Infection, Genetics and Evolution 11 (2011) 1616–1623

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Variation in European harbour seal immune response genes and susceptibility to phocine distemper virus (PDV)

Alex J. McCarthy [a,1], Marie-Anne Shaw [a], Paul D. Jepson [b], Sophie M.J.M. Brasseur [c], Peter J.H. Reijnders [c,d], Simon J. Goodman [a,*]

[a] Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
[b] Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK
[c] Ecosystems Department, Institute for Marine Resources and Ecosystem Studies, P.O. Box 167, 1790 AD Den Burg, The Netherlands
[d] Aquatic Ecology and Water Quality Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 11 March 2011
Received in revised form 2 June 2011
Accepted 3 June 2011
Available online 25 June 2011

Keywords:
Phocine distemper virus
SLAM
Association study
Disease susceptibility
Morbillivirus
Host genetics

ABSTRACT

Phocine distemper virus (PDV) has caused two mass mortalities of European harbour seals (*Phoca vitulina*) in recent decades. Levels of mortality varied considerably among European populations in both the 1988 and 2002 epidemics, with higher mortality in continental European populations in comparison to UK populations. High levels of genetic differentiation at neutral makers among seal populations allow for the possibility that there could be potential genetic differences at functional loci that may account for some of the variation in mortality. Recent genome sequencing of carnivore species and development of genomic tools have now made it possible to explore the possible contribution of variation in candidate genes from harbour seals in relation to the differential mortality patterns. We assessed variation in eight genes (*CD46*, *IFNG*, *IL4*, *IL8*, *IL10*, *RARa*, *SLAM* and *TLR2*) encoding key proteins involved in host cellular interactions with *Morbilliviruses* and the relationship of variants to disease status. This work constitutes the first genetic association study for *Morbillivirus* disease susceptibility in a non-model organism, and for a natural mortality event. We found no variation in harbour seals from across Europe in the protein coding domains of the viral receptors *SLAM* and *CD46*, but SNPs were present in SLAM intron 2. SNPs were also present in *IL8 p2* and *RARa* exon 1. There was no significant association of *SLAM* or *RARa* polymorphisms with disease status implying no role of these genes in determining resistance to PDV induced mortality, that could be detected with the available samples and the small number of polymorphisms indentified. However there was significant differentiation of allele frequencies among populations. PDV and other morbilliviruses are important models for wildlife epidemiology, host switches and viral evolution. Despite a negative result in this case, full sequencing of pinniped and other 'non-model' carnivore genomes will help in refining understanding the role of host genetics in disease susceptibility for these viruses.

1. Introduction

The *Morbillivirus* phocine distemper virus (PDV) caused mass mortalities among European seal populations in 1988 and 2002 (Heide-Jørgensen et al., 1992; Härkönen et al., 2006). Both outbreaks arose in spring and originated at the Isle of Anholt, Denmark, a haul out site shared between European harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*). It is thought the virus is endemic in arctic seal species, and that grey seals may act as shuttle vector, transferring the virus into more susceptible harbour seal populations. On both occasions PDV spread throughout Europe, killing up to 60% of individuals in local populations, together with a smaller number of grey seals. In total PDV accounted for around 30,000 and 23,000 harbour seal deaths in 1988 and 2002 respectively (Heide-Jørgensen et al., 1992; Hall et al., 2006; Härkönen et al., 2006).

Abbreviations: ACN, acetonitrile; CD, cluster designation; CDV, canine distemper virus; dHLPC, denaturing high performance liquid chromatography; F_{IS}, *F* statistic for inbreeding coefficient of individuals relative to the subpopulation; GWA, genome-wide association; H_E, expected heterozygosity; H_O, observed heterozygosity; H–W, Hardy–Weinberg equilibrium; IFN, interferon; IgC, immunoglobulin constant domain; IgV, immunoglobulin variable domain; IL, interleukin; MCP, membrane cofactor protein; MHC, major histocompatibility complex; MV, measles virus; PDV, phocine distemper virus; RARa, retinoic acid receptor alpha; SLAM, signalling lymphocyte activation molecule; SSPE, subacute sclerosing panencephalitis; TEAA, triethylammonium ion; TLR, toll-like receptor.

* Corresponding author. Tel.: +44 (0)113 3432561; fax: +44 (0)113 3432835.
E-mail address: s.j.goodman@leeds.ac.uk (S.J. Goodman).
[1] Present address: Centre for Infection, Department of Cellular and Molecular Medicine, St. George's, University of London, Cranmer Terrace, London SW17 0RE, UK.

1567-1348/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2011.06.002

Mortality levels in *P. vitulina* differed across European populations. High levels of mortality were recorded in continental European populations, ranging from 22% to 60% (Heide-Jørgensen et al., 1992; Härkönen et al., 2006). Lower mortality levels were recorded in UK populations (Thompson and Miller, 1992; Härkönen et al., 2006). Interestingly, the mortality level of most individual populations remained relatively unchanged between epidemics. The main exception was the East coast of England, where mortality fell from 50% in 1988 to 22% in 2002 (Thompson et al., 2005). Viral attenuation, varying exposure to immunosuppressive persistent organic pollutants (POPs), epidemiological factors, and variation in genetic resistance loci have been suggested as the main potential factors accounting for this variation in mortality (Hall et al., 2006; Härkönen et al., 2006). Viral attenuation and variation in POP burden have largely been excluded (Hall et al., 2006; Müller et al., 2004; Nielsen et al., 2009), while epidemiological modelling suggests that variation in the arrival time of the virus, in combination with differential exposure rates due to variation in the sex/age-class make up of seal haul out groups in different locations can potentially explain much of the mortality pattern observed (Grenfell et al., 1992; Härkönen et al., 2007; Harris et al., 2008; Klepac et al., 2009; Lonergan et al., 2010). However, the potential contribution of population genetic variation remains to be examined in detail. Understanding the roles of ecological, epidemiological and genetic factors in this mortality pattern will provide new insights into the disease biology of morbilliviruses, and may even suggest novel opportunities for the control of this important family of pathogens.

Past population genetic studies using microsatellite markers and mitochondrial DNA sequence variation have shown the European harbour seal metapopulation to be highly structured, with at least six genetically differentiated populations (Iceland, Scotland–Ireland, English East Coast, Wadden Sea, Western Scandinavia and East Baltic) with F_{ST} values ranging from 0.061 to 0.403 (Stanley et al., 1996; Goodman, 1998). Genetic diversity plays an important role in safeguarding host populations against pathogens (Haldane, 1947; Altizer et al., 2003). Differences in mortality levels among populations could be explained by differential distributions of disease resistance alleles in a structured population (Harding et al., 2005). Of particular interest are genes at the host–pathogen interface, including genes encoding viral receptors and proteins that mediate and regulate immune responses (Hall et al., 2006; Acevedo-Whitehouse and Cunningham, 2006). The only immune response genes that have been assessed in harbour seals to date are within the major histocompatibility complex (MHC), where levels of variation are in Class I loci appear comparable to those of other carnivores (Goodman, 1995), suggesting that a simple hypothesis of limited MHC variation does not account for the observed PDV susceptibility of European harbour seals. However, to date there has been no association study performed between MHC alleles and PDV susceptibility, nor any evaluation of the role of other genes involved in host interactions with morbilliviruses.

A precedent has been set from human genetics for the involvement of viral receptor alleles in disease resistance (Liu et al., 1996; Glass et al., 2006). The known cellular receptors of morbilliviruses are signalling lymphocyte activation molecule (*SLAM*, also known as CD150) and CD46 (membrane cofactor protein, MCP). SLAM is the primary cellular receptor and morbilliviruses interact with the immunoglobulin variable (IgV) domain and an immunglobulin constant domain (IgC) of the extracellular region (Tatsuo et al., 2001). CD46 is a secondary receptor though its role in infection is less clear (Dörig et al., 1993; Naniche et al., 1993). Immune detection molecules of innate immunity include Toll-like receptor 2 (TLRs) that interact with morbilliviruses (Bieback et al., 2002). Cytokines that regulate the immune response through cellular signalling could also have a role in disease resistance; variants of interferon gamma (*IFNG*), interleukin-4 (*IL4*), *IL8* and *IL10* have previously been associated with susceptibility to mammalian infectious diseases (Pacheco et al., 2008). Host levels of vitamin A may also be important in *Morbillivirus* infections (Semba, 2003; Rodeheffer et al., 2007). Recently, it was found that individuals in a ferret model of canine distemper virus (CDV) that are vitamin A supplemented do not display disease symptoms (Rodeheffer et al., 2007). Hence the vitamin A receptor (*RARa*) is also a candidate of interest as variation could impact vitamin A uptake. Genomic approaches have been used to identify successfully resistance alleles for infectious diseases in humans and, more recently, animals. The release of animal genome sequences, such as the cat (Pontius et al., 2007), dog (Lindblad-Toh et al., 2005) and giant panda (Li et al., 2010), together with the development of new bioinformatics tools are providing novel opportunities to investigate genetic variation in carnivore species (Kohn et al., 2006). These resources allow identification of conserved locations for primer design and development of assays for variation in functional genes in non-model species lacking comprehensive sequence coverage.

In the present study firstly we identify variation in genes encoding *Morbillivirus* receptors (*SLAM* and *CD46*), proteins involved in immune detection (*TLR2*), proteins involved in immune regulation (*IFNG*, *IL4*, *IL8*, *IL10*) and proteins involved in disease physiology (*RARa*). We subsequently genotype individuals of the European harbour seal metapopulation (including survivors and non-survivors of the epizootics) to determine differences in distribution of alleles between populations and perform an association study to test the relationship between gene variants and PDV mortality.

2. Materials and methods

2.1. Tissue and blood samples

A collection of harbour seal skin, tissue and blood samples was built up from material in the archives of European marine mammal stranding programmes, seal rehabilitation centres, or were donated by collaborating researchers. Samples were collected during both the 1988 and 2002 PDV epidemics from harbour seal carcasses. Blood and skin samples were also collected from healthy individuals during the PDV epidemic periods and post-epidemic periods (1989–1993, 2003–2006). Samples were genotyped from the following populations, English East Coast, Scottish East Coast, Iceland, Wadden Sea (Dutch and German Wadden Sea) and Swedish west coast (Skagerrak, Kattegat, West Baltic), which correspond to the populations identified in previous genetic studies (Goodman, 1998).

2.2. Primer design

Primers for *IFNG*, *IL4* and *IL10* were designed for amplification of canine sequences at the University of Manchester (Short et al., 2007). For all other genes there is no genomic DNA sequence available from *P. vitulina*. Canine genomic and mRNA sequence was aligned with mRNA sequence from other mammalian species in ClustalX in order to identify exon boundaries and identify conserved mammalian gene regions (Thompson et al., 1997). Primers were designed in webprimer (http://seq.yeastgenome.org/cgi-bin/web-primer) on dog sequences to amplify exons and introns for receptor genes, and promoter gene regions for cytokine genes. All primers were produced by Sigma–Genosys UK. Details of primer sequences are shown in Supplementary Table 1 and Fig. 1.

2.3. PCR, purification and sequencing

DNA was extracted from samples using a standard phenol: chloroform extraction protocol for both tissue and blood samples.