Knockdown of two Cadherin genes confers resistance to Cry2A and Cry1C in *Chilo suppressalis*

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*Bacillus thuringiensis* (Bt) Cry toxins play an important role in the management of insect pests. Resistance to Bt toxins has been reported in many pest insects but the mechanism responsible for this resistance in rice crop pests remains largely unknown. Cadherin is one of several Bt toxin receptors. At present, only one cadherin gene, *CsCAD1*, has been documented in the striped rice stem borer, *Chilo suppressalis*. We amplified a nearly full-length transcript of another *C. suppressalis* cadherin gene, *CsCAD2*, and found that it has a different expression pattern to *CsCAD1*. *CsCAD1* was highly expressed in fifth and sixth instar larvae, especially in the midgut, while the expression levels of *CsCAD2* were equally in each developmental stage. Newly hatched larvae were fed on rice smeared with synthesized siRNA to knockdown either *CsCAD1* or *CsCAD2*, and then were fed transgenic rice expressing either the Cry2A or Cry1C toxins. The siRNA-treatment groups had lower mortality and higher survival rates than the control group, suggesting that reduced expression of *CsCAD1* or *CsCAD2* increased resistance to Cry2A and Cry1C. We conclude that *CsCAD1* and *CsCAD2* interact with Bt toxins in *C. suppressalis* and that this interaction could be the mechanism underlying Bt resistance in this insect.

Insecticidal Cry proteins from *Bacillus thuringiensis* (Bt) have been widely used to develop transgenic crops that have become an important part of agricultural pest management. Cry toxins are ingested by digestive proteases in the midgut of insects where the activated toxins interact with midgut brush border membrane proteins, including cadherin, ABC type C transporters (ABCCs), alkaline phosphatase (ALP), and aminopeptidase N (APN). Cry toxins are integrated into the membrane, leading to pore formation, cell lysis and insect death. However, the development of resistance to Bt toxins in many pest insects threatens to make transgenic Bt crops redundant.

The development of resistance to Bt toxins in insects has been associated with mutation, down-regulation, or deletion, of Bt receptors. Cadherin, a calcium-dependent cell adhesion protein, is thought to be one of several such receptor proteins that bind to Cry toxins. The first such cadherin protein to be identified was the Cry1A toxin-binding protein in *Manduca sexta*, which was then found to be involved in binding Cry toxins in other Lepidopteran, coleopteran and dipteran insects. However, the affinity of cadherins for different Cry toxins varies in different insects. For example, some Cry toxins are not lethal to the Coleoptera or Lepidoptera.

The striped rice stem borer, *Chilo suppressalis* Walker, is one of the most destructive rice pests in China and other Asian countries. Transgenic rice strains expressing the Cry toxins Cry2A and Cry1C have been developed to protect rice crops from this notorious pest. However, it is likely that *C. suppressalis* will develop resistance to these toxins once transgenic rice becomes more widely grown. It is, therefore, important to understand the mechanisms that confer resistance to Cry toxins in this species.

A cadherin-like *C. suppressalis* gene (*CsCAD1*, AY118272) has been deposited in the NCBI GenBank. We used Rapid-amplification of cDNA ends (RACE) to clone another *C. suppressalis* cadherin gene, which we named *CsCAD2*, and investigated the expression of both genes in different *C. suppressalis* developmental stages and tissues. We found that knockdown of these genes reduced sensitivity to both Cry2A and Cry1C in *C. suppressalis*.

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Results

Amplifying the CsCAD2 gene. We obtained a fragment of a cadherin gene from the transcriptome of C. suppressalis and, after mapping this fragment onto the C. suppressalis genome, found that this gene had not previously been reported. We named this newly discovered gene CsCAD2. By amplifying the transcript with RACE and incorporating information from the C. suppressalis genome, we obtained a nearly full-length transcript of CsCAD2, including a 5' untranslated coding region (UTR), open reading frame (ORF). The CsCAD2 ORF was 4,912 bp, encoding 1,493 amino acids. The CsCAD2 protein sequence had high identity with other insect cadherins, for example, 88% with that of Bombyx mori and 86% with that of Plutella xylostella.

