資料２－２

Ａ　研究報告（詳細版）

平成２８年３月９日

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医薬品 研究報告 調査報告書

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一般的名称	解凍人赤血球液		研究報告の公表状況	Perumpail RB, Ahmed A, Higgins JP, So SK, Cochran JL, Drobeniuc J, Mixson-Hayden TR, Teo CG. Emerg Infect Dis. 2015 Sep;21(9):1679-81.	公表国 米国	
販売名（企業名）	解凍赤血球液-LR「日赤」（日本赤十字社） 照射解凍赤血球液-LR「日赤」（日本赤十字社）					

研究報告の概要

○HEV Genotype 4に感染後、急速に進行した致死的肝硬変
患者は1985年からカリフォルニア州に居住する68歳の中国系男性、16年前にB型肝炎による肝硬変のため肝移植を受け、それ以降は抗ウイルス薬と免疫抑制剤を続けたが、定期的な肝機能検査に異常はなかった。2013年4月に香港で軽度の黄疸の治療を受け、米国帰国時（黄疸発症3週間後）の検査では、HBV DNAは検出されなかった。3ヶ月後の生検ではグレード3の肝炎が確認され、血液検体はHEV RNA陽性となった。治療の4ヶ月後に腹水が認められ、血液検体のHEV RNAは陰性となったが、HEV IgM抗体およびIgG抗体が検出された。発症の8ヶ月後に顕著な肝不全となり肝移植を受けたが、手術中に死亡した。この際の肝生検により、ステージIVの線維化を伴う小葉の激しい炎症が確認された。
直近の香港渡航期間とHEVの潜伏期間が重なることから、患者は香港訪問中にHEV Genotype 4に感染した可能性が最も高い。中国国内では過去10年間にわたってHEV感染の報告数は上昇しており、2013年の報告症例数は28,232例であった。同様の傾向にある香港では、ほぼ全てのHEV感染例においてHEV Genotype 4との関連が認められている。患者が感染したHEVのサブゲノム配列と、中国本土および香港から報告されたヒト/ブタHEV Genotype 4の配列との間には密接な関連が認められた。
HEV Genotype 3に感染した固形臓器移植のレシピエントにおける、肝硬変へ急速進行する慢性肝炎は過去に報告されているが、HEV Genotype 4については報告例がない。患者の肝生検により、肝炎の持続と増悪、並びに線維化の発症と急激な悪化が確認された。E型肝炎を早期診断して、抗ウイルス剤の迅速な投与、並びに適切な免疫抑制剤投与計画につなげていく必要があると考える。

使用上の注意記載状況・その他参考事項等

解凍赤血球液-LR「日赤」
照射解凍赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
免疫抑制剤を投与されていた患者が、HEV Genotype 4の流行国にて当該ウイルスに感染した後に急速に肝硬変が進行して死亡したという報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎（A型及びE型）の感染防止、病態解明、治療等に関する研究」の一環として、重症化が懸念されるHEV Genotype 4の輸血感染報告があった北海道赤十字血液センターで輸血用血液について試行的個別NATを実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

of healing of suppurative nodes and is the only evidence-based effective treatment (*9*). Surgical excision remains controversial because of potentially high rates of significant scarring (*10*). For nonsuppurative lymphadenitis, a watch-and-wait approach is recommended because most resolve rapidly (*8*).

Given our findings, the National TB Program in Georgia subsequently created a management protocol. This protocol recommends no intervention for nonsuppurative lymphadenitis and needle aspiration for suppurative local lymphadenitis.

In summary, we found an increasing rate of BCG-associated lymphadenitis after a shift to exclusive BCG SSI vaccine use in Georgia. Countries with a BCG vaccination policy should have a clear protocol on management of BCG vaccine–related adverse events to avoid inappropriate treatment in children.

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Fatal Accelerated Cirrhosis after Imported HEV Genotype 4 Infection

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To the Editor: Hepatitis E is a viral hepatitide that is endemic in many developing countries. In its classic form, it results from ingesting fecally contaminated water that carries hepatitis E virus (HEV), and it frequently resolves without treatment. When hepatitis E is imported to the United States, it originates mainly from persons who have acquired HEV genotype 1 infection from South Asia (*1*). We report imported HEV genotype 4 infection (Technical Appendix Figure, panel A) in a patient during which cirrhosis and fatal hepatic decompensation ensued.

The patient was a 68-year-old man of Chinese ethnicity who had been a California resident since 1985. He sought treatment for mild jaundice in April 2013 in Hong Kong, where he had been staying for 7 weeks. Sixteen years before, he had undergone orthotopic liver transplantation at Stanford University Medical Center (Palo Alto, California, USA) for hepatitis B cirrhosis. Since then, he had received entecavir and tacrolimus for maintenance and had been vaccinated against hepatitis A virus. Until his current illness, routine liver function tests had not indicated hepatic dysfunction (values in November 2012: alanine aminotransferase 2 IU/L, aspartate aminotransferase 24 IU/L, alkaline phosphatase 67 IU/L, total bilirubin 0.5 mg/dL).

When the patient returned to the United States, 3 weeks after onset of jaundice, the initial work-up showed the following values: alanine aminotransferase 149 IU/L, aspartate aminotransferase 59 IU/L, alkaline phosphatase 193 IU/L, total bilirubin 2.8 mg/dL (online Technical Appendix Figure, panel B, http://wwwnc.cdc.gov/EID/article/21/9/15-0300-Techapp1.pdf). Hepatitis B virus DNA and antinuclear antibodies were not detected, and the tacrolimus level was stable. Ultrasound revealed a normal transplanted liver. A liver biopsy specimen showed mild portal, biliary, and lobular inflammation and early biliary injury (Figure,

