Linking Allele-Specific Expression And Natural Selection In Wild Populations

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Abstract

Allele-specific expression (ASE) is now a widely studied mechanism at cell, tissue and organism levels. However, population-level ASE and its evolutive impacts have still never been investigated. Here, we hypothesized a potential link between ASE and natural selection on the cosmopolitan copepod *Oithona similis*. We combined metagenomic and metatranscriptomic data from seven wild populations of the marine copepod *O. similis* sampled during the *Tara* Oceans expedition. We detected 587 single nucleotide variants (SNVs) under ASE and found a significant amount of 152 SNVs under ASE in at least one population and under selection across all the populations. This constitutes a first evidence that selection and ASE target more common loci than expected by chance, raising new questions about the nature of the evolutive links between the two mechanisms.

Introduction

Allele-specific expression (ASE), or allelic imbalance, refers to difference of expression between two alleles of a locus in a heterozygous genotype due to genetic or epigenetic polymorphism. Through DNA methylation or histone modifications, epigenetics could repress a disadvantageous or a specific parental allele, leading in some cases to monoallelic expression, as demonstrated in a variety of organisms including mouse, maize or bumblebee [1–4]. On the other hand, ASE may have a genetic origin through, for example, mutations in transcription factor binding sites [5,6], or post-transcriptional mechanisms like non-sense mediated decay [7–9]. Recently, several studies led to a better understanding of ASE thanks to the development of advanced tools allowing their detection at the individual, tissue and cell levels[7,9–15]. ASE has been investigated in the context of *cis-* and *trans*-regulation of gene expression[16], expression evolution [17] and association between gene expression and human diseases [18]. First approaches in natural populations of primates and flycatchers have been undertaken with individual-level data [19–21]. Moreover, studies began to question the relative contribution of genetics and environment on gene expression using ASE in human [22–25] and fruit flies[26].

However, population-level ASE in several wild populations of one species and its potential evolutive origins and consequences remain largely uninvestigated. The need for numerous individual RNA-seq and whole-genome genotyping data constitutes the main obstacle for population-scale analyses. Today, the advances of next-generation sequencing technologies allow integrating large metagenomic and metatranscriptomic data from environmental samples, and new approaches can now be considered using whole population information.

In natural populations, we expect most loci to be under neutral evolution and balanced expression (Fig. 1a)[27,28]. When selection occurs on a specific locus, the selected allele tends to homozygosity

creating a specific population-level expression pattern of the selected allele (Fig.1b).In the

absence of selection, if the same allele is favored by ASE in most of the individuals, the observed

population-level expression pattern (Fig. 1c) will be similar to the one observed in the case of

selection (Fig.1b).Considering that both mechanisms impact fitness, we hypothesized that ASE

and natural selection could preferentially target the same loci, in different populations, showing a

possible link between the two mechanisms.

In this study, we focus on the widespread epipelagic, temperate and cold water small-sized

copepod, *Oithona similis* (Cyclopoida, Claus 1866), notably known to be highly abundant in

Arctic[30–33]. Copepods, and particularly *Oithona*, are small crustaceans forming the most abundant

metazoan on Earth, reflecting strong adaptive capacities to environmental fluctuations[34–36].They

play a key ecological role in biogeochemical cycles and in the marine trophic food chain[37];

therefore copepods constitute an ideal model to study wild population evolution[38–41]

The first goal of our study was to identify loci under selection before demonstrating that

population-level ASE can be detected with metagenomic and metatranscriptomic data collected

by the *Tara* Oceans expedition[42] during its Arctic phase. Then we provided evidence of a

quantitative link between ASE and natural selection.

Material and Methods

Material sampling, mRNA extraction and transcriptome sequencing

Oithona similis specimens were sampled at the North of the Large Bay of Toulon, France (Lat

43°06' 02.3" N and Long 05°56' 53.4"E). Sampling took place in November 2016. The samples

were collected from the upper water layers (0-10m) using zooplankton nets with a mesh of 90µm

and 200µm (0.5 m diameter and 2.5 m length). Samples were preserved in 70% ethanol and

stored at -4°C. From the Large Bay of Toulon samples, *O. similis* individuals were isolated under

the stereomicroscope. We selected two different development stages: four copepodites (juveniles)

and four adult males. Each individual was transferred separately and crushed, with a tissue

grinder (Axygen) into a 1.5 mL tube (Eppendorf). Total mRNAs were extracted using the 'RNA

isolation' protocol from NucleoSpin RNA XS kit (Macherey-Nagel) and quantified on a Qubit

2.0 with a RNA HS Assay kit (Invitrogen) and on a Bioanalyzer 2100 with a RNA 6000 Pico

Assay kit (Agilent). cDNA were constructed using the SMARTer-Seq v4 Ultra low Input RNA

kit (ClonTech). The libraries were constructed using the NEBNext Ultra II kit, and were

sequenced with an Illumina HiSeq2500 (Supplementary Fig. 1).

Transcriptomes assembly and annotation

Each read set was assembled with Trinity v2.5.1[43] using default parameters and transcripts were

clustered using cd-hit v4.6.1[44] (Supplementary Table 1).To ensure the classification of the

sampled individuals, each ribosomal read set were detected with SortMeRNA [45] and mapped with

bwa v0.7.15using default parameters [46] to 82 ribosomal 28S sequences of *Oithona* species used in

Cornils et al., 2017 (Supplementary Fig. 2). The transcriptome assemblies were annotated with

Transdecoder v5.1.0[43] to predict the open reading frames (ORFs) and protein sequences

(Supplementary Table 1). In parallel, homology searches were also included as ORF retention

criteria; the peptide sequences of the longest ORFs were aligned on *Oithona nana* proteome [40]

using DIAMOND v0.9.22[48]. Protein domain annotation was performed on the final ORF

predictions with Interproscan v5.17.56.0[49] and a threshold of e-value $<10^{-5}$ was applied for Pfam

annotations. Finally, homology searches of the predicted proteins were done against the nr NCBI

database, restricted to Arthropoda (taxid: 6656), with DIAMOND v0.9.22 (Supplementary Fig.

1).

Variant calling using *Tara* Oceans metagenomic and metatranscriptomic data

We used metagenomic and metatranscriptomic reads generated from samples of the size fraction

20–180 µm collected in seven *Tara* Oceans stations TARA_155, 158, 178, 206, 208, 209 and 210

(Supplementary Table 2), according to protocols described in Alberti et al. 2017 [50].

The reference-free variant caller *DiscoSNP++* [51,52] was used to extract SNVs simultaneously

from raw metagenomic and metatranscriptomic reads, and ran using parameters –b 1. Only SNVs

corresponding to biallelic loci with a minimum of 4x of depth of coverage in all stations were

initially selected. Then, SNVs were clustered based on their loci co-abundance across samples

using density-based clustering algorithm implemented in the R package dbscan [53,54] and ran with

parameters epsilon = 10 and minPts = 10. This generated three SNVs clusters, the largest of

which contained 102,258 SNVs. To ensure the presence of *O. similis* SNVs without other

species, we observed the fitting of the depth of coverage to the expected negative binomial

distribution in each population (Supplementary Fig. 3). For each variant in each population, the

B-allele frequency (BAF) and the population-level B-allele relative expression (BARE) were

computed; $BAF = \frac{G_B}{G_B + G_A}$ and $BARE = \frac{T_B}{T_B + T_A}$, with G_A and G_B the metagenomic read counts of

the reference and alternative alleles respectively, T_A and T_B the metatranscriptomic read counts of

the reference and alternative alleles respectively.

Variant filtering and annotation

SNVs were filtered based on their metagenomic coverage. Those with a metagenomic coverage

lying outside a threshold of median $\pm$ 2 σ in at least one population, with a minimum and

maximum of 5x and 150x coverage were discarded. To keep out rare alleles and potential calling

errors, only variants characterized by a BAF comprised between 0.9 and 0.1, and a BARE

between 0.95 and 0.05 in at least one population were chosen for the final dataset resulting in

25,768 SNVs (Supplementary Fig. 4).

The variant annotation was conducted in two steps. First, the variant sequences were relocated on

the previously annotated *O.similis* transcripts using the "VCF_creator.sh" program of

DiscoSNP++. Secondly, a variant annotation was carried with SNPeff [55] to identify the location

of variants within transcripts (i.e., exon or UTR) and to estimate their effect on the proteins

(missense, synonymous or nonsense).The excess of candidate variant annotations was tested in

the following classes: missenses, synonymous, 5' and 3'UTR. A significant excess was

considered for a hypergeometric test p-value < 0.05.

Genomic differentiation and detection of selection

The differentiation among the seven populations was investigated through the computation of the

F_{ST} metric or Wright's fixation index [56,57].For each locus, global F_{ST} including the seven

populations and pairwise-F_{ST} between each pair of population was computed, using the

corresponding BAF matrix. For the global F_{ST} computation, a Hartigans' dip test for unimodality

was performed [58]. We retained the median pairwise-F_{ST} as a measure of the genomic

differentiation between each population. The *pcadapt* R package v4.0.2 [59] was used to detect

selection among populations from the B-allele frequency matrix. The computation was run on

"*Pool-seq*" mode, with a minimum allele frequency of 0.05 across the populations, and variants

with a corrected Benjamini and Hochberg [60] p-value < 0.05 were considered under selection.

Population-level ASE detection using metagenomic and metatranscriptomic data

In each population, we first selected variants for BAF $\neq$ {0,1}. Then, we computed $D = BAF$-

$BARE$, as the deviation between the BAF and the BARE. In the absence of ASE, D is close to 0,

as most of the biallelic loci are expected to have a balanced expression[7,28,61] we expect the D

distribution to follow a Gaussian distribution centered on 0. We estimated the Gaussian

distribution parameters and tested the probability of a variant to belong to this distribution ("D-

test" or "deviation test"). Given the large number of tests, we applied the Benjamini and

Hochberg approach to control the False Discovery Rate (FDR).

We also computed a "low expression bias" test by comparing the read counts T_A and T_B to the

observed metagenomic proportion 1-BAF and BAF respectively with a chi-square test and

applied the Benjamini and Hochberg correction for multiple testing. These two tests were applied

to BAFs, BAREs and read counts of the seven populations separately and the seven sets of

candidate loci targeted by ASE (deviation test q-value < 0.1 and low expression bias test q-

value< 0.1) were crossed to identify loci under ASE in different populations, or shared ASEs.

To identify alleles targeted by both ASE and selection, the set of variants under ASE in each

population was crossed with the set of loci detected under selection. The size of the intersection

was tested by a hypergeometric test, Hypergeometric(q,m,n,k), with q being number of alleles

under ASE in the population and under selection (size of intersection), m being the total number

of alleles under selection, n being the total number of variants under neutral evolution, and k

being the total number of alleles under ASE in the tested population. We considered that, in a

given population, the number of alleles under both ASE and selection was significantly higher

than expected by chance for p-value < 0.05.

Gene enrichment analysis

To identify specific biological function or processes associated to the variants, a domain-based

analysis was conducted. The Pfam annotation of the transcripts carrying variants targeted by ASE

and selection was used as entry for dcGO Enrichment [62]. A maximum of the best 300 GO-terms

were chosen based on their z-score and FDR p-value ($<10^{-3}$) in each ontology category. To

reduce redundancy, these selected GO-terms were processed using REVIGO[63], with a similarity

parameter of 0.5 against the whole Uniprot catalogue under the SimRel algorithm. To complete

the domain-based analysis, the functional annotations obtained from the homology searches

against the nr were manually curated.

Results

Oithona similis **genomic differentiation and selection in Arctic Seas**

From metagenomic and metatranscriptomic raw data of seven sampling stations (Fig. 2a), we

identified 25,768 expressed variants. Among them, 97% were relocated on *O. similis*

transcriptomes.

The global distribution of F_{ST} of the seven populations was unimodal (Hartigans' dip test,

D=0.0012, p-value=0.99) with a median-F_{ST} at 0.1, confirmed by the pairwise-F_{ST} distributions

(Supplementary Fig. 5).The seven populations were globally characterized by a weak to moderate

differentiation, with a maximum median pairwise-F_{ST} of 0.12 between populations from

TARA_210 and 155/178 (Fig. 2c,d). Populations from stations TARA_158 (Norway Current),

206 and 208 (Baffin Bay) were genetically closely-related, with the lowest median pairwise F_{ST}

(0.02), despite TARA_158 did not co-geolocalize with the two other stations. The four other

populations (TARA_155, 178, 209, and 210) were equally distant from each other (0.1-0.12).

Finally, TARA_158, 206 and 208 on one side, and TARA_155, 178, 210 and 209 on the other

side showed the same pattern of differentiation (0.05-0.07).

The PCA decomposed the genomic variability in six components; the first two components

discriminated TARA_155 and 178 from the others (32% and 28.1% variance explained

respectively, Fig. 2b), and the third component differentiated TARA_210 and 209 (19.5%). The

fourth principal component separated TARA_209 and 210 from 158/206/208 (11.3 %), with the

last two concerning TARA_158/206/208 (Supplementary Fig. 5).Globally, these results

dovetailed with the F_{ST} analysis, with details discussed later. Finally, we detected 674 variants

under selection, representing 2.6% of the dataset (corrected p-value < 0.05).

Loci targeted by population-level ASE and selection in Arctic populations

The number of SNVs tested for ASE varied between 13,454 and 22,578 for TARA_210 and 206

respectively. As expected, the D deviation, representing the deviation between B-allele frequency

and B-allele relative expression, followed a Gaussian distribution in each population (Fig. 3a and

Supplementary Fig. 6). Variants under ASE (i.e. having a D significantly higher or lower than

expected) were found in every population, ranging from 26 to 162 variants for TARA_178 and

206 respectively (Table 1). Overall, we found 587 variants under ASE, including 535 population-

specific ASEs, and 52 ASEs shared by several populations (Fig.3b). Remarkably, 30 ASEs out of

the 52 were present in the populations from TARA_158, 206 and 208 that correspond to the

genetically closest populations. The seven sets of variants under ASE were crossed with the set of

variants under selection, as illustrated for TARA_209 (Fig. 3c). The size of the intersection

ranged from 5 to 42 variants (TARA_155 and 210/206) and was significantly higher than

expected by chance for all the populations (hypergeometric test p-value < 0.05). It represented a

total of 152 unique variants under selection and ASE in at least one population (Table 1,

Supplementary Table 3), corresponding to 23% and 26% of variants under ASE and under

selection respectively.

214   Functional analysis of genes targeted by ASE and selection

Among the 152 loci targeted by ASE and selection, 145 were relocated on *O. similis* transcripts

(Supplementary Table 4). Amid these transcripts, 137 (90%) had a predicted ORF, 97 (64%)

were linked to at least one Pfam domain and 90 (59%) to a functional annotation. Fifteen SNVs

were missense variations, 59 synonymous, 31 and 29 were located in 5' and 3' UTR, without any

significant excess (Supplementary Table 4 and 5). Based on homology searches (Supplementary

Table 4), eight genes were linked to nervous system (Table2). Among them, two genes were

involved in glutamate metabolism (omega-amidase NIT2 and5-oxoprolinase), three were

predicted to be glycine, γ-amino-butyric acid (GABA) and histamine neuroreceptors. Finally,

four were also implicated in arthropods photoreceptors. The domain-based analysis confirmed

these results, with an enrichment in GO-terms biological process also linked to nervous system

(Supplementary Fig. 7).

Discussion

O. similis **populations are weakly structured within the Arctic Seas**

Global populations of *O. similis* are known to be composed of cryptic lineages[47]. Thus the

assessment that the seven populations used in our study belong to the same *O. similis* cryptic

lineage was a prerequisite for further analyses. The high proportion of variants mapped on the

Mediterranean transcriptomes (97%) showed that the variant clustering method was efficient to

regroup loci of an *O.similis* cryptic species. Plus, the unimodal distribution of F_{ST} showed that

these populations of *O. similis* belong to the same polar cryptic species.

Secondly, we see that the seven populations examined are well connected with low median

pairwise-F_{ST}, despite the large distances separating them. Weak genetic structure in the polar

region was already highlighted for other major Arctic copepods like *Calanus glacialis*[64], and

Pseudocalanus species[65]. The absence of structure was explained by ancient diminutions of

effective population size due to past glaciations[65–67], or high dispersal and connectivity between

the present-day populations due to marine currents[64].

Going into details, three different cases can be described. First, the differentiation of populations

from TARA_155 and 178 compared to the others could be explained by isolation-by-distance.

Secondly, the geographically close populations from TARA_210 and 209 present higher

differentiation (median pairwise-F_{ST} of 0.11). This could be explained by the West Greenland

current acting as a physical barrier between the populations, which could lead to reduced gene

flow[68]. At last, the strong link between TARA_158 from Northern Atlantic current and

TARA_206/208 from the Baffin Bay is the most intriguing. Despite the large distances that

separate the first one from the others, these three populations are well connected.

Metagenomic data enable to draw the silhouette of the population genetics but lacks resolution

when dealing with intra-population structure. However, our findings are concordant with

previous studies underpinning the large-scale dispersal, interconnectivity of marine zooplankton

populations in other oceans, at diverse degrees[38,69–71].

Toward the link between ASE and natural selection

Usually, at the individual level, the ASE analyses are achieved by measuring the difference in

RNA-seq read counts of a heterozygous site. But at the population level, obtaining a large

number of individuals remains a technical barrier especially for uncultured animals, or when the

amount of DNA retrieved from a single individual is not sufficient for high-throughput

sequencing. Here, the detection of ASE at population level was possible by comparing the

observed frequencies of the alleles based on metagenomic and metatranscriptomic data, which by

passes the obstacles previously described.

In our study, the amount of detected ASE in each population was always lower than 1% of tested

heterozygous variants, which altogether correspond to 2% of the total set of variants. In humans

[72],baboons [21] and flycatchers [19], 17%, 23% of genes and 7.5% of transcripts were affected by

ASE respectively. The difference with our results can be explained by one main reason. The

detection of population-level ASE identifies only the ASE present in a large majority of

individuals, which can be considered as "core ASEs".

These core ASEs constitute the majority of detected ASEs and are population-specific, meaning

the main drivers of this expression pattern are local conditions like different environmental

pressures or population dynamics including, for example, the proportion of each developmental

stage and sex, known to vary between populations and across seasons [30,73]. Another result is the

presence of a small amount of variants affected by ASE in different populations. Most of these

variants are under ASE in at least two of the three closest populations from TARA_158, 206 and

208. First, the genetic closeness and large geographic distances between these three populations

suggest that their shared ASEs are under an environmental independent genetic control.

Secondly, the number of variants tested for ASE is higher in these three populations than the

others, leading to a greater proportion of ASEs detected which also elevates the chances for a

variant to be declared under ASE in several populations.

A significant amount of SNVs (152) were subject to selection among the seven populations and

to ASE in at least one population. We confirmed our first hypothesis (Fig. 1), as exampled with

the variant 841109 (Fig. 3d), characterized by an ASE in favor of the B-allele in TARA_209 and

fixation of this allele in TARA_210. Three main features of ASE can be under selection. First,

the observed variant can be in linkage with another variation in upstream *cis*-regulatory elements

like transcription factors fixation sites, or epialleles[7]. Secondly, the annotation of candidate

variants with SNPeff revealed a majority of variants located in 5' and 3'UTRs, which are

variations known to both affect transcription efficiency through mRNA secondary structures,

stability and location[74–76]. For variants located in exons, a majority were identified as

synonymous mutations, growingly described as potential target of selection by codon usage bias,

codon context, mRNA secondary structure or transcription and translation dynamics [77,78]. Finally,

fifteen missense mutations were spotted, but with moderate predicted impact on protein amino

acid composition. However, we did not find premature nonsense mutation, even if variants under

ASE has been described to trigger or escape potential nonsense-mediated decay [28,61,79], but the

possibility that the causal variation is located in introns cannot be ruled out.

The process of adaptation through gene expression was suspected in human populations and

investigated thanks to the large and accessible amount of data. In a first study, a link has been

established between gene expression and selection, affecting particular genes and phenotypes,

looking at *cis*-acting SNPs[80]. In a second study, the team was able to detect ASE in different

populations and to quantify genetic differentiation and selection[61]. They found particularly one

gene that shows strong differentiation between European and African populations and under ASE

in Europeans and not in Africans. However, they did not quantify this phenomenon. Both

emphasized the impact of selection on gene expression. In the same way, another approach

showed that ASE or expression variations with high effect size were rare in the populations,

based on intra-population analyses in *Capsella grandiflora* and human [28,81]. This situation is

presumably encountered in our analysis, as exampled with the B-allele of variant 20760212,

under ASE and with a low genomic frequency in TARA_210, but fixed in the others (Fig. 3d).

Our results complete previous analyses, as they quantify the link between ASE and selection in

populations and reveal the evolutive potency of ASE, for the first time at the population-level. It

remains to understand the nature of the association between ASE and selection. To address this

question, we formulate the hypothesis that they impact chronologically the same loci, following

constant or increasing selective pressure as well as environmental changes (Fig. 4).

Nervous system and visual perception are important targets of the natural selection and

ASE in *O. similis*

This evolutive link between ASE and selection is supported by the biological functions associated

to the targeted genes, which are involved notably in the copepods nervous system in two ways.

The first result is the presence of genes implicated in glutamate metabolism and glycine and/or

GABA receptors. Glutamate and GABA are respectively excitatory and inhibitory

neurotransmitters in arthropods motor neurons [82]. Plus, glycine and GABA receptors have

already been described as a target of selection in *O. nana* in Mediterranean Sea[40,41]. Secondly, the

functional analysis revealed also the importance of the eye and visual perception in the *O. similis*

evolution.

Copepod nervous system constitutes a key trait for its reproduction and survival, and based on

our data, a prime target for evolution, allowing higher capacity of perceiving and fast reacting

leading to more efficient predator escape, prey catching and mating. This can explain the great

evolutive success of these animals [35,83,84].

Conclusion

Gene expression variation is thought to play a crucial role in evolutive and adaptive history of natural populations. Herein, we developed proper methods integrating metagenomic and metatranscriptomic data to detect ASE at the population-level for the first time. Then, we demonstrated the link between ASE and natural selection by providing a quantitative observation of this phenomenon and its impact on specific biological features of copepods. In the future, we will try to generalize these observations to other organisms. Then, we will understand the nature of the link between ASE and natural selection by questioning the chronology between the two mechanisms.

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Author's contributions

Individuals for transcriptome production were sampled by J-LJ and KS. KS extracted RNA, EP and KL prepared the libraries and sequencing, MAM assembled the reads and RLJ annotated transcriptomes. PP and CA gave expertise support on *DiscoSNP++* and statistical framework respectively. RLJ and MAM performed the analyses and wrote the manuscript. MAM designed and supervised the study. J-LJ and PW offered scientific support.

Competing interests

The authors declare no competing interests.

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Tables

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Table 2: Functional annotations of variants targeted by ASE and selection implicated in nervous
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Supplementary Table 1: *Oithona similis* Mediterranean transcriptomes summary

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Figure 2: Genomic differentiation of *O. similis* populations from Arctic Seas. **a,** Geographic
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Supplementary Fig 1: Method pipeline overview

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Supplementary Fig 3: *Oithona similis* depth of coverage of biallelic loci in seven *Tara* Oceans
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Supplementary Fig 5: Genomic differentiation of Arctic Seas *Oithona similis* populations

Supplementary Fig 6: Allele-specific expression detection

Supplementary Fig 7: Functional analysis of *O. similis* transcripts targeted by ASE and
selection

Table 1: Allele-specific expression detection and link with selection by population

Population	Genomic median depth of coverage	Number of tested variants	Number of variants under ASE	Number of variants under ASE and selection	Hypergeometric test p-value
TARA_155	25	18,812	91	6	9.89E-3*
TARA_158	35	21,476	131	29	5.06E-20*
TARA_178	24	18,145	26	9	5E-11*
TARA_206	55	22,578	162	42	4.82E-31*
TARA_208	48	21,469	133	14	2.2E-6*
TARA_209	12	13,956	62	24	1.05E-23*
TARA_210	14	13,454	69	42	8.89E-51*
Overall	-	25,768	587 (2.3%)	152 (0.59%)	-

625 **Table 2:** Functional annotations of variants targeted by ASE and selection implicated in nervous system

VarID	Ref	Alt	Homology search	Pfam	SnpEff Localization	SnpEff Impact	References
722267	A	G	histamine H1 receptor	PF00001	3 ' UTR	MODIFIER	85–87
9665345	T	G	chaoptin	PF13306 | PF13855	synonymous variant	LOW	88,89
15623788	G	A	eye-specific diacylglycerol kinase	PF13637	synonymous variant	LOW	90,91
23795359	A	T	vang-like protein 2-B	PF06638	synonymous variant	LOW	92,93
1276227	C	T	glycine receptor subunit alpha-2 / gamma-aminobutyric acid receptor subunit alpha-6	PF02932 | PF2931	3 ' UTR	MODIFIER	-
1404415	G	C	omega-amidase NIT2	PF00795	3 ' UTR	MODIFIER	-
11174785	A	G	5-oxoprolinase	PF02538 | PF05378 | PF01968	5 ' UTR	MODIFIER	-
11690229	A	T	glycine receptor subunit alpha-2	PF02931	synonymous variant	LOW	-

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