Gene structure and phylogenetic analysis of CsCAD1 and CsCAD2. The nucleic acid sequences of CsCAD1 and CsCAD2 were aligned with the genome of C. suppressalis to obtain the structures of both genes (Fig. 1A). The two genes are located in different scaffolds of the genome. CsCAD1 had 41 exons and 40 introns and a length of 44,762 bp, whereas CsCAD2 had only 24 exons and 23 introns and a length of 316,095 bp. Surprisingly, the first CsCAD2 intron was very long, 253,600 bp. Conserved domain analysis indicates that both CsCAD1 and CsCAD2 have characteristics that are conserved in other cadherin proteins. CsCAD1 had eight cadherin repeat domains, two Ca²⁺ binding sites and one trans-membrane region, whereas CsCAD2 had seven cadherin repeat domains, five Ca²⁺ binding sites and one trans-membrane region (Fig. 1B). Comparison of CsCAD1 and CsCAD2 with nine other cadherin genes of three well-studied insects; B. mori, P. xylostella, and D. melanogaster, indicated that CsCAD1 was conserved in Lepidopteran. However, CsCAD2 was not clustered with other Lepidopteran CADs but was adjacent with Drosophila CADs, which is worthy of further investigations (Fig. 1C).

Temporal and spatial expression of CsCAD1 and CsCAD2. The expression patterns of both CsCAD1 and CsCAD2 were compared using quantitative PCR (qPCR). The house-keeping gene E2F and the G3PDH gene were used as internal controls. qPCR analyses of the expression of CsCAD1 and CsCAD2 in first to sixth instar larvae, and in adults, indicated that CsCAD1 expression peaked in 6th instar larvae and decreased markedly in adults. In contrast, CsCAD2 was expressed in all developmental stages without significant difference (Fig. 2). CsCAD1 expression was almost exclusively confined to the midgut and was detected only at very low levels in other tissues, including the head, epidermis and fat body (Fig. 3A). In contrast, although CsCAD2 expression was also highest in the midgut, it was also highly expressed in the head and epidermis. Furthermore, although CsCAD2 expression was lowest in the fat body, its expression in that organ was nearly half that in the midgut (Fig. 3B). This indicates that although CsCAD1 expression is largely confined to the midgut, CsCAD2 was expressed in all tested tissues. This was consistent with phylogenetic analysis, indicating that CsCAD1 was conserved in Lepidopteran insects, while CsCAD2 was a more ancient protein and conserved in almost all insects.
Figure 2. Relative abundance of mRNA of the *Chilo suppressalis* cadherin genes (A) CsCAD1 and (B) CsCAD2 in different larval instars and adults. Results are means ± SE. Bars with the same lowercase letter are not significantly different.

Figure 3. Relative abundance of mRNA of the *Chilo suppressalis* cadherin genes (A) CsCAD1 and (B) CsCAD2 in different body parts. Results are means ± SE. Bars with the same lowercase letter are not significantly different.
Knockdown of CsCAD1 and CsCAD2 by RNA interference. In order to investigate the functions of CsCAD1 and CsCAD2 we used siRNAs to silence both genes and a random sequence siRNA without any targets in C. suppressalis as a negative control (siNC). Newly hatched larvae were put into petri dishes containing rice stems smeared with siRNAs and the expression levels of CsCAD1 and CsCAD2 were measured with qPCR after 48 hrs (Fig. 4). The relative abundance of target gene transcripts was 40% of that in the control group, indicating that both target genes were successfully downregulated. The expression of non-target homolog genes was not affected, indicating that RNAi knockdown of CsCAD1 and CsCAD2 was achieved without influencing non-target genes.

Silencing CsCAD1 and CsCAD2 reduced sensitivity to Bt toxins. The RNAi-treated larvae were transferred to feed on one of three rice varieties; transgenic Cry2A rice, transgenic Cry1C rice, and non-transgenic Minghui 63 (negative control).

The death rate of the siNC treatment group was about 50% after feeding on transgenic Cry2A rice two days. In contrast, the mortality of the siCAD1 treatment group was just 10%, and that of siCAD2 treatment group only 5%, after two days (p < 0.05, Tukey’s HSD). Over the following three days, significant differences in mortality were observed between the two siRNA treatment groups and the siNC group. Over 70% of the siNC group were dead after 5 days whereas <30% of both siRNA treatment groups died over the same time period (Figs 5A and 6).