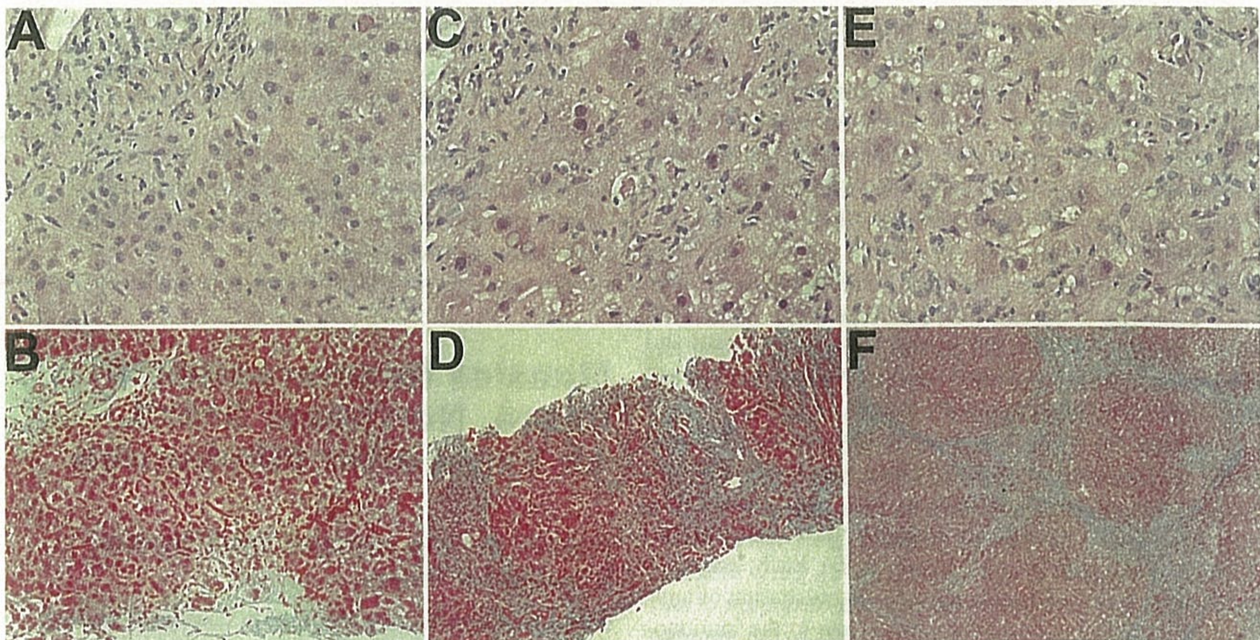

Figure. Serial histologic changes in liver of the patient who received a diagnosis of hepatitis E after a visit to Hong Kong in 2013 (A and B: at first biopsy; C and D: second biopsy; E and F: third biopsy. A) Mild mixed portal infiltration; minimal lobular inflammation; acidophil body present at upper right; and bile duct showing injury with lymphocytic infiltration (original magnification ×400). b) Mild portal inflammation; some interface activity; and portal tracts not showing increased fibrosity (original magnification ×200). C) Mononuclear infiltration of portal tract at upper right with bile duct/ductular infiltration and injury; lobular changes more severe, showing more inflammation, acidophil bodies and reactive nuclear change in hepatocytes with ballooning of some hepatocytes (original magnification ×400). d) Portal and lobular inflammation; and marked increase in fibrosis with bridging and regenerative nodule formation (original magnification ×100). E) Extensive lobular inflammation and reactive hepatocytic changes with nuclear enlargement, prominent nucleoli, and ballooning (original magnification ×400). F) Well-developed cirrhosis (original magnification ×40). Hematoxylin and eosin staining (A, C, E); Masson trichrome staining. (B, D, F).

panels A, B; a color version of this figure is available online [http://wwwnc.cdc.gov/EID/article/21/9/15-0300-F.htm]). The prednisone dosage was escalated, and mycophenolate mofetil was added. Liver enzyme activity showed some improvement, but the bilirubin level continued to rise (online Technical Appendix Figure, panel B).

A biopsy specimen taken 3 months later showed grade 3 hepatitis with bile ductular reaction, bridging hepatocytic necrosis and fibrosis, and regenerative nodule formation (Figure, panels C, D). A blood sample taken about this time tested positive for HEV RNA. The patient was then given ribavirin (1,000 mg/d). Before hepatitis E was diagnosed, tacrolimus was given (1 mg 2×/d); when the diagnosis was confirmed, the tacrolimus dose was reduced to 0.5 mg every other day. Four months after the patient sought treatment, ascites was noted. Ribavirin was stopped because of pancytopenia. Blood samples subsequently tested negative for HEV RNA, but HEV IgM and IgG were found. Hepatic function did not improve.

Eight months after onset of the patient's condition, marked hepatic decompensation occurred (online Technical Appendix Figure), culminating in esophageal variceal hemorrhage. The patient was placed on a waiting list and then underwent liver transplantation, but he died during the operation from complications of hemorrhage. Biopsy of the liver explant revealed intense lobular inflammation with the hepatocellular reactive changes persisting and stage IV fibrosis (Figure, panels E, F).

The patient had lived and worked in Hong Kong before he became a resident of the United States. He had not visited Hong Kong in the 3 years preceding his most recent trip, nor had he traveled to Europe. Review of his medical records revealed no evidence of hepatic dysfunction after his previous travels. Considering that his most recent visit to Hong Kong coincided with the incubation period of hepatitis E (*2*), he most likely acquired HEV genotype 4 infection during that visit.

In China over the past decade, national notifications of HEV infection have risen, with 28,232 cases reported in 2013 (*3*). In Hong Kong, where a rising trend in hepatitis E notifications also has been observed (150 cases reported in 2012 [*4*]), HEV infections are almost all associated with HEV genotype 4 (*5*).

This patient's HEV subgenomic sequence was closely related to human and porcine HEV genotype 4 sequences

reported from mainland China and Hong Kong (online Technical Appendix Figure, panel A). Porcine liver has been implicated as a possible HEV transmission vehicle in that region (*6*); although we do not know whether the patient ate food that carried HEV, the possibility underscores the importance of avoiding eating inadequately cooked animal-derived food products during international travel (*2*).

Chronic hepatitis with accelerated cirrhosis has been reported in solid-organ transplant recipients infected with HEV genotype 3, but not with genotype 4 (*7*). Serial liver biopsy specimens from the patient showed persistent and worsening hepatitis and rapid onset of fibrosis that intensified (online Technical Appendix Figure, panel B).

Testing for HEV infection is recommended during initial assessments of posttransplant hepatic dysfunction because histologic appearances in liver biopsy specimens may not clearly distinguish between graft rejection and acute viral hepatitis (Figure, panels A, B). Early diagnosis of hepatitis E should lead to prompt administration of antiviral therapy and appropriate adjustments to the immunosuppressant drug regimen, particularly because some drugs can exert opposing effects on HEV replication (*8*).

Acknowledgments

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Measles Reemergence in Ceará, Northeast Brazil, 15 Years after Elimination

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To the Editor: Measles was endemic in Brazil before 2000 and caused large outbreaks every 2 or 3 years (*1*). Although measles was eliminated in Brazil in 2000, cases have continued to be imported (*2*,*3*). During 2001–2014, the median annual number of measles cases reported in Brazil was 50 (range 2–712). The median annual number of Brazilian states with reported cases was 2.5 (range 1–7). Since elimination, the highest numbers of cases reported in Brazil occurred in 2013 (220) and in 2014 (712) (*3*–*5*). According to the Pan American Health Organization, endemic transmission is reestablished when epidemiologic and laboratory evidence indicate that a chain of transmission of a virus strain has continued uninterrupted for ≥12 months in a defined geographic area (*6*).

