No association of prion protein gene (PRNP) polymorphisms with susceptibility to the pandemic 2009 swine flu

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Abstract

Background The pandemic 2009 swine flu is a highly infectious respiratory disorder caused by H1N1 influenza A viruses. A recent study reported that knockout of the prion protein gene (PRNP) induced susceptibility and lethality in influenza A virus-infected mice.

Objective Thus, we examined the association between genetic variations of the PRNP gene and susceptibility to pandemic 2009 swine flu.

Results We did not find an association between PRNP polymorphisms and susceptibility to pandemic 2009 swine flu.

Conclusions To the best of our knowledge, this was the first evaluation of the association between PRNP polymorphisms and vulnerability to pandemic 2009 swine flu.

Keywords Influenza · Pandemic · H1N1 · PRNP · SNP · Susceptibility · Prion disease

Introduction

Influenza A viruses are negative-sense single-stranded RNA viruses causing respiratory disorders in humans and animals. Since the genome of influenza A viruses is segmented RNA, the genome can be reassorted through the life cycle of various types of influenza A viruses and cause antigenic shifts (Javanian et al. 2021; Moghadami 2017). The pandemic influenza A H1N1 2009 virus is caused by an antigenic shift mediated by quadruple reassortments of viral genomes, including swine, avian, and human influenza A viruses (Allen et al. 2017; Everitt et al. 2012; Girard et al. 2010; Kim et al. 2020; Schnitzler and Schnitzler 2009). In response to the invasion of influenza A viruses, macrophages are activated to clear pathogens. However, macrophages are also the major reservoir of reactive oxygen species (ROS). The generation of excessive ROS results in the destruction of epithelial cell layers, which are considered the first line of antiviral defense for the host (Lin et al. 2016; Reshi et al. 2014; Kim et al. 2021).

The prion protein (PrP), encoded by the prion protein gene (PRNP), is a glycosylphosphatidylinositol (GPI)-anchored multifunctional protein composed of a nonsstructural octapeptide repeat domain and a globular C-terminal domain (Kim et al. 2021a, b). Previous studies have reported that PrP plays a pivotal role in the protection of oxidative stress in several organs, including the brain, heart and lungs (Castle and Gill 2017). In addition, deficiency of the PRNP gene confers susceptibility and lethality in influenza A virus-infected mice (Chida et al. 2018). Thus, we postulated that genetic variations in the PRNP gene, which are related to the function and expression level of PrP, may be associated with susceptibility to influenza A viruses.

To investigate the association between PRNP polymorphisms and vulnerability to pandemic 2009 swine flu, we investigated the genotype and allele frequencies of the PRNP polymorphisms located on the open reading frame (ORF) and transcriptional regulatory region in 97 healthy control subjects and 30 pandemic 2009 swine flu-infected patients using direct sequencing. We evaluated an association between PRNP polymorphisms and susceptibility to pandemic 2009 swine flu infection by comparing the genotype
and allele frequencies of the \textit{PRNP} polymorphisms between these two groups.

\section*{Materials and methods}

\subsection*{Ethics statements}

All samples were collected with informed consent under institutional review board-approved protocols. All experimental procedures were approved following the guidelines of the institutional review board (IRB) of Jeonbuk National University and the 1964 Helsinki Declaration and its later amendments (approval number: JBNU 2017-08-009). All the samples and information were anonymized prior to study.

\subsection*{Subject}

Detailed information on all subjects was explained in a previous study (Kim et al. 2020). In brief, healthy controls and pandemic 2009 swine flu-infected patients have no underlying disease and co-morbidity (Table 1).

\subsection*{Genomic DNA extraction}

Genomic DNA was isolated from 200 μl of peripheral blood using the Blood Genomic DNA Isolation Kit (Qiagen, Valencia, California, USA) following the manufacturer’s protocol.

\begin{table}[h!]
\centering
\begin{tabular}{lcc}
\hline
\textbf{Characteristics} & \textbf{Patients} & \textbf{Controls} \\
\hline
Number & 30 & 97 \\
Age & 55.27 ± 17.88 & 61.27 ± 8.46 \\
Sex (n, %) & & \\
\quad Male & 11 (36.67) & 32 (32.99) \\
\quad Female & 19 (63.33) & 65 (67.01) \\
No. of ICU admissions & 1 & NA \\
\hline
\end{tabular}
\caption{Detailed information on the study population}
\end{table}

\textit{ICU} intensive care unit, \textit{NA} not applicable

\begin{table}[h!]
\centering
\begin{tabular}{lccc}
\hline
\textbf{Target} & \textbf{Primers} & \textbf{Annealing temperature (°C)} & \textbf{Product size (bp)} \\
\hline
Open reading frame region & Forward & CAACCGCTACCCACCTCAG & 58 & 564 \\
& Reverse & AGGAGCATGCTCGATCTCTC & & \\
Transcription regulatory region & Forward & GAGAAACCTTGCAGTCA & 56 & 586 \\
& Reverse & AAGGTGACAAAAAGATGGGC & & \\
\hline
\end{tabular}
\caption{Detailed information on the primers used in this study}
\end{table}

\subsection*{Genetic analysis}

The \textit{PRNP} gene was amplified by polymerase chain reaction (PCR) using gene-targeted primers. Detailed information on the primers and experimental conditions are described in Table 2. PCR was carried out using GoTaq® DNA Polymerase (Promega, Fitchburg, Wisconsin, USA) and an S-1000 Thermal Cycler (Bio–Rad, Hercules, California, USA) following the manufacturer’s protocol. The PCR products were directly analyzed with an ABI 3730 automatic sequencer (ABI, Foster City, California, USA) and the results were annotated by Finch TV software (Geospiza Inc, Seattle, USA).

\subsection*{Statistical analysis}

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., USA).

\section*{Results}

\subsection*{Investigation of polymorphisms of the \textit{PRNP} gene}

To investigate the genotype and allele frequencies of \textit{PRNP} polymorphisms in the Korean population, we performed direct sequencing in 97 healthy controls and 30 pandemic 2009 swine flu-infected patients. The sequenced products were homologous to the \textit{PRNP} gene of \textit{Homo sapiens} registered in GenBank (Gene ID: 5621). We performed genotyping for 2 SNPs, M129V and E219K, located on the ORF of the \textit{PRNP} gene (Table 3), and 3 SNPs, c.1368 T > C, c.1380 T > C and c.1424G > A, located on the transcriptional regulatory region of the \textit{PRNP} gene (Table 4).

\subsection*{Evaluation of an association between \textit{PRNP} polymorphisms and vulnerability to pandemic 2009 swine flu}

To examine an association between the genetic distribution of the \textit{PRNP} gene and susceptibility to pandemic 2009 swine flu...
flu infection, we compared the genotype and allele frequencies of the PRNP gene polymorphisms between the healthy control subjects and pandemic 2009 swine flu-affected patients.

Interestingly, there is no association of the genotype and allele distributions of 2 SNPs, M129V and E219K, of the PRNP gene located on the ORF with vulnerability to pandemic 2009 swine flu (Table 3). In addition, there is no association of the genotype and allele distributions of 3 SNPs, c.1368 T > C, c.1380 T > C and c.1424G > A, located in the transcriptional regulatory region of the PRNP gene with susceptibility to pandemic 2009 swine flu (Table 4).

**Discussion**

Previous studies have reported that nonsynonymous genetic polymorphisms of the PRNP gene modulate structural alterations of PrP and are related to susceptibility to several types of prion diseases, including Creutzfeldt–Jakob disease (CJD) in humans and chronic wasting disease (CWD) in elk and deer. In humans, genetic variations of the human PRNP gene at codons 129 and 219 play a major role in susceptibility to CJD (Alperovitch et al. 1999; Jeong et al. 2005; Lee et al. 2001; Lloyd et al. 2011; Vollmert et al. 2006). In addition, nonsynonymous SNPs of the cervid PRNP gene at codons 95, 96, and 132 are also associated with vulnerability to CWD (Arifin et al. 2021; Johnson et al. 2006; Robinson et al. 2012). However, we did not find an association between functional genetic variations, including M129V and E219K, and vulnerability to pandemic 2009 swine flu (Table 3). In addition, previous studies have reported that genetic polymorphisms found in the promoter of the PRNP gene are involved in susceptibility to CJD and bovine spongiform encephalopathy (BSE). In humans, c.1368 T > C (rs1029273), located in the promoter region plays a pivotal role in susceptibility to sporadic CJD in British and German populations (Bratosiewicz-Wasik et al. 2012; Mastrianni 2010; Mead et al. 2001). In addition, 23- and 12-bp insertion/deletion polymorphisms located in the transcription regulatory region of the bovine PRNP gene are related to the expression level of the bovine PRNP gene and affect vulnerability to BSE (Haase et al. 2007; Murdoch and Murdoch 2015). Notably, we found no association between promoter polymorphisms of the PRNP gene and vulnerability to the pandemic 2009 swine flu in the present study (Table 4). It indicates that PRNP polymorphisms do not affect the infection mechanism of the pandemic 2009 swine flu. However, since this evaluation has been performed in relatively small cohorts, further confirmation in large cohorts would be highly advantageous in the future. In addition, Koreans have highly heterogeneous genetic background of the PRNP gene compared to other ethnic groups, and further investigation of the association analysis in other ethnic groups is needed in the future. In the present study, only one patient was admitted to the ICU (Table 1). Thus, we could not perform the association analysis between the disease severity and the PRNP polymorphisms. Since the PrP is related to lethality in
influenza A virus-infected mice, PRNP polymorphisms may be related to the disease severity. Thus, further association analysis stratified by severity, including the entrance of the intensive care unit and/or pneumonia, is needed to validate the association between entire PRNP polymorphisms and the clinical outcome of the pandemic 2009 swine flu-infected patients (Chida et al. 2018).

In a recent study, the MX1 protein, which showed potent antiviral activity, played a pivotal role in blocking H7N9 influenza viruses (Chen et al. 2021). Furthermore, genetic variations of the MX1 gene are associated with susceptibility to influenza viruses. Since H7N9 influenza viruses showed a similar pathomechanism to H1N1 influenza viruses, further investigation between MX1 polymorphisms and susceptibility to pandemic 2009 swine flu is highly desirable in the future.

Conclusions

In this study, we investigated genetic variations of the PRNP gene in healthy control subjects and the pandemic 2009 swine flu-infected patients using direct sequencing. We evaluated an association between PRNP polymorphisms and susceptibility to the pandemic 2009 swine flu infection. However, we did not find an association between these polymorphisms and susceptibility to pandemic 2009 swine flu.

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Author contributions YCK, SYW and BHJ conceived and designed the experiment. YCK and SYW performed the experiments. YCK, SYW and BHJ analyzed the data. YCK and BHJ wrote the paper. All authors read and approved the final manuscript.

Data availability statement All data are available from the corresponding authors upon reasonable request.

Declarations

Conflict of interest Yong-Chan Kim declares that he/she has no conflict of interest. Sae-Young Won declares that he/she has no conflict of interest. Byung-Hoon Jeong declares that he/she has no conflict of interest.

Ethical approval All samples were collected with informed consent under institutional review board-approved protocols. All experimental procedures were approved following the guidelines of the institutional review board (IRB) of Jeonbuk National University and the 1964 Hel-sinki Declaration and its later amendments (approval number: JBNU 2017-08-009). All the samples and information were anonymized prior to study.

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