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医薬品　研究報告　調査報告書

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報告番号・報告回数		報　告　日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	乾燥イオン交換樹脂処理人免疫グロブリン	研究報告の公表状況	鳥インフルエンザウイルスの血液感染の可能性について、Emerging Infections Diseases/WWW.cdc.gov/eid/Vol.12, No.6, June 2006 に掲載があった。	公　表　国	
販売名（企業名）	ガンマガード（バクスター株式会社）				

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点（血液感染の可能性に関連した鳥インフルエンザウイルスの伝播） 研究報告の題目：H5N1 インフルエンザＡウイルスと感染人血漿　タイ、2006 年 2005 年 12 月にタイにおいて鳥インフルエンザウイルス（H5N1）に感染し、死亡した症例の血漿から鳥インフルエンザウイルス（H5N1）が分離された。本ウイルスは遺伝子解析の結果、A/Thailand/NK165/05 accession no. DQ 372591-8 株として同定され、発育鶏卵培養により増殖能も確認された。 報告者は、患者の血漿から増殖能のあるウイルスを検出したことから、人の血液を介しての伝播についての可能性を提起し、H5N1 鳥インフルエンザに感染していると疑われる症例の血清あるいは血漿サンプルを処理して輸送する場合の取り扱いについて、注意すべきと報告している。 詳細は添付文献のとおり。	２．重要な基本的注意 (1) 本剤の原材料となる血漿については、FDA で認可された方法で HBs 抗原、抗 HCV 抗体、抗 HIV-1 及び HIV-2 抗体陰性であることを確認し、かつ ALT（GPT）値でスクリーニングを実施している。さらに、プールした試験血漿については、HBV-DNA、HCV-RNA、HIV-1-RNA、HIV-2-RNA 及び HAV-RNA について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。同様に、ヒトパルボウイルス B19-DNA についてはプールした試験血漿で核酸増幅検査（NAT）を実施し、10^5IU/mL 以下であることを確認した健康人血漿を用いている。本剤は、Cohn の低温エタノール分画法によって得られた免疫グロブリン画分を、TNBP／TritonX-100／Tween80 処理することによりエンベロープを有するウイルスを不活化し、さらにイオン交換樹脂処理により夾雑たん白やウイルスを排除する工程を施しているが、ウイルス等の感染

報告企業の意見	今後の対応
本剤の製造工程におけるウイルス不活化／除去工程において、当該ウイルスと同科ではないが、同じ RNA ウイルスであるフラビウイルス科の C 型肝炎ウイルス（HCV）の不活化・除去バリデーション試験に用いられるモデルウイルスであるウシ下痢性ウイルス（BVDV）は、不活化・除去されることが示されていることより、輸血に関連した血漿分画製剤による感染の可能性は極めて小さいと考える。	当該感染症に関し、引き続き、情報の収集を行っていく。 また、同様に同一生物種等から人に感染すると認められる疾病に関する情報の収集に努める

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		1 性を完全には否定できないので、投与に際しては、次の点に十分注意すること。 1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。 2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

感染症の用語は、MedDRA/J　version（9.0）を使用。

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H5N1 Influenza A Virus and Infected Human Plasma

To the Editor: Since January 2004, a total of 22 persons have been confirmed infected with avian influenza A virus (H5N1) in Thailand; 14 of these patients died. Three waves of outbreaks occurred during the past 2 years. The last patient of the third wave was a 5-year-old boy whose symptoms developed on November 28, 2005; he was hospitalized on December 5 and died 2 days later. The child resided in the Ongkharak District, Nakhon Nayok Province, 70 km northeast of Bangkok. Villagers informed the Department of Livestock after the patient's illness was diagnosed. Five dead chickens had been reported in this area from November 28 to December 1, 2005. Samples from these chickens could not be obtained, thus, no H5N1 testing was performed. The boy had fever, headache, and productive cough for 7 days before he was admitted to the Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center. Clinical examination and chest radiograph showed evidence of lobar pneumonia. He was treated with antimicrobial drugs (midecamycin and penicillin G) and supportive care, including oxygen therapy. On December 7, the patient's condition worsened, and severe pneumonia with adult respiratory distress syndrome developed. Laboratory tests showed leukopenia (2,300 cells/mm^3), acidosis, and low blood oxygen saturation by cutaneous pulse oximetry (81.6%). Oseltamivir was administered after his parents informed hospital staff about the boy's contact with the dead chicken. However, the boy died the same day; no autopsy was performed. On December 9, the cause of death was declared by the Ministry of Public Health to be H5N1 influenza virus.