From December 2, 2013, through December 31, 2014, in the state of Ceará, Brazil, 681 measles cases were reported. A measles case was considered confirmed when a patient exhibited fever, rash, and ≥1 of 3 symptoms and signs (i.e., cough, runny nose, conjunctivitis); was positive for IgM and negative for IgG against measles virus; and had not been vaccinated in the previous 21 days. D8 genotype, the same virus genotype that was circulating in Europe, was the only genotype identified, and how the virus was introduced into the region was not clear (*4*,*5*). From 2000 to 2013, vaccine coverage among children 12 months of age remained >95% in Ceará, although that coverage was not homogeneous for the whole state. In 14.7% (27/184) of municipalities, the vaccination coverage was much lower

医薬品　研究報告　調査報告書

識別番号・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
			2015年11月30日	該当なし。	
一般的名称	別紙のとおり。	研究報告の公表状況	ProMED-mail 20151126.3819995	公表国 ブラジル	
販売名（企業名）	別紙のとおり。				

研究報告の概要

問題点：ブラジルにおいて、妊婦のジカウイルス罹患が起因と推測される新生児の小頭症が多く報告されている。

2015年11月21日までに、ブラジルの9州の160の市町村において739症例の小頭症症例が報告された。報告例数は、Pernambuco州487例、Paraiba州96例、Sergipe州54例、Rio Grande do Norte州47例、Piaui州27例、Alagoas州10例、Ceara州9例、Bahia州8例、Goias州1例であった。Rio Grande do Norte州において、死亡症例も報告されているが、死因は調査中である。

9州で報告された小頭症の増加の原因はまだ確認できていないが、11月17日にOswaldo Cruz研究所において超音波検査で胎児が小頭症と確認された二人の妊婦の羊水においてジカウイルスのRNAの存在をリアルタイムRT-PCRで確認した。

ヒトにおけるジカウイルス感染に関する重要な科学的発見ではあるが、現在のデータではヒトでのジカウイルス感染を小頭症の原因と関連付けることはできない。

使用上の注意記載状況・その他参考事項等

記載なし。

報告企業の意見	今後の対応
別紙のとおり。	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

MedDRA/J ver.18.1

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子*、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第Ⅹ因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロン－Ｉ静注用 500mg、⑧献血ベニロン－Ｉ静注用 1000mg、⑨献血ベニロン－Ｉ静注用 2500mg、⑩献血ベニロン－Ｉ静注用 5000mg、⑪ベニロン*、⑫注射用アナクトＣ2,500 単位、⑬コンファクトＦ注射用 250、⑭コンファクトＦ注射用 500、⑮コンファクトＦ注射用 1000、⑯ノバクトＭ静注用 400 単位、⑰ノバクトＭ静注用 800 単位、⑱ノバクトＭ静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン "化血研"、㉔献血トロンビン経口・外用５千「化血研」、㉕献血トロンビン経口・外用１万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンＰ500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20％化血研*、㉛アルブミン 5％化血研*、㉜静注グロブリン*、㉝アンスロビンＰ1500 注射用、㉞バイクロット配合静注用、㉕ノバクトＭ静注用 500 単位、㉖ノバクトＭ静注用 1000 単位、㉗ノバクトＭ静注用 2000 単位
報告企業の意見	ジカウイルスは、日本脳炎ウイルス、デングウイルス、ウエストナイルウイルスなどと同属のフラビウイルス属の蚊媒介性感染症ウイルスとして知られており、一本鎖 RNA 及びエンベロープを有するウイルスである。今回の報告は、ジカウイルスに罹患した妊婦から胎児への感染を示唆するものであり、蚊による感染経路以外の新たな感染経路が示唆される。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年８月30日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したジカウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程の BVDV クリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるジカウイルスへの感染報告例は無い。 以上の点から、上記製剤はジカウイルスに対する安全性を確保していると考える。

*：現在製造を行っていない

INF2015-005

Published Date: 2015-11-26 11:48:16
Subject: PRO/EDR> Zika virus - Brazil (18): microcephaly, RFI
Archive Number: 20151126.3819995

ZIKA VIRUS - BRAZIL (18): MICROCEPHALY, REQUEST FOR INFORMATION
****
A ProMED-mail post
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http://www.isid.org

Date: Tue 24 Nov 2015
Source: Secretariat of Health Surveillance, Ministry of Health of Brazil [in Portuguese, trans. Mod.TY, summ. & edited]
http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/noticias-svs/20929-ministerio-divulga-boletim-epidemiologico-sobre-microcefalia

Up to 21 Nov 2015, 739 suspected cases of microcephaly were reported, identified in 160 municipalities in 9 states of Brazil, according to the 2nd edition of the epidemiological report on microcephaly, released on [Tue 24 Nov 2015]. The government continues to make every effort to monitor and investigate the increasing number of cases of microcephaly in the country as a priority.

Pernambuco state remains with the highest number of cases (487); it was the first to identify an increase of microcephaly in its area and has had the collaboration of a Ministry of Health team since [22 Oct 2015]. Next are the states of Paraiba (96), Sergipe (54), Rio Grande do Norte (47), Piaui (27), Alagoas (10), Ceara (9), Bahia (8) and Goias (1). Among the total cases, a suspicious death was reported in the state of Rio Grande do North. This case is under investigation to determine the cause of death.

Case investigations are being done by the Ministry of Health, integrated with the state and municipal secretariats, and with the help of national and international institutions. Committees of specialists are supported by the Ministry of Health in epidemiological and laboratory analysis, as well as case monitoring.

Since the cases appeared, the Ministry of Health sent to the state secretariats orientation for the process of notification, surveillance, and assistance for pregnant women and their babies affected with microcephaly. This information is constantly updated.

It is not yet possible to ascertain the cause of the increase in cases of microcephaly that has been recorded in 9 states. All hypotheses are being thoroughly analyzed by the Ministry of Health and any conclusion at this point is premature. Analyses were not completed and, therefore, are still ongoing.

The Flavivirus Laboratory of the Instituto Oswaldo Cruz (Fiocruz) in Rio de Janeiro participates in the investigations and concluded on [17 Nov 2015] a diagnostic test established the presence of the Zika virus genome in samples from 2 pregnant women from Paraiba, whose fetuses had microcephaly confirmed by ultrasound examination. The genetic material (RNA) of the virus was detected in samples from amniotic fluid, using real-time RT-PCR.

Despite being an important scientific finding for understanding of Zika virus infection in humans, the current data do not allow one to correlate unequivocally, infection by Zika virus as a causal factor of microcephaly. Such an explanation will be made by studies coordinated by the Ministry and other institutions involved in research into the causes of microcephaly in the country.

