seen from Table 7 that the expression levels of the target genes XM_001863852 and XM_001845881 were similar between the sensitive and resistant strains of *Culex pipiens pallens*; the fold changes in expression were 0.177 and 0.548, respectively. The expression level of the target gene XM_001845883.1 in the resistant strain was higher than that in the sensitive strain, and the fold change in expression was 2.281.

Discussion

It has been reported that at least 504 insect species worldwide exhibit chemical insecticide resistance [38], that more than 109 (subspecies) vector mosquitoes are resistant to one or more insecticides [39], and that the number of species and the quantities of resistant insects have increased year to year. *Culex pipiens pallens* is an important vector for lymphatic filariasis and Japanese encephalitis virus. It is one of the main stinging and harassing mosquito species in cities and towns. Chemical pesticides have played an important role in the prevention and treatment of malaria and filariasis in Shandong Province. However, in recent years, due to the need to establish healthy and civilized cities, the large, continuous

and irregular use of pesticides has led to the development of drug resistance and caused environmental pollution, while the degree of resistance by mosquitoes has also shown an increasing trend. The occurrence and development of resistance in mosquitos has limited the further application of chemical pesticides and has become a prominent problem in the prevention and control of insect-borne diseases in China and abroad. Based on the high chemical resistance of *Culex pipiens pallens* in Shandong Province[40], this study used high-throughput transcriptome sequencing technology to qualitatively and quantitatively study cypermethrin-resistant strains of *Culex pipiens pallens* and identified the cuticle protein genes responsible for the drug-resistant phenotype. Real-time quantitative PCR were used to verify the identified cuticle protein genes and to confirm whether there is a high correlation between cuticle protein genes and the drug resistance phenotype.

Mosquito resistance generally includes 4 types: metabolic resistance, target resistance, cuticle resistance, and behavioural resistance. In 1963, a survey of houseflies (*Fannia canicularis*) identified the cause of insecticide resistance. It was found that the penetration of chemical pesticides in resistant lines was slower than in sensitive lines, suggesting that slower penetration could be the cause for dichlorodiphenyltrichloroethane (DDT) and pyrethroid resistance [41]. Studies have shown that pyrethroid-resistant female *Anopheles sinensis* have thicker cuticles than do sensitive female *Anopheles sinensis* and that female mosquitoes also have thicker cuticles than do male mosquitoes [42]. In addition, Lily et al. [43] confirmed that cuticle thickening is present in pyrethroid-resistant strains of *Cimex lectularius*. Cuticle analysis by electron microscopy and characterization of lipid extracts showed that resistant mosquitoes had a thicker outer skin layer and a higher hydrocarbon content (approximately 29%) [44]. Multiple studies have confirmed that the cuticle of resistant lines is much thicker than that of sensitive lines [43, 44].

The cuticular protein (CP) family was first discovered in 2007 by tandem mass spectrometry analysis of epidermal exfoliation from *Anopheles gambiae* [45]. Most gene family members have the prefix CPLC (cuticular protein of low complexity), and such members often play an important role in protein-protein interaction networks [46, 47].

The above evidence suggests that insect cuticle proteins play an indispensable role in mosquito resistance. The expression of the target genes XM_001863852 and XM_001845881 in the *Culex pipiens pallens* laboratory resistant strain was lower than that in the laboratory sensitive strain, and the fold changes in expression were 0.177 and 0.548, respectively, while the expression of XM_001845883.1 in the resistant strain was higher than that in the sensitive strain, and with a 2.281-fold change in expression. GO function analysis indicated that all 3 were genes responsible for cuticle structural components, and an NR database comparison also showed that these genes code cuticle proteins in *Culex pipiens*. In fact, when we screened the genes, we initially identified 3 different genes in the XM_001845 series: XM_001845880, XM_001845881 and XM_001845883.1. However, when designing the primers, the primers designed for these 3 were not ideal, and there were varying degrees of non-specific amplification. We compared the designed primer fragments to the BLAST database and found that the primers were highly consistent with an unknown conserved hypothetical protein. We questioned if the 3

different genes in the XM_001845 series were different splicing isoforms of the same gene, and if so, whether such frequent splicing could promote resistance in mosquitoes. After considering the experimental cost, experimental significance and feasibility of the experiment, we selected 2 of the genes to conduct further validation, and the experimental results for XM_001845881 and XM_001845883.1 were opposite to each other. Therefore, we questioned whether the 2 genes had antagonistic effects in the genetic pathways leading to the formation of cuticle resistance in mosquitoes; for example, XM_001845883.1 is responsible for promoting the formation of cuticle resistance, while XM_001845881 is responsible for regulating the expression of other upstream or downstream cuticle protein genes, preventing the overexpression of other related cuticle proteins. Because the 3 identified target genes in our study are novel, the original identification process was relatively complicated and innovative; however, the specific regulatory networks and the in vivo function of the respective genes need to be further explored.

Chemical control has become the main means of controlling disease vectors and reducing vector infectious diseases, including the indoor and outdoor use of adult mosquito insecticides and the use of larval insecticides at mosquito breeding sites. However, the emergence and spread of pesticide resistance seriously affects disease vector control. Due to the lack of knowledge about the geographical distribution of potential pesticide resistance mechanisms, it is difficult to develop effective pesticide resistance management strategies. In addition, the spatiotemporal principles for the selection and use of pesticides also need further planning and implementation.

Conclusions

These data provide transcriptomic information related to the resistance of *Culex pipiens pallens* and preliminarily verify the relationship between the identified cuticle protein genes and mosquito drug resistance, partially explaining the specific mechanism of mosquito cuticle resistance, providing a scientific basis for the study of new target insecticides, and providing new ideas for the prevention and control of mosquitoes.

Abbreviations

RNA-seq	RNA sequencing
CPGs	cuticular protein genes
CP	cuticular protein
DEGs	differentially expressed genes
GO	Gene Ontology
PCR	Polymerase Chain Reaction

WNV	West Nile Virus
SLE	St. Louis encephalitis
SINV	Sindbis virus
RVFV	Rift Valley fever
JEV	Japanese encephalitis
<i>Vssc</i>	voltage sensitive sodium channel
IRS	indoor residual spraying
ITNS	insecticide-treated nets
ncRNAs	noncoding RNAs
sRNAs	small RNAs
CDC	Centers for Disease Control and Prevention
EP	Eppendorf
dscDNA	double strands cDNA
FPKM	fragment per kilobase exon model
REST	reliability simulation tool
lncRNAs	long noncoding RNAs
GATK	Genome Analysis Toolkit
SNPs	single nucleotide polymorphisms
INDEL	insertion and deletion
VCF	variant call format
KEGG	Kyoto Encyclopedia of Genes and Genomes
DDT	dichlorodiphenyltrichloroethane
CPLC	cuticular protein of low complexity
WHO	World Health Organization

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Authors' contributions

QQS, PC, CXZ, IJL, XS, XXG, HFW, YW, HML provided the samples collection and wrote the manuscript. HWW and MQG reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Distribution of SNP sites Up2k refers to the area within 2000 bp upstream of a gene, and Down2k refers to the area within 2000 bp downstream of a gene.

Figure 4

Distribution of INDEL sites Up2k refers to the area within 2000 bp upstream of a gene, and Down2k refers to the area within 2000 bp downstream of a gene.

Statistic of Differently Expressed Genes

Figure 6

Comparison of DEGs between groups The abscissa indicates the pairs of samples for differential comparison, and the ordinate indicates the corresponding number of DEGs.

Figure 8

GO function classification map of DEGs The X axis represents the number of DEGs, and the Y axis represents GO function classification.

Figure 10

Pathway classification of DEGs The X axis represents the number of DEGs, and the Y axis represents the KEGG pathway.

Supplementary Files

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