A blood sample was collected from the patient on December 7; anticoagulation was accomplished with ethylenediaminetetraacetic acid (EDTA) for repeated biochemistry analysis and complete blood count. The plasma from the EDTA blood sample was separated 2 days later and stored at –20°C for 12 days. The sample was subsequently given to the Center of Excellence in Viral Hepatitis, Faculty of Medicine, Chulalongkorn University, for molecular diagnosis and then stored at –70°C, where specific precautions implemented for handling highly infectious disease specimens such as H5N1 influenza virus were observed. Plasma was examined by multiplex reverse transcription–polymerase chain reaction (RT-PCR) (*1*) and multiplex real-time RT-PCR (*2*), both of which showed positive results for H5N1 virus. The virus titer obtained from the plasma was 3.08×10^3 copies/mL. The plasma specimen was processed for virus isolation by embryonated egg injection, according to the standard protocol described by Harmon (*3*). Briefly, 100 µL 1:2 diluted plasma was injected into the allantoic cavity of a 9-day-old embryonated egg and incubated at 37°C. The infected embryo died within 48 hours, and the allantoic fluid was shown to contain 2,048 hemagglutinin (HA) units; also, subtype H5N1 was confirmed (*1,2*). Whole genome sequencing was performed and submitted to the GenBank database under the strain A/Thailand/NK165/05 accession no. DQ 372591-8. The phylogenetic trees of the HA and neuraminidase (NA) genes were constructed by using MEGA 3 (*4*) for comparison with H5N1 viruses isolated from humans, tigers, and chickens from previous outbreaks in 2004 and 2005 (Figure). The sequence analyses of the viruses showed that the HA cleavage site contained SPQRERRKKR, which differed from the 2004 H5N1 virus by an arginine-to-lysine substitution at posi-

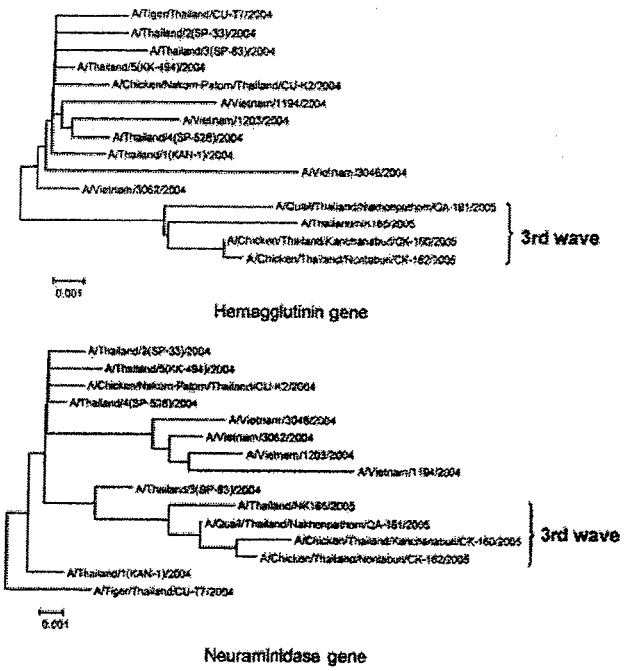

Figure. Phylogenetic analysis of the hemagglutinin and neuraminidase genes of H5N1 from study patient compared with sequences from previous outbreaks (2004–2005).

tion 341. That finding had also been observed in wild bird species during earlier outbreaks in Thailand in 2004 (*5*). Similar to the 2004–2005 H5N1 isolates from Thailand, a 20–amino acid deletion at the NA stalk region was observed. Moreover, the amino acid residues (E119, H274, R292, and N294) of the NA active site were conserved, which suggests that the virus was sensitive to oseltamivir. In addition, a single amino acid substitution from glutamic acid to lysine at position 627 of PB2 showed increased virus replication efficiency in mammals (*6*).

Observing live influenza virus in human serum or plasma is unusual. However, in 1963, low quantities of virus were isolated from blood of a patient on day 4 of illness (*7*), and in 1970, the virus was cultivated from blood specimens from 2 patients (*8*). Recently, a fatal case of avian influenza A (H5N1) in a Vietnamese child was reported. The diagnosis was determined by isolating the virus from cerebrospinal fluid, fecal, throat, and serum specimens (*9*); viral RNA was found in 6 of 7 serum specimens 4–9 days after the onset of illness (*10*). In this case, the H5N1 virus could be isolated from plasma on day 10 after symptoms developed. This case showed the virus in the patient's blood, which raises concern about transmission among humans. Because probable H5N1 avian influenza transmission among humans has been reported (*11*), this case should be a reminder of the necessity to carefully handle and transport serum or plasma samples suspected to be infected with H5N1 avian influenza. Because viable virus has been detected in blood samples, handling, transportation, and testing of blood samples should be performed in a biosafety (category III) containment laboratory to prevent the spread of the virus to healthcare and laboratory workers.

We express our thanks to the Thailand Research Fund (Senior Research Scholar), Royal Golden Jubilee PhD Program and Center of Excellence in Viral Hepatitis Research, and Prasert Auewarakul for their generous support of our study.

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識別番号・報告回数			報告日	第一報入手日 2006年4月21日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	BMJ Online doi:10.1136/bmj.38804.511644.55	公表国 イギリス	
販売名（企業名）	①テタノブリンーIH（ベネシス） ②テタノブリン（ベネシス）					

研究報告の概要

＜目的＞
プリオン陽性と判定された虫垂検体から抽出されたDNAのプリオン蛋白遺伝子コドン129の分析
＜調査対象の検体＞
英国のvCJD発生調査の一つとして回顧的にプリオンについて試験を実施した12,674の虫垂及び扁桃の検体から陽性と判定された3つの虫垂検体。これら検体が得られた患者の年齢は外科手術時点で20-29歳であり、これら手術が行われたのは1996-9年である。
＜結果＞
3検体の内2検体は十分な量のDNAが利用可能であり、両方共がプリオン蛋白遺伝子コドン129はバリンのホモ接合体であった。
＜結論＞
これが、プリオン蛋白におけるコドン129のバリンのホモ接合体がvCJDに対する感受性が強い証拠を初めて示したものである。試験が行われたvCJDの臨床例はこれまで全て、メチオニンのホモ接合体に起こっている。そして、医原性vCJDであることがほぼ間違いない単一例が一人の患者に見つかっているが、この患者はこの遺伝子座にメチオニン／バリンのヘテロ接合体を有していた。バリンのホモ接合体のコドン129プリオン蛋白遺伝子を有するvCJD感染者は長い潜伏期間を有している可能性があり、この間に水平感染が血液ドナー又は無症候期におけるこれらの感染者に使用された汚染手術用具のいずれかから起きる可能性がある。

報告企業の意見	今後の対応
プリオンについて試験を実施した12,674の虫垂及び扁桃の検体から陽性と判定された3つの虫垂検体のうち2つの虫垂検体のプリオン蛋白遺伝子コドン129がバリンのホモ体であったことから、バリンのホモ接合体のコドン129プリオン蛋白遺伝子を有するvCJD感染者は長い潜伏期間を有している可能性があり、この潜伏期間中に血液ドナー又は無症候期におけるこれらの感染者に使用された汚染手術用具のいずれかから水平感染が起きる可能性があることを示唆する報告である。 これまで血漿分画製剤によってvCJDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンーIHの記載を示す。

2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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BENE2006-012

Research

Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study

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Abstract

Objective To perform prion protein gene (*PRNP*) codon 129 analysis in DNA extracted from appendix tissue samples that had tested positive for disease associated prion protein.
Design Reanalysis of positive cases identified in a retrospective anonymised unlinked prevalence study of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom.
Study samples 3 positive appendix tissue samples out of 12 674 samples of appendix and tonsil tested for disease associated prion protein. The patients from whom these samples were obtained were aged 20-29 years at the time of surgery, which took place in 1996-9.
Setting Pathology departments in two tertiary centres in England and Scotland.
Results Adequate DNA was available for analysis in two of the three specimens, both of which were homozygous for valine at codon 129 in the *PRNP*.
Conclusions This is the first indication that the valine homozygous subgroup at codon 129 in the *PRNP* is susceptible to vCJD infection. All tested clinical cases of vCJD have so far occurred in the methionine homozygous subgroup, and a single case of probable iatrogenic vCJD infection has been identified in one patient who was a methionine/valine heterozygote at this genetic locus. People infected with vCJD with a valine homozygous codon 129 *PRNP* genotype may have a prolonged incubation period, during which horizontal spread of the infection could occur either from blood donations or from contaminated surgical instruments used on these individuals during the asymptomatic phase of the illness.

Introduction

In a prevalence study for variant Creutzfeldt-Jakob disease (vCJD), we identified three appendixes that stained positively for disease associated prion protein (PrP). We looked at 12 674 specimens (11 109 appendixes, 1565 tonsils) removed between 1995 and 2000. Most of the patients (83%) were aged 10-30 years at the time of operation.[1 2] This number of positive results is greater than would be predicted from the number of patients diagnosed with vCJD in United Kingdom (161 to date). Furthermore, the annual incidence of new cases of vCJD has declined from a peak in 1999. As all patients with vCJD belong to the methionine homozygous subgroup, determined by the codon 129 polymorphism in the prion protein gene (*PRNP*),[2] one possible explanation for this apparent discrepancy could be a different *PRNP* genotype in the three positive cases (the prevalences of *PRNP* codon 129 genotypes in the general UK population are about 40% methionine homozygous, 10% valine homozygous, and 50% heterozygous). This possibility was supported by a slightly different pattern of immunoreactivity in the second and third positive appendix cases in comparison with clinical cases of vCJD.[2] We recently identified a case of asymptomatic vCJD infection that seemed to have been transmitted by red cell transfusion in a *PRNP* codon 129 heterozygote, demonstrating that the methionine homozygous genotype is not uniquely susceptible to vCJD infection.[3]

Methods

We analysed the *PRNP* codon 129 polymorphism in the three samples of appendix tissue embedded in paraffin that stained positively for disease associated prion protein in the prevalence study. In the first case, a transmission study is currently under way using material from the remaining unstained sections. This meant that only immunostained sections were available for genotype studies and the extracted DNA was not good enough for further analysis. In the two remaining cases, as there was not sufficient material available for both transmission studies and genotype studies, and in view of possible *PRNP* influences on the staining pattern of disease associated prion protein in these cases, we used the remaining material for DNA analysis. A single 6 μm unstained paraffin section was available from each case, and these were de-paraffinised and scraped into individual microcentrifuge tubes for DNA extraction with the Puregene DNA Purification Kit (Gentra Systems, USA). Pelleted DNA was rehydrated for one hour at 65°C and then used as a template for amplification by the polymerase chain reaction (PCR), along with positive and negative control samples. PCR primers used were specific for a 506 bp region of *PRNP* containing the polymorphic sequence for the codon 129 residue. PCR products were digested at 37°C with the restriction enzyme Nsp1 (New England Biolabs, UK), which specifically recognises changes at the *PRNP* codon 129 polymorphic DNA sequence. Digest products were analysed on 1.5% agarose gels with positive controls for the codon 129 variants (MM, MV, VV).

Results

For both cases the genotype was confirmed as homozygous for the valine allele (VV) (figure). This method has been previously validated[4 5] and was controlled in our laboratory by studying the

PRNP codon 129 genotype in both paraffin embedded sections and frozen tissues from 25 other cases.

Discussion

These results give the first indication that *PRNP* codon 129 valine homozygotes may be susceptible to vCJD infection. Though the immunohistochemical technique used in our earlier study seems to be specific for disease associated prion protein,[6] it is unlikely to be 100% sensitive, suggesting that the true prevalence of vCJD infection in the UK population may be even higher than earlier estimated (3/12 674).[2] Genetic studies of kuru, another orally transmitted human prion disease, found that *PRNP* codon 129 MV and VV genotypes were associated with longer incubation periods than the MM genotype.[7] As the ethical approval for our study placed restraints on the identification of individual cases, we are not able to state with certainty the age of the patients in the positive cases at the time of surgery. We can, however, state that they were aged 20-29 years at the time of surgery, which took place in 1996-9. No clinical cases of vCJD at any age have yet been identified in *PRNP* codon 129 valine homozygotes, indicating the need for continued surveillance of all cases of vCJD in the UK.

Though it is inadvisable to overinterpret the data from only three positive cases in this study, it is perhaps surprising (given the relative prevalences of *PRNP* codon 129 genotypes in the general population) that both the positive cases analysed here were valine homozygotes. Though this may represent a chance finding, we should consider the possibility of differences in the peripheral pathogenesis of vCJD that depend on the *PRNP* codon 129 genotype. The patient who developed asymptomatic vCJD infection after red blood cell transfusion was a codon 129 heterozygote in whom both tonsil and appendix tissues were negative on staining for disease associated prion protein with methods identical to those used in this study, though the spleen and lymph nodes gave positive results.[5] *PRNP* polymorphisms in sheep infected with scrapie also have a major influence on the incubation period and timing and distribution of disease associated prion protein in lymphoid tissues during the incubation period.[8]

A prolonged incubation period after infection with vCJD is likely to result in an asymptomatic carrier state (which cannot yet be identified), which represents a potential risk for horizontal transmission of vCJD infection by blood transfusion, blood products, or contaminated surgical instruments. These uncertainties further underline the need for continued surveillance of vCJD in the UK (including surveillance for subclinical or asymptomatic infection[9]), a requirement to continue to reduce the possibility of secondary iatrogenic transmission, and the inclusion of carrier states and susceptibility to vCJD infection in all *PRNP* codon 129 genotypes in future disease modelling.

Contributors: JWI and DAH were responsible for the prevalence study and the analysis of the results, including the selection of the cases for analysis, and drafted and modified the manuscript. MTB established the methods for DNA extraction and analysis, designed and executed the validation study, and drafted and modified the manuscript. KC and DH performed the DNA extraction on the test materials and in the validation study, and modified the manuscript. MLeG, SL, DLR, and LMcC identified cases for the validation study and prepared the paraffin sections for DNA analysis and modified the manuscript. JWI is guarantor.

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Competing interest: None declared.

Ethical approval: The prevalence study received approval from the South and West multi-centre research ethics committee (MREC reference 99/6/32) and for each of the centres included, appropriate local research ethics committee approval.

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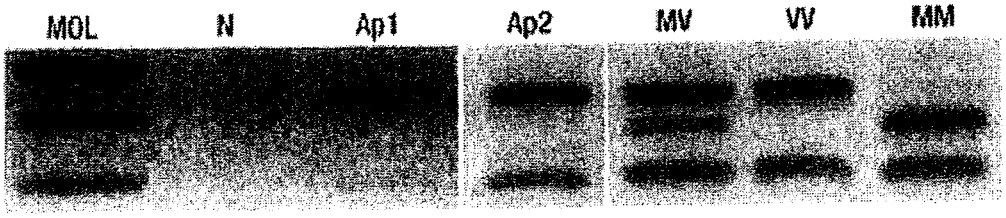

Restriction digest pattern for *PRNP* codon 129 genotype analysis in two paraffin section tissue samples (shown combined). The test sample results clearly show banding patterns equivalent to the VV genotype control (Mol=molecular weight ladder, N=PCR negative control, Ap1=appendix tissue from positive case 2, Ap2=appendix tissue from positive case 3, positive control samples from *PRNP* codon 129 MM, MV, and VV genotypes)

What is already known on this topic

A recent prevalence study of accumulation of prion protein (as a marker for variant Creutzfeldt-Jakob disease) in appendix and tonsil specimens in the UK found three cases in12 674 samples, which is more than expected from the current number of clinical cases of vCJD

What this study adds

Analysis of DNA from two of the three positive samples found they were valine homozygotes at codon 129 in the prion protein gene, indicating that this genetic subgroup (which is a different subgroup to that in which all cases of vCJD so far have occurred) is susceptible to vCJD infection

Individuals with this genotype may have a prolonged incubation period with subclinical infection and could cause secondary spread of vCJD by blood transfusion or surgery