For managers and health professionals, the Ministry of Health directs that all cases of microcephaly be communicated immediately, electronically. Also, that prevention efforts be strengthened and that vector control in urban and peri-urban areas, conform to the Guidelines of the National Dengue Control Program.

It is important that pregnant women maintain monitoring and prenatal medical consultations, with the completion of all tests recommended by their doctors. The Ministry of Health reinforces the guidance to not consume alcohol or any other type of drugs, not use drugs without medical prescription, and avoid contact with people with fever or infections.

It is important, also, that pregnant women adopt measures to reduce the presence of mosquitoes that transmit pathogens, by the elimination of breeding sites and to protect themselves from exposure to mosquitoes, such as keeping doors and windows closed or screened, wear long pants and long-sleeved shirts, and use [mosquito] repellants that are approved for use by pregnant women.

Microcephaly is not a new problem. It is a congenital malformation in which the brain does not develop properly. In the current situation, the investigation of the cause has the health authorities worried. In this case, babies are born with head circumference (PC) lower than normal, which is usually greater than 33 cm [13 in]. This defect can

be a congenital effect of a number of factors from different sources, such as chemicals, biological (infectious) agents such as bacteria, viruses, and radiation.

Cases of microcephaly under investigation [state / number of cases]:
Pernambuco / 487
Paraiba / 96
Sergipe / 54
Rio Grande do Norte / 47
Piaui / 27
Alagoas / 10
Ceara / 9
Bahia / 8
Goias / 1

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Communicated by:
ProMED-PORT
<promed-port@promedmail.org>

[Concerning the data, now updated, of microcephaly in Brazil: indeed, there was a significant increase in the number of cases -- 399 cases up to 17 Nov 2015 to 739 on 24 Nov 2015. There is still little information available or reported on the results of clinical, epidemiological, and laboratory investigations conducted to date. Reportedly, albeit in general terms, the most common causes of congenital infections are toxoplasmosis, cytomegalovirus, as well as environmental causes, etc., that apparently already have been (or are being) investigated. Early on, there are no findings or conclusive results.

The identification of the Zika virus genome in the amniotic fluid of 2 pregnant women who showed clinical signs compatible with those expected/described for symptomatic infection with Zika virus, and whose fetuses showed signs of microcephaly in prenatal exams, points to the ability of the virus to cross the placental barrier. That is, one of the conditions for fetal infection to occur has been demonstrated. However, although it is a quite significant finding, it is subject to corroboration for the possible elucidation of the (or a few) cases of microcephaly in the northeast region; the results are still not definitive, that is, a causal relationship has not yet been (and can not) be definitively proven.

What now? There is a temporal association between increased incidence of microcephaly and the length and geographic distribution of transmission (and the exposure of pregnant women) to Zika [virus] in the northeast states. A large proportion of mothers whose newborns now have microcephaly at birth, mentioned a clinical presentation during pregnancy that would be compatible with infection (symptomatic) caused by Zika [virus]. Identification of the genome of Zika [virus in] amniotic fluid indicates that the virus is capable of crossing the placental barrier. In short, evidence adds up to, but does not establish for now, a causal relationship.

The surveillance system is on alert. Clinical and epidemiological investigations must (or should) be conducted. The research protocol proposed by the Ministry of Health (see http://portalsaude.saude.gov.br/images/pdf/2015/novembro/18/microcefalia-nota-informativa-17nov2015-c.pdf; in Portuguese) contribute to standardization and uniformity of definition of criteria and prospective investigation of upcoming cases. But until final and conclusive results are obtained, all caution should be taken in order to prevent alarmism, panic, or extreme measures.

ProMED-PORT takes the opportunity to thank Dr Ricardo Albernaz <ricmanga@gmail.com> for his submitted comments that are in line with comments previously stated in the current and previous post, and that point to "the self-evident severity of the event," to the fact that the data available [still] do not prove a causal relationship between microcephaly and Zika [virus infections], to the lack of "detailed information on the cases, and to the need for appropriate strategies for research and health risk communication from the public agencies. - Mod.RNA

Establishing a causal relationship between Zika virus infections during gestation and microcephaly will not be easy. If there is retrospective study to determine what proportion of the mothers of microcephalic infants have antibodies to Zika virus, ProMED-mail would be interested in the results.

Prospective studies, as mentioned above, have a greater possibility of establishing the Zika virus-microcephaly link. If Zika virus continues to spread in in Brazil, there may be a sufficiently large pool of cases for a significant study. Now that there is a Zika virus outbreak going on in Colombia, it would be prudent for physicians there to watch for an increase in microcephaly cases in infected pregnant women. Ideally, there should be close communication and collaboration between the public health authorities in both countries to employ standardized approaches to obtain comparable results.

A HealthMap/ProMED-mail map of Brazil can be accessed at http://healthmap.org/promed/p/6. - Mod.TY]

See Also

Zika virus - Brazil (17): microcephaly 20151121.3808514
Zika virus - Brazil (16): (PE) microcephaly cause undetermined 20151118.3799192
Zika virus - Brazil (15): (AL) RFI 20151117.3799132
Zika virus - Brazil (14): (BA) microcephaly susp, RFI 20151106.3767857
Zika virus - Brazil (13): (MT, AL) 20151014.3714950
Zika virus - Brazil (12): (MT) 20150921.3660532
Zika virus - Brazil (11): (AL) 20150903.3621836
Zika virus - Brazil (10): (PR) 20150830.3611318
Zika virus - Brazil (09): conf., Guatemala susp. 20150723.3531482

Zika virus - Brazil (08): 20150716.3513770
Zika virus - Brazil (07): 20150630.3473420
Zika virus - Brazil (06): (BA) 20150619.3449500
Zika virus - Brazil (05) 20150612.3431148
Zika virus - Americas: PAHO alert, country alerts, Brazil update 20150609.3422423
Zika virus - Brazil (04): (RJ): 20150608.3420363
Zika virus - Americas: PAHO alert, country alerts, Brazil update: 20150609.3422423
Zika virus - Brazil (03): (RR) 20150604.3408349
Zika virus - Brazil (02): (SP) 20150524.3382529
Zika virus - Brazil: confirmed 20150519.3370768
Undiagnosed illness - Brazil (02): Zika virus conf 20150515.3364149
Undiagnosed illness - Brazil: (Northeast, RJ) Zika virus susp, RFI 20150501.3334749
....jw/ty/mj/lm

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医薬品　研究報告　調査報告書

識別番号・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	－		研究報告の公表状況	World J Virol 2015 May 12; 4(2): 113-123 ISSN 2220-3249 (online)	公表国：仏国	
販売名(企業名)	－					

