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Genome-wide Association Mapping of Response to Infection by the Aleutian Mink Disease Virus

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ABSTRACT: Aleutian mink disease virus (AMDV) causes the most important health issue for the mink industry worldwide. With no treatment or vaccine, and failure of viral eradication attempts from most regions of the world, genetic selection for tolerance is a possibility. The genomes of 95 black mink that were inoculated with AMDV and showed varying degrees of response to infection were analyzed using next generation sequencing. A total of 1713 high quality single nucleotide polymorphisms (SNPs) were identified and analyzed for their associations with response to infection. Some SNPs were significantly associated with severity of disease symptoms in kidneys and presence of AMDV in blood and organs. Only weak associations were discovered for antibody titre (0 to 1024) and severity of the disease symptoms in liver and lung. These findings provide the first step towards marker-assisted breeding for mink tolerant of AMDV.
Keywords: Mink; Aleutian mink disease virus; Genome-wide association mapping

Introduction

Aleutian disease (AD) is the most important health issue for the mink industry in almost all mink producing regions. Infection with the Aleutian mink disease virus (AMDV) often causes economic losses (Hansen and Lund, 1988; Broll and Alexandersen, 1996). There is no vaccine or treatment for this disease, and testing of the mink for antibodies against the virus by countercurrentimmunoelectrophoresis (CIEP) and the elimination of infected animals has not been effective in complete eradication of AMDV from many ranches in Nova Scotia (Farid et al., 2012) and elsewhere. The development of AD symptoms in mink depends on both host and viral factors, and some mink can tolerate the infection, i.e. do not develop the disease symptoms after infection (An and Ingram 1977; Hadlow et al. 1985). Utilizing the natural differences that exist among animals in response to infection by AMDV to create a resistant population is a logical approach. It is not feasible for ranchers to select mink for increased resistance to the disease using traditional animal breeding techniques, because it is a prohibitively expensive undertaking. DNA markers would greatly facilitate selection for increased resistance to AMDV infection.

Genome-wide association (GWA) and genomic selection (GS) are increasingly being used to establish genotype-phenotype relationships across diverse species. GWA and GS require genome-wide genotype data in large numbers of samples. Genotyping-by-sequencing (GBS) uses next generation sequencing to generate genome-wide genotype data. As it is far faster and more cost effective than genotyping microarrays, it is becoming the method of choice across a diverse range of organisms for the purposes of GWA and GS. The GBS technique enables the discovery of large numbers of single nucleotide polymorphisms (SNPs) by sequencing whole genomes of many individuals simultaneously at a relatively low cost (Elshire et al., 2011).

We have access to a population of black mink that have been inoculated with a single strain of the AMDV in 2010, and their responses to infection have been regularly monitored. To date, we have identified animals with diverse response to infection. The objective of this research was to use this valuable animal population to uncover SNPs in the genomes of mink differing in response to infection by the AMDV, and compute the associations between the SNPs and the responses to infection.

Materials and Methods

Source of animals and sampling. All protocols were approval by the institutional Animal Care and Use Committee. Approximately 1400 black mink were inoculated with a local strain of AMDV during the fall of 2010, 2011 and 2012, of which 644 were euthanized in February each year. Samples of serum and seven organs (spleen, liver, kidney, lungs, bone marrow, lymph nodes, small intestine) were collected after euthanasia. Samples from four organs (liver, heart, kidney, lungs) were also collected and stored in 10% formalin for histopathology. The number of inoculated mink that died or were terminated because of sickness was 392 during 2010 to 2013.

Laboratory activities. The presence of antibodies against the virus on days 35, 120 and 350 post-inoculation (pi) was determined in the plasma of all inoculated animals by CIEP. Antibody titer of the 644 animals that were euthanized was determined by CIEP using 11 two-fold serial dilution of plasma samples (1/1 to 1/1024). Antibody titer was recorded as the reciprocal of highest level of dilution that resulted in positive CIEP test.

DNA was extracted from plasma of all inoculated animals on days 35, 120 and 350 pi, at termination time and from cell-free tissue suspensions using Dynabeads Silane viral nucleic acid extraction kit (Invitrogen, Burlington, ON). Presence of AMDV was tested in triplicate by the polymerase chain reaction (PCR) as previously explained (Farid, 2013) using extracted DNA. Severity of the disease symptoms was determined by histological examination of

the four organs by an experienced pathologist at the Pathology Laboratory, NS Department of Agriculture.

Selection of experimental animals. A total of 70 inoculated mink with a wide range of anti-AMDV antibody titers, viral replication status and severity of AD symptoms were selected for this experiment. In addition, 25 mink from a ranch that have never been exposed to the virus were used as controls. High molecular weight genomic DNA was extracted from the spleens of the animals. Purity and concentration of DNA samples were evaluated and genomic DNA was digested by the restriction enzyme ApeKI (G↓CWGC) prior to sequencing to reduce genome complexity.

Two different adapters were used, one of which contained the bar code unique to each individual mink and the other was the standard Y-adaptor used to generate DNA sequencing libraries for the Illumina HiSeq sequencer. After digestion with the restriction enzyme, the adapters were quantified, added to 96-well plates, genomic DNA was added (100 ng in 10 µL) and adapters were ligated to the ends of genomic DNA fragments. Aliquots of each of the 95 samples were pooled and excess adapters were removed. DNA fragments were amplified by PCR using primers complementary to the ligated adapters. PCR products were cleaned, fragment sizes were checked, and libraries with no or minute amounts of adapter-dimers were used for DNA sequencing.

DNA sequencing and sequence analysis. The samples were analyzed at the Genome Quebec sequencing centre and 206,625,585 reads, each with a length of 100 bp, for a total of over 20 billion bp of DNA sequence were generated. Of these sequences, 95.1% (196,581,081) had acceptable quality and passed filtering steps, and only 4.9% were dropped due to absence of an identifiable barcode or restriction site residue at the beginning of the read. These figures suggest that library preparation and sequencing were performed satisfactorily. Also 13.8% of the reads (28,415,267) had to be adapter trimmed because they were less than 100 bp in length, and the sequencer read into the common GBS adapter, indicating that the ApeKI enzyme worked reasonably well for the mink genome. The average number of high quality reads of the 95 mink was 2,069,249, ranging from 692,225 to 4,778,284 reads. The distribution of reads across samples was relatively uniform and no samples were removed due to low sequencing coverage.

Genetic marker identification. A custom bioinformatics pipeline was developed for processing the resulting DNA sequence data. The pipeline takes raw DNA sequence data as input and outputs a table of genetic markers for each DNA sample that are of sufficient quality for the purposes of genetic mapping. Our analysis is restricted to one type of genetic marker, namely single nucleotide polymorphisms (SNPs). Because there is no reference genome available for mink, we aligned the mink DNA sequences to the ferret genome, the most closely related species for which a draft reference genome is available. In addition, we processed the GBS data through UNEAK (Lu et al. 2013) which does not require the use of a reference genome. We performed the downstream analysis using both sets of genetic markers. As part of the UNEAK GBS pipeline, all the reads were trimmed to 64 base pairs in length during the analysis. The program was set to filter out SNPs with more than 20% missing genotype calls and a minor allele frequency of less than 5%.

Data analysis. The reads that passed filtering steps (196,581,081) were used for downstream GBS analysis. We sought to identify statistical associations between SNPs and traits of interest by performing a GWA analysis. We used the software TASSEL and the efficient mixed model for association (EMMA), which accounts for the confounding effects of relatedness among individuals. The associations between SNPs and the nine phenotypic traits ($\log_{10}$ of CIEP titer, severity of lesions in the kidney, liver and lung, presence of the virus in blood on days 35, 120 and 350 pi, as well as during the entire life, and the presence of the virus in organs) were analyzed. Presence of the virus on final sampling date was not analyzed because too few individuals (6) had the virus in the samples.

Results and discussion

Distribution of phenotypic data. The inoculated mink that were used in this study survived until sampling at 16 months pi. All animals became CIEP positive by day 35 pi, and antibody titer varied between zero and 1024 at termination time. The number of PCR negative animals was seven (10%) on day 35 pi, but increased to 60 (86%) by day 350 pi and to 70 (100%) by termination time, indicating that viremia was short-lived and viral replication was transient in these animals. The virus was, however, sequestered in most animals as only seven mink did not have a detectible level of the virus in any of the seven organs at the termination time. These animals were infected because they were all CIEP positive by day 35 pi, but showed very limited period of viral replication. Of the seven mink that were PCR negative on day 35 pi, six remained PCR negative in all blood tests (D35, D120, D350, termination). The number of mink with no lesions, minor, moderate, severe and very severe lesions in any of the four organs were 11, 21, 24, 11 and 3, respectively, when terminated.

Number of SNPs detected. A total of 1713 high quality SNPs were identified in the 95 mink. Assuming that the size of the mink genome is similar to the ferret genome (2.7 Gb, http://www.genomesize.org), this number shows a SNP density of approximately one per 1.5 Mb, which is very low and may be due to the low level of genetic variability of black mink. Although relatedness among animals contributes to the low level of genetic variability, animals that were genotyped in this study originated from several ranches, and those from one of the ranches were not related to those from other ranches for at least two decades.

Associations between SNPs and phenotypes. The efficient mixed model for association identified several suggestive associations between specific SNPs and a few traits. The five most strongly associated SNPs with each of the phenotypes had P values ranging from 0.0043 to 0.0000039 (Table 1). However, only one SNP reached statistical significance after Bonferonni correction (0.05/1713= 0.0000291) (association between SNP in TP369238 and severity of AD symptoms in the kidney). Most of the SNPs listed in Table 1 had low minor allele frequencies (i.e. < 0.1) and need additional data for confirmation.

Table 1: The top 12 markers with the highest association with phenotypic traits

Trait	Marker	Probability	R^2
Virus in organs	TP873113	5.51E-05	0.55
Severity of AD symptoms in kidney	TP369238	3.95E-06	0.50
Virus in blood, day 35 pi	TP68499	4.90E-04	0.48
Virus in blood during lifetime	TP68499	1.78E-04	0.48
Virus in blood, day 35 pi	TP23435	2.04E-04	0.40
Severity of AD symptoms in kidney	TP585879	1.35E-04	0.39
Virus in blood, day 350 pi	TP1033312	8.08E-04	0.38
Severity of AD symptoms in kidney	TP641596	4.35E-05	0.37
Virus in organs	TP424318	3.99E-05	0.36
Severity of AD symptoms in kidney	TP655889	1.34E-04	0.34
Virus in organs	TP379233	1.87E-03	0.33
Severity of AD symptoms in kidney	TP305055	1.40E-04	0.31

Of the top five SNPs associated with the phenotypic traits, only four SNPs each appeared twice, all were associated with presence of the virus in blood on day 35 pi and during lifetime. The highest degrees of associations were with the presence of virus in organs (R^2=0.55), lesion scores in the kidney (R^2=0.51), presence of virus in blood during lifetime (R^2=0.48) and on day 35 pi (R^2=0.47 and 0.40 for two SNPs) (Table 1). Lesion scores in the kidney had the highest associations with the top five SNPs (R^2 ranged between 0.31 and 0.50), followed by presence of the virus in blood on day 35 pi (R^2 ranged between 0.23 and 0.47). Associations between the top five SNPs and CIEP-titer, severity of the disease symptoms in liver and lung and presence of the virus in blood on day 120 pi were all lower than 0.30.

Conclusion

The number of SNPs detected in these animals was smaller than expected, and there is no published information to compare these results with. Considering the low sample size and SNP density, these GWA results should be interpreted with caution. Additional animals need to be tested to obtain more powerful SNP-trait associations that can then be followed up for the purposes of marker-assisted breeding.

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