Similar results were obtained in experiments in which larvae were fed transgenic rice expressing the Cry1C toxin. In this case mortality in the siCAD1 and siCAD2 treatment groups were 10% and 20%, respectively, significantly lower than in the NC group (p < 0.05, Tukey’s HSD) (Figs 5B and 6). Interestingly, knockdown of CsCAD2 conferred higher resistance to the Cry2A toxin than knockdown of CsCAD1 (Fig. 5A). Conversely, silencing CsCAD1 conferred higher resistance to the Cry1C toxin than to CsCAD2 (Fig. 5B). This suggests that these two cadherin genes have different binding affinities for different Bt toxins.

Discussion

Phylogenetic analysis of a newly cloned C. suppressalis cadherin gene, CsCAD2, suggests that this is most closely related to D. melanogaster cadherin genes. In contrast to CsCAD1, which was almost exclusively expressed in the midgut, CsCAD2 were highly expressed in a number of different organs. This suggests that these two genes have different functions.

Recent studies have confirmed that Cry1A toxins interact with at least one type of receptor in the midgut. After the Cry1A toxin has been activated by enzymes in the midgut, it binds to the first receptor (midgut cadherin) with high affinity, which facilitates oligomerization of the toxins via a proteolytic process resulting in cell lysis. Previous studies have already indicated that cadherin is a receptor of Cry toxins in M. sexta, B. mori, Diatraea saccharalis and Heliothis virescens. However, most studies have focused on cadherins that are specifically expressed in the midgut. Our results demonstrate that CsCAD2 is both expressed in organs other than the midgut, and that knockdown of this gene increased resistance to both Cry1C and Cry2A. This suggests that this widely expressed gene is also probably a Bt toxin receptor.

We found that silencing either CsCAD1 or CsCAD2 reduced the sensitivity of C. suppressalis to Bt toxins, suggesting that both genes interact with Bt toxins in C. suppressalis. However, their differential expression in different tissues suggests that they could have different functions. This hypothesis is supported by the results of the RNAi experiment. The mortality of the siCAD1 treatment group on transgenic Cry2A rice was slightly higher than that of the siCAD2 treatment group, suggesting that CsCAD2 has higher affinity for Cry2A than CsCAD1. However, when larvae were fed on transgenic Cry1C rice the mortality of the siCAD2 treatment group was higher than that of the siCAD1 treatment group, suggesting that the affinity of CsCAD1 for Cry1C was higher than that of CsCAD2.

Materials and Methods

Insects and rice. C. suppressalis larvae of a strain susceptible to Bt toxins were collected in Wenzhou, Zhejiang province, China. Larvae were raised on rice seedlings in a laboratory at 25 ± 1 °C, 16/8 h light/dark and

Figure 4. Relative expression of the Chilo suppressalis cadherin genes CsCAD1 and CsCAD2 24 hrs after siRNA treatment. Results are means ± SE. Bars with the same lowercase letter are not significantly different; NC = negative control, siCAD1 = CsCAD1 knockdown treatment group, siCAD2 = CsCAD2 knockdown treatment group.
80% humidity. The midguts of fourth instar larvae were dissected, immediately frozen in liquid nitrogen and stored at −70 °C until required for RACE.

Two transgenic rice strains, one expressing Cry2A and the other Cry1C, were used in experiments. These strains were derived from the same parental strain, Minghui 63, which was used as the negative control. Rice seeds were soaked for three days and then germinated in petri dishes on wet filter paper. All rice seeds were kindly provided by Prof. Yong-Jun Lin of Huazhong Agricultural University.

Total mRNA isolation and cDNA synthesis. Whole bodies of different developmental stages of C. suppressalis (2nd to 6th instar larvae and adults), and specific body parts (head, midgut, epidermis, fat body), were first homogenized in a tissue grinder. TRIzol reagent (GIBCO, USA) was then used to isolate total mRNA from these samples according to the manufacturer’s protocol. Genomic DNA was removed from total RNA with a DNA-free kit (Ambion, USA). The integrity of the RNA obtained was checked by electrophoresis on a 1.5% agarose gel. The 260/280 nm absorbance ratios of all RNA samples were between 1.8 and 2.2. First strand cDNA was synthesized using M-MLV reverse transcriptase (Takara, Japan) with Oligo (dT18) as the anchor primer. The reaction mixtures were incubated at 70 °C for 10 min followed by 42 °C for one hour and 70 °C for 15 min. The cDNA was stored at −20 °C for further use.

Quantitative real-time PCR. Quantitative real-time PCR (qPCR) was carried out with a SYBR Premix Ex Taq kit (Takara) using an ABI Prism 7300 (Applied Biosystems, USA) to detect the expression of GsCAD1 and GsCAD2 in the midgut of different development stages, and in different body parts. Primers were designed with Beacon Designer 7 (Table S1) and dissolution curves and gel electrophoresis were used to determine primer specificity. The amplification efficiency of all primers was checked with a cDNA dilution gradient, after which 2 μL of cDNA template was used in the PCR reaction according to the PCR kit’s protocol. The qPCR began at 95 °C for 30 secs, followed by 40 cycles of 95 °C for 5 secs, annealing at 60 °C for 31 sec, ending with cycles of 95 °C for 15 sec, 60 °C for 60 sec, and 95 °C for 15 sec. The specificity of the qPCR reactions was monitored with melting curve analysis using SDS software (version 1.4) and gel electrophoresis. Amplification efficiencies were determined by a series of template dilutions. All experiments were repeated in triplicate. The raw Ct values were obtained using ABI 7300 SDS software (Version 1.4). The standard Delta-Delta-Ct method was used to analysis the qPCR

Figure 5. Mortality of Chilo suppressalis larvae treated with siRNA designed to silence the cadherin genes GsCAD1 or GsCAD2, after feeding on transgenic rice expressing (A) Cry2A or (B) Cry1C; siNC = negative control, siCAD1 = GsCAD1 knockdown treatment group, siCAD2 = GsCAD2 knockdown treatment group.
data. The housekeeping genes E2F and GAPDH (GenBank No.: DQ311161.1 and AB262581.1) were used as the internal controls. Significant differences among multiple means were determined using Tukey’s HSD (P < 0.05).

**RACE amplification of CsCAD2.** Total RNA was extracted from the midgut of the 3rd instar larvae. RACE amplification was carried out with a SMARTer RACE cDNA Amplification Kit (Takara) according to the manufacturer’s protocol. Fragments of CsCAD2 cDNA were obtained from the transcriptome data used in previous studies. The primers CTCATTACCTCCCTCCCACTCGGCAG (5′ RACE) and TGACAATCCACCACATTTCACGCAGG (3′ RACE) were designed, based on the sequences obtained, to amplify the full-length of the CsCAD2 gene. The end-to-end primers (5′ AAACTTAATAGGCTTACTCGTTCTACC and 3′ GCTGTTCCCTGTCAAATGTCAC) were designed to amplify the full length of the CsCAD2 gene. PCR products were inserted into vector (Takara, Dalian, China) and sequenced by the Nanjing Genscript Company, China. The transcriptome and genome data were used to obtain the full-length transcript of CsCAD2. The resultant sequence was submitted to GenBank (Accession No. JQ747493).

**RNA interference of cadherins.** Two types of siRNA, siCAD1 and siCAD2, were used to silence the CsCAD1 and CsCAD2 genes, respectively, and a random sequence siRNA was included as a negative control (siNC) (Table S2). All siRNAs were synthesized by the GenePharma Company. Larvae were treated with siRNAs in petri dishes with wet filter paper on the bottom. The siRNA was smeared onto 4 cm-long sprouts of non-transgenic rice and about 1000 newly hatched larvae were then put in the petri dishes to feed on the treated rice sprouts. Rice sprouts were replaced every 4 hrs for three days. All experiments were conducted at 27 °C and were repeated in triplicate so that there about 3000 insects were used per treatment and nearly 10 thousand in total.

Larvae from each treatment were used to assess both susceptibility to Bt transgenic rice and investigate gene expression. Susceptibility to transgenic rice was assessed in a randomly selected group of 90 larvae. These were subdivided into three groups of 30 which were randomly assigned to feed on either transgenic rice expressing Cry2A toxin, transgenic rice expressing Cry1C toxin, or non-transgenic rice. For another part, to examine the gene expression change, 50 insects were randomly selected at the 2nd day after the insects were fed on transgenic rice. All experiments were repeated in triplicate.

**Gene evolution and domain analysis.** Phylogenetic analysis was conducted in MEGA (v6.0) using the Neighbor-joining method with 1000 bootstrap replicates. Domain structures of candidate cadherin genes were analyzed using CD Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Signal peptides were predicted with SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/) and transmembrane domains with TMPred (http://www.ch.embnet.org/software/TMPRED_form.html). Cox’ proportional hazard model implemented in
Program R (R Core Team R version 3.2.3) was used to analyze changes in survival from the 2nd to 5th day after larvae had commenced feeding on transgenic rice plants.

References

1. Prado, J. R. et al. Genetically engineered crops: from idea to product. *Annual review of plant biology* 65, 769–790, doi:10.1146/annurev-arplant-050213-040039 (2014).
2. Pardo-Lopez, L., Soberon, M. & Bravo, A. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. *FEBS microbiology reviews* 37, 3–22, doi:10.1111/j.1574-6976.2012.00341.x (2013).
3. Gao, Y., Oppert, B., Lord, J. C., Liu, C. & Lei, Z. *Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris* quatuordecimpunctata to *Beauveria bassiana* infection. *J Invertebr Pathol* 109, 260–263, doi:10.1016/j.jip.2011.12.003 (2012).
4. Gahan, L. J., Gould, F. & Heckel, D. G. Identification of a Gene Associated with Bt Resistance in *Heliotis virescens*. *Science* 293, 857–860 (2001).
5. Gahan, L. J., Pauchet, Y., Vogel, H. & Heckel, D. G. An ABC Transporter Mutation Is Correlated with Insect Resistance to *Bacillus thuringiensis* Cry1Ac Toxin. *Plos Genetics* 6, e1001248 (2010).
6. Park, Y. et al. ABC transporters mediate insect resistance to multiple Bt toxins revealed by bulk segregant analysis. *BMC Biology* 12, 1–15 (2014).
7. Jurafuentes, J. L. & Adang, M. J. Characterization of a Cry1Ac-receptor alkaline phosphatase in susceptible and resistant *Heliotis virescens* larvae. *European Journal of Biochemistry* 271, 3127–3135 (2004).
8. Guo, Z. et al. MAPK signaling pathway alters expression of midgut ALP and ABCG2 genes and causes resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamondback moth. *Insect Science* 11, e1005124 (2015).
9. Wang, X. Y. et al. RNAi in the striped stem borer, *Chilo suppressalis*, establishes a functional role for aminopeptidase N in Cry1Ab intoxication. *J Invertebr Pathol* 143, 1–10, doi:10.1016/j.jip.2016.11.004 (2017).
10. Adang, M. J., Crickmore, N. & Jurat-Fuentes, J. L. Chapter Two - Diversity of *Bacillus thuringiensis* Crystal Toxins and Mechanism of Action. (Elsevier Science & Technology, 2014).
11. An, J., Gao, Y., Lei, C., Gould, F. & Wu, K. Monitoring cotton bollworm resistance to Cry1Ac in two counties of northern China during 2009–2013. *Pest Management Science* 71, 377–382 (2014).
12. Jin, L. et al. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. *Nat Biotechnol* 33, 169–174, doi:10.1038/nbt.3100 (2015).
13. Zhang, H. et al. Diverse genetic basis of field-evolved resistance to Bt cotton in cotton bollworm from China. *Proc Natl Acad Sci USA* 109, 10275–10280, doi:10.1073/pnas.1200156109 (2013).
14. Bravo, A. *et al*. *Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Bacillus thuringiensis* to *Ostrinia nubilalis*. *Insect Biochemistry & Molecular Biology* 35, 33–40 (2005).
15. Hua, G., Zhang, R., Abdullah, M. A. F. & Adang, M. J. Anopheles gambiae Actin genes in Helicoverpa armigera. *Journal of Biological Databases & Curation* 6, 1–10, doi:10.1038/nbt.3100 (2015).
16. Jin, L. *et al*. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. *Nat Biotechnol* 33, 169–174, doi:10.1038/nbt.3100 (2015).
17. Hua, G., Park, Y. & Adang, M. J. Cadherin mutation linked to resistance to Cry1Ac affects male paternity and sperm competition in *Helicoverpa armigera*. *Journal of Insect Physiology* 70, 67–72 (2014).
18. Vladamudi, R. K., Weber, E., Ji, L., Ji, T. H. & B. L. Jr. Cloning and Expression of a Receptor for an Insecticidal Toxin of *Bacillus thuringiensis*. *Journal of Biological Chemistry* 270, 5490 (1995).
19. Nagamatsu, Y. et al. Cloning, sequencing, and expression of the *Bombyx mori* receptor for *Bacillus thuringiensis* insecticidal Cry1A(a) toxin. *Bioscience Biotechnology & Biochemistry* 62, 727–734 (1998).
20. Xu, X., Yu, L. & Wu, Y. Disruption of a cadherin gene associated with resistance to Cry1Ac (delta)-endotoxin of *Bacillus thuringiensis*. *Helicoverpa armigera*. *Applied & Environmental Microbiology* 71, 948–954 (2005).
21. S., M. *et al*. Three cadherin alleles associated with resistance to *Bacillus thuringiensis* in pink bollworm. *Proceedings of the National Academy of Sciences* 100, 5004 (2003).
22. Flannagan, R. D. et al. Identification, cloning and expression of a Cry1Ab cadherin receptor from European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). *Insect Biochemistry & Molecular Biology* 35, 33–40 (2005).
23. Hua, G., Zhang, R., Abdullah, M. A. F. & Adang, M. J. Anopheles gambiae Cadherin ActC1 binds the Cry4Ba Toxin of *Bacillus thuringiensis* cry4Ba and a Fragment of ActC1 synergizes Toxicity. *Insect Biochemistry & Molecular Biology* 35, 2003.
24. Hua, G., Park, Y. & Adang, M. J. Cadherin AdCad1 in *Alphitobius diaperinus* larvae is a receptor of Cry3Bb toxin from *Bacillus thuringiensis*. *Insect Biochemistry & Molecular Biology* 45, 11–17 (2013).
25. Tan, S. Y. et al. RNAi induced knockdown of a cadherin-like protein (EF531715) does not affect toxicity of Cry34/35A1B or Cry3Aa to *Diabrotica virgifera virgifera* larvae (coleoptera: chrysomelidae). *Insect Biochemistry & Molecular Biology* 73, 117 (2016).
26. Guo, Z. et al. The midgut cadherin-like gene is not associated with resistance to *Bacillus thuringiensis* toxin Cry1Ac in *Plutella xylostella* (L.). *Journal of Invertebrate Pathology* 126, 21–30 (2015).
27. Hong, K. *et al*. Gene cloning and expression of aminopeptidase N and cadherin from midgut of the rice stem borer, *Chilo suppressalis*. *Insect Science* 17, 393–399 (2010).
28. Alcántara, E. P., Aguda, R. M., Curtiss, A., Dean, D. H. & Cohen, M. B. *Bacillus thuringiensis* Cry3Bb –endotoxin binding to brush border membrane vesicles of rice stem borer (p 169–177). *Archives of Insect Biochemistry & Physiology* 55, 169–177 (2010).
29. Avisar, D. et al. The *Bacillus thuringiensis* delta-endotoxin Cry1C as a potential bioinsecticide in plants. *Plant Science* 176, 315–324 (2009).
30. Yin, C. et al. *ChiloDR*: a genomic and transcriptome database for an important rice insect pest *Chilo suppressalis*. *Database the Journal of Biological Databases & Curation* 2014, 92–108 (2014).
31. Bravo, A. *et al*. Oligomerization triggers binding of a *Bacillus thuringiensis* Cry1Ab pore-forming toxin to aminopeptidase N receptor leading to insertion into membrane microdomains. *Biochimica Et Biophysica Acta* 1667, 38–46 (2004).
32. Pigott, C. R. & Ellar, D. J. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. *Microbiology & Molecular Biology Reviews* 71, 255–281 (2007).
33. Yang, Y. et al. Down regulation of a gene for cadherin, but not alkaline phosphatase, associated with Cry1Ab resistance in the sugarcane borer *Diatraea saccharalis*. *Plos One* 6, e25783 (2011).
34. Ma, W. et al. Correction: Exploring the Midgut Transcriptome and Brush Border Membrane Vesicle Proteome of the Rice Stem Borer, *Chilo suppressalis* (Walker). *Plos One* 7, e38151 (2012).

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Author Contributions
Z.Z. analyzed the data, made the figures and tables and wrote the manuscript. X.L.T. carried out experiments and analyzed the data. W.H.M. contributed to the discussion. F.L. designed the project, analyzed the data and also contributed to writing the manuscript.

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