研究報告の概要

デング熱は、4つの血清型を持ちエンベロープを有するRNAウイルスであるデングウイルス(DENV)によって発症する疾患であり、主にヤブカ属（ネッタイシマカとA.ヒトスジシマカ）に咬刺されることにより感染する。毎年5000万人～1億人が感染しており、死亡例はおおよそ2万人にのぼる。無症候又は限定的な軽い発熱をみるのが一般的だが、特に貧困層の小児においてショックや死亡につながる出血性の合併症に連続する重篤な症状の原因となる。現在までに5例(3報告)の輸血によるデング感染例が報告されている。

第1報告は2002年の香港で重症貧血に対し輸血が実施された76歳、女性において報告され、2日後に軽度の発熱を認めたが、自然に解熱した。供血者が血清学的に特徴的なデング感染を示したことから、二次感染例とされた。血液製剤に対する分子テストにおいて、DEN-1が陽性であった。輸血から2ヵ月後には受血者にIgMが検出された。

第2報告は2008年のシンガポールにおける3例の報告である。供血後に発熱と筋肉痛を認めた供血者由来の血液製剤を投与された3例の受血者のうち、2例において感染症状を認めたが自然に軽快した。3例において急性デング感染の血清学的証拠が示された。また、PCRにて供血者及び症状を認めた2例の受血者の血液サンプルからDEN-2が検出された。

第3報告は2007年のプエルトリコにおけるデング熱のアウトブレイク時の報告である(公表は2012年)。15,350例の供血者サンプルを後方視的に検査したところ、29例においてDENVが陽性であった。それらの供血者から供血を受けた受血者のうち3例においてNATが実施され、1例が陽性を示した。受血者は輸血から3日後にデング出血熱を発症した。供血者及び受血者の両方から同じエンベロープ配列を有するウイルスが認められた。本症例が血液製剤による重症デング感染を認めた初めてのケースであった。

2009年、米国血液バンク協会は、北米において将来の輸血受血者の安全性に潜在的インパクトを与える新たな感染性因子における最上位にDENVを据えた。これはバベシア(寄生虫症)やvCJDと同レベルである。効果的な予防的ワクチンの開発が待たれるが、血液製剤の安全性を高めるための効率的な措置の発展が重要である。

使用上の注意記載状況・その他参考事項等

重要な基本的注意

［患者への説明］

本剤の投与又は処方にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

報告企業の意見	今後の対応
デングウイルスの輸血用血液製剤による感染報告である。 現時点までに血漿分画製剤から伝播が疑われた報告は入手していない。	今後とも デングウイルスの血液による感染報告等に関する情報に留意していく。

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MINIREVIEWS

Is transfusion-transmitted dengue fever a potential public health threat?

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Author contributions: Pozzetto B conceived the review and wrote the paper; Memmi M updated the bibliography, took care of the figures, contributed to the redaction of the manuscript and approved it; Garraud O improved substantially the content of the manuscript and approved it.

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Abstract

Dengue is an arboviruses due to single-stranded enveloped ribonucleic acid viruses, named dengue viruses (DENV), that include four serotypes and are mainly transmitted *via* the bite of mosquitoes of the genus *Aedes* (*A. aegypti* and *A. albopictus*). The distribution of the disease was historically limited to intertropical areas; however, during the last thirty years, the perimeter of the disease extended considerably and temperate areas are now at risk of outbreaks. The present global burden of dengue is considerable: 2.5 billion people over more than 100 countries are concerned; 50 to 100 million infections occur every year, with a number of fatal cases of approximately 20000. Although frequently asymptomatic or limited to a mild fever, dengue is responsible for severe cases mainly consecutive to the occurrence of hemorrhagic complications that can lead to shock and death, notably in children from poor-resource settings. The place of DENV as a transfusion-transmitted pathogen has been recognized only in 2008. At the present time, only five cases of transfusion-transmitted dengue, including one case of dengue hemorrhagic fever, have been formerly documented. This review provides a general overview of dengue, its viruses and their vectors. It replaces the disease in the context of other viral diseases transmitted by arthropods. It discusses the threat of dengue on the supply of blood products in endemic and non endemic areas. Finally, it describes the specific and non specific measures available for improving the security of blood products with regards to this emerging risk. Interestingly, in 2009, the American Association of Blood Banks placed DENV in the highest category of emerging infectious agents for their potential impact on transfusion recipient safety for the next years in North America.

Key words: Dengue; Dengue viruses; *A. aegypti*; *A. albopictus*; Transfusion-transmitted virus; Blood safety

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Core tip: The place of dengue viruses as transfusion-transmitted pathogens has been recognized only in 2008. By now, only five cases of transfusion-transmitted dengue, including one case of dengue haemorrhagic fever, have been formerly documented. This review provides a general overview of dengue, its viruses and their vectors. It replaces the disease in the context of other viral diseases transmitted by arthropods. It discusses the threat of dengue on the supply of blood products in endemic and non-endemic areas. Finally, it describes the specific and non-specific measures available for improving the security of blood products concerning this emerging risk.

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INTRODUCTION

Dengue is an arboviruses mainly transmitted by mosquito bite that constitutes a major public health concern: two-fifths of the world's population, mainly located in the intertropical regions, are exposed to the risk of infection. According to the World Health Organization (WHO), an estimated 500000 people with severe dengue require hospitalization each year, a large proportion of whom are children; about 2.5% of those affected die[1]. Despite the large distribution of this "old" infection and the fact that the virus can be present for about one week in the blood of infected patients, the risk of dengue as a transfusion-transmitted disease emerged very recently (first publications in 2008). An attempt to explain this paradox is proposed later in this review. After a few recalls concerning dengue, its viruses and their vectors, the disease is replaced in the larger context of arboviruses associated to a demonstrated or possible risk of transmission *via* blood products. The third part of the manuscript intends to answer the question formulated in the title of the paper: "Is transfusion-transmitted dengue fever a potential public health threat?" The last part of the study describes the measures available for reducing this risk.

RECALLS ON DENGUE, ITS VIRUSES AND THEIR VECTORS

Dengue viruses

Dengue viruses (DENV) are single-stranded ribonucleic acid (RNA) viruses, 40 to 60 nm in size, belonging to the *Flaviviridae* family (Table 1) and exhibiting an icosahedral capsid and a lipid envelope. The viral genome codes for ten viral proteins: three structural (core, membrane-associated and envelope) and seven non structural ones. The envelope protein is responsible for the specific recognition of host cells and for the development of protective neutralizing antibodies. Non structural proteins have been associated with the pathogenesis of severe forms of the disease. Dengue viruses include four serotypes entitled DEN-1, DEN-2, DEN-3 and DEN-4. The infection by one serotype confers a strong protection against the corresponding serotype but only a partial immunity against the three other ones, which explains that an individual can be infected several times during life by DENV. It is worthwhile to note that a fifth dengue serotype has been identified on virus samples that were collected during an outbreak in Malaysia in 2007[2]. More data are awaited about the epidemiological significance of this observation.

Vectors of DENV

The main vectors of DENV are mosquitoes of the *Aedes* genus (also called *Stegomya*).

The most common vector of dengue viruses is *Aedes aegypti* whose distribution is very large in intertropical regions of the world (Figure 1). In the Americas, discontinuation of *Aedes aegypti* control efforts in the mid-20th century has led to a resurgence of dengue throughout South and Central America, resulting in hundreds of thousands of dengue cases in these areas. In October 2012, an outbreak of DEN-1 infection was documented for the first time in the Portuguese island of Madeira[3]; the viral strain was shown to be very close to a virus strain originated from Venezuela[4].

Aedes albopictus (the tiger mosquito) is also involved in dengue outbreaks or isolated cases, notably in temperate regions as Europe where the mosquito is able to survive in cooler environment and expended very quickly (Figure 1) from Asia following the international trade in used tyres and other goods such as lucky bamboo. In 2010, an autochthonous outbreak of dengue was documented in Croatia[5] and two sporadic cases were identified in Nice city in the South-East of France[6].

A third species, *Aedes polynesiensis*, has been involved in rare cases. *Aedes* mosquitoes are highly domesticated mosquitoes that are able to grow in urban environment, notably in human-made containers filed with stagnant water (*e.g.*, water storage tanks, subterranean pits, flowerpot trays). Interestingly, when both viruses are present in the same area, *Aedes albopictus* is able to displace *Aedes aegypti* from competing environment, which would facilitate the dissemination of DENV into temperate regions that are refractory to colonization by *Aedes aegypti*[7].

Routes of transmission of DENV

Dengue is mainly a mosquito-borne infectious disease. Besides the sylvatic reservoir that involves not human primates with occasional contamination of humans, the human cases are mostly related to the urban or

Table 1 Main arboviruses exhibiting a potential or demonstrated transfusion-associated risk

	Dengue virus	West Nile virus	Saint-Louis encephalitis virus	Tick-borne encephalitis virus	Chikungunya virus	Colorado tick fever virus
Family	*Flaviviridae*	*Flaviviridae*	*Flaviviridae*	*Flaviviridae*	*Togaviridae*	*Reoviridae*
Virus characteristics						
Nucleic acid	ssRNA	ssRNA	ssRNA	ssRNA	ssRNA	dsRNA
Envelope	Yes	Yes	Yes	Yes	Yes	No
Vectors	Mosquitoes (*Aedes aegypti* and *Aedes albopictus*)	Mosquitoes (genus *Culex* but also Aedes albopictus)	Mosquitoes (genus *Culex*)	Ticks (genus *Ixodes*)	Mosquitoes (*Aedes aegypti*, *Aedes albopictus*)	Ticks (*Dermacentor andersoni*)
Usual vertebrate hosts	Humans	Birds	Birds	Rodents	Humans, primates	Humans
Geographical distribution	World (mainly intertropical regions)	Asia, Africa, Europe, Americas	Americas	Europe, Asia	Africa, Asia, West Pacific, Europe,	Western USA and Canada
Clinical features						
Incubation period in days	2-14	2-14	4-21	7-14	1-12	3-6
Asymptomatic forms	75%	80%	> 99%	80%	15%	low%
Clinical manifestations	DF-DHF-DSS	Fever-encephalitis	Fever-encephalitis	Fever-encephalitis	Fever-joint pains	Fever-encephalitis
Vaccine	Phase III trials	No	No	Yes	No	No
Demonstrated transfusion-transmitted cases	Yes	Yes (high number)	No	Yes	No	Yes

ssRNA: Single-stranded RNA; dsRNA: Double-stranded RNA; DF: Dengue fever; DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome; CHIKV: Chikungunya virus.

peri-urban cycle where human beings are the main amplifying host for DENV (Figure 2). Female mosquitoes get infected by biting infected humans during their viremic phase; after 7 to 14 d of incubation, the mosquito is able to transmit the virus *via* blood feeding. Besides mosquito biting, DENV may be accidentally acquired after vertical transmission, especially in near-term pregnant women through the placenta[8], *via* the organ transplantation process[9,10], after needle-stick injury[11] and, as evidenced below, after transfusion of blood products.

Clinical presentation

The infection occurs after an incubation period of 3-14 d (average 3-7 d). Approximately 75% of all DENV infections are asymptomatic, notably in adults. The common symptomatic infection, which appears as a mild febrile illness associated or not with more evocative symptoms, represent approximately 20% of DENV infections. In endemic areas, about 5% of all acute febrile illnesses can be related to DENV[12]. Severe forms may represent up to 5% of symptomatic infections; they are more frequent at the two extremes of life (very young children and elderly) and in patients with diabetes mellitus, hypertension and renal insufficiency[13]. As shown in Figure 3, the classification of dengue presentations evolved through time[14]. According to the WHO classifications of 1975 and 1997, symptomatic dengue was divided in undifferentiated fever, dengue fever (DF) and dengue hemorrhagic fever (DHF) ranging from mild hemorrhagic symptoms (grade I) to dengue shock syndrome (DSS) (grades III and IV). In 2009, WHO proposed a new simplified classification in two presentations: dengue (without or with warning signs) and severe dengue (Figure 3). The latter classification is more adapted to clinical evaluations in primary care or resource-limited settings; however, it does not differentiate hemorrhagic forms from other severe presentations. A trend to capillary fragility together with the risk of thrombocytopenia is common features of all dengue cases, even those without hemorrhagic complications. It can be searched for by the tourniquet test that consists in applying and inflating a blood pressure cuff to the midpoint between the systolic and diastolic blood pressures for five minutes. The test is positive if more than 10 to 20 petechiae per square inch develop.

Pathophysiology

From a pathophysiological point of view, many aspects of disease remain unsolved (for a review, see[15]). The fist targets of DENV after mosquito bite seems to be Langerhans cells, dermal cells and interstitial dendritic cells, but many other cells can replicate the virus, including hepatocytes, lymphocytes, endothelial cells, neuronal cells and muscle satellite cells[16]. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN)[17] and the mannose receptor (CD206)[18] have been described as potential host receptors for virus entry. As for other flaviviruses, both signal transducer and activator of transcription 1 and 2 possess the ability to independently limit the severity of DENV pathogenesis. When these signalling pathways are inactivated, notably within the hepatosplenic compartment, the deregulation of cell-mediated immunity may lead to the activation of CD4+ and CD8+ T cells, which results in a "cytokine/chemokine storm" that plays an important role in the vascular permeability leading to leakage of plasma into the extravascular compartment seen in DHF. The resulting

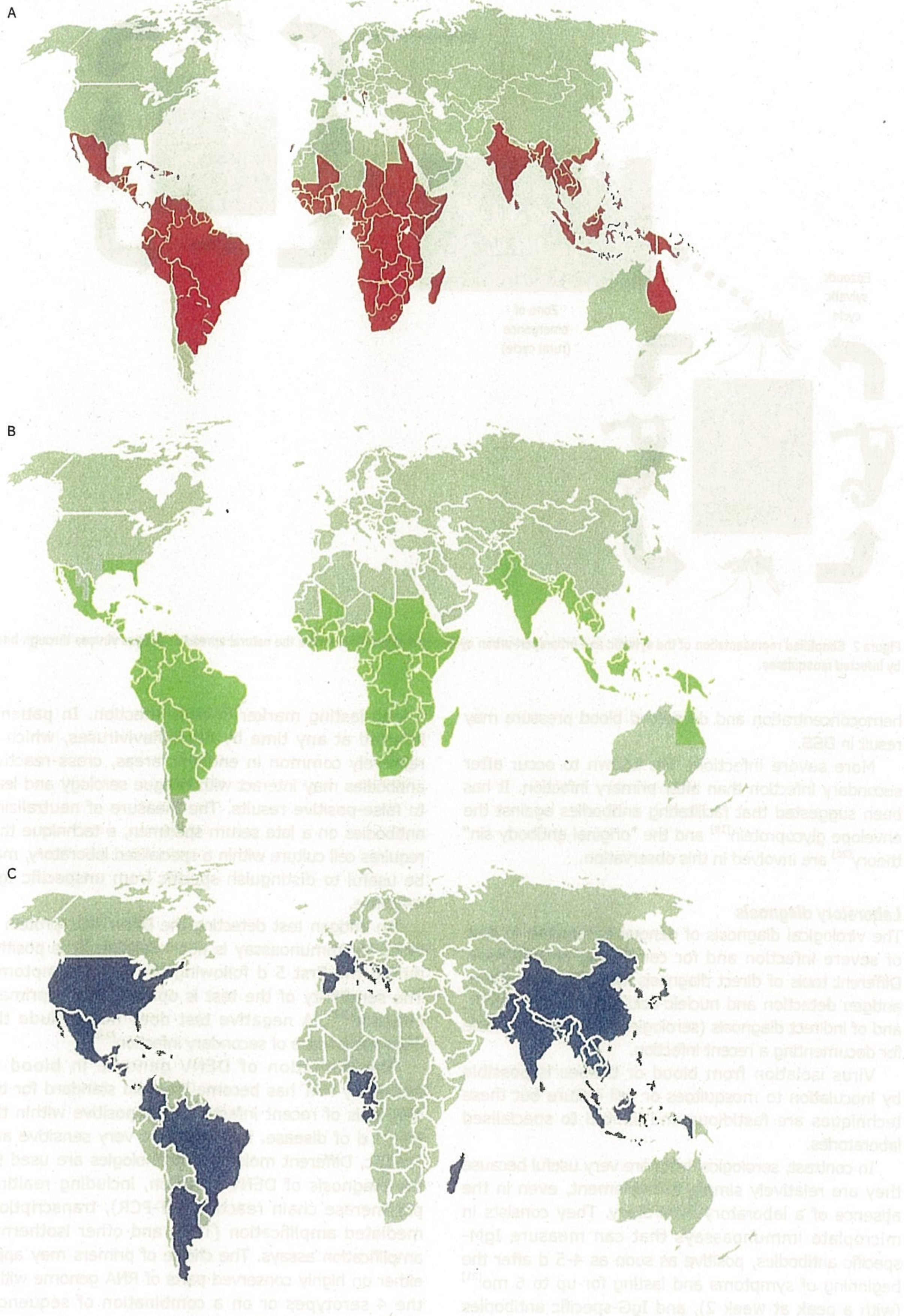

Figure 1 Overall distribution of dengue cases (endemic or epidemic) worldwide (A) and perimeter of expansion of the two main vectors of dengue viruses, *Aedes aegypti* (B) and *Aedes albopictus* (C).

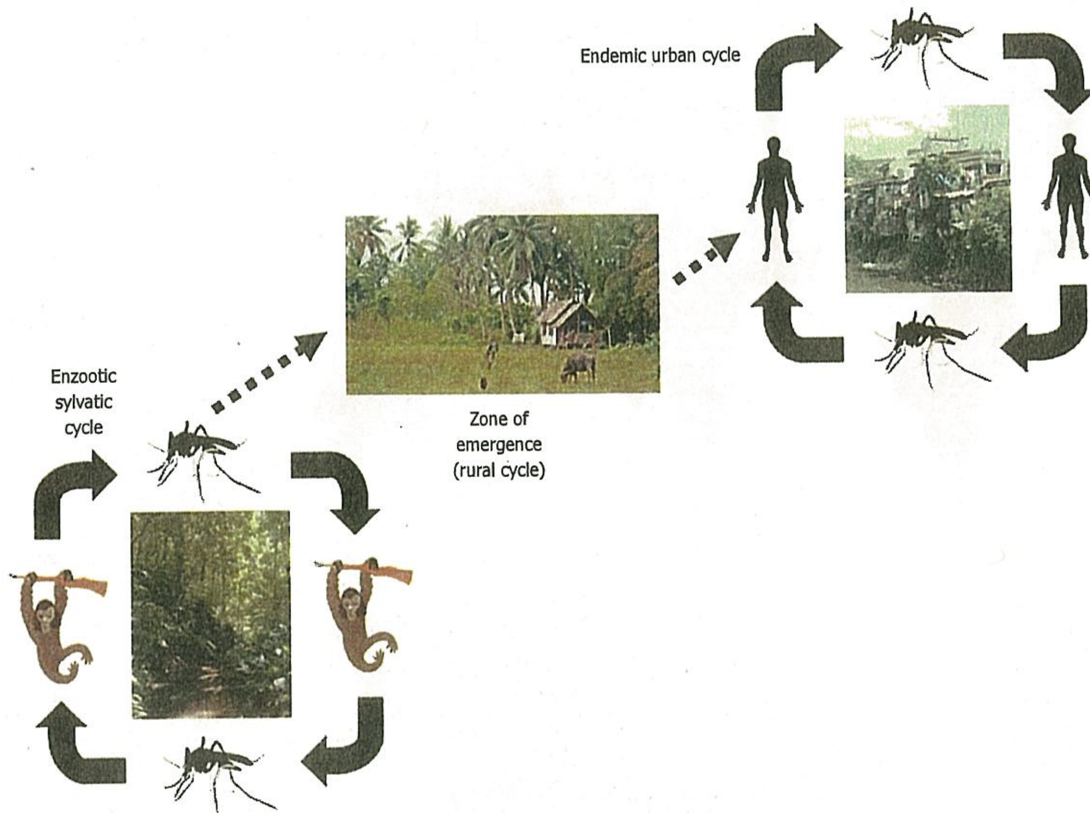

Figure 2 Simplified representation of the sylvatic and urban/peri-urban cycles of dengue that models the natural spread of dengue viruses through bites by infected mosquitoes.

hemoconcentration and decreased blood pressure may result in DSS.

More severe infections are known to occur after secondary infection than after primary infection. It has been suggested that facilitating antibodies against the envelope glycoprotein[19] and the "original antibody sin" theory[20] are involved in this observation.

Laboratory diagnosis

The virological diagnosis of dengue is required in case of severe infection and for confirming an outbreak. Different tools of direct diagnosis including cell culture, antigen detection and nucleic acid technologies (NAT), and of indirect diagnosis (serological tests) are available for documenting a recent infection.

Virus isolation from blood or tissues is possible by inoculation to mosquitoes or cell culture but these techniques are fastidious and limited to specialised laboratories.

In contrast, serological tests are very useful because they are relatively simple to implement, even in the absence of a laboratory of virology. They consists in microplate immunoassays that can measure IgM-specific antibodies, positive as soon as 4-5 d after the beginning of symptoms and lasting for up to 6 mo[21] (with a peak at week 2), and IgG-specific antibodies that become positive a few days after IgM and are a long-lasting marker of past infection. In patients infected at any time by other flaviviruses, which is relatively common in endemic areas, cross-reactive antibodies may interact with dengue serology and lead to false-positive results. The measure of neutralizing antibodies on a late serum specimen, a technique that requires cell culture within a specialised laboratory, may be useful to distinguish specific from unspecific IgM response.

An antigen test detecting the DENV NS1 protein in blood by immunoassay is now available. It is positive during the first 5 d following the initial symptoms. The sensitivity of the test is optimal during primary infection[22]. A negative test does not exclude the diagnosis in case of secondary infection[23,24].

The detection of DENV genome in blood or tissues by NAT has become the gold standard for the diagnosis of recent infection. It is positive within the first 5 d of disease. NAT tests are very sensitive and specific. Different molecular technologies are used for the diagnosis of DENV infection, including realtime polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA) and other isothermal amplification assays. The choice of primers may apply either on highly conserved parts of RNA genome within the 4 serotypes or on a combination of sequences specific of each of the 4 serotypes.

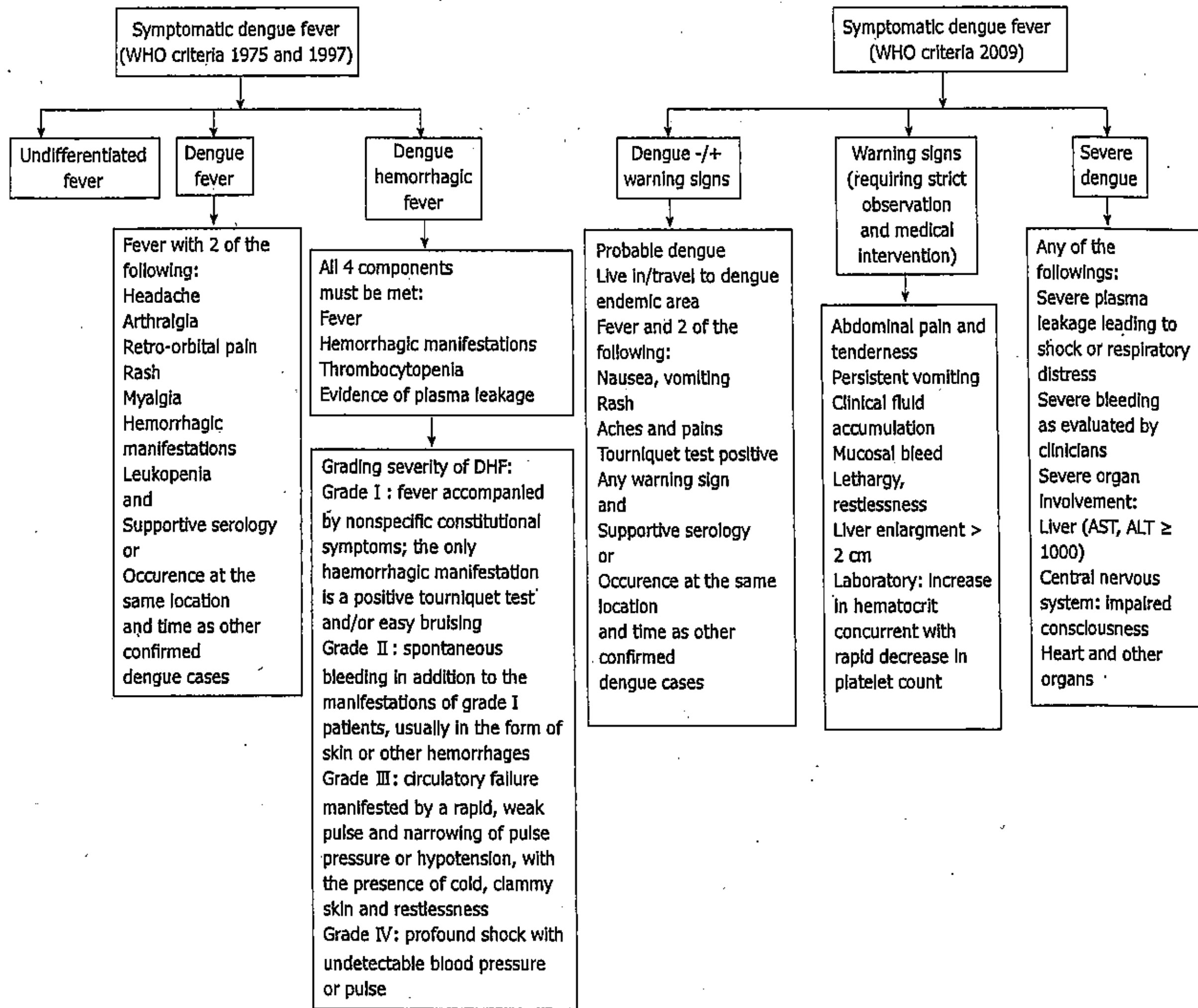

Figure 3 Successive classifications of dengue clinical presentations according to the World Health Organisation definitions. WHO: World Health Organisation. AST: Aspartate transaminase; ALT: Alanine transaminase.

Prevention

At the individual level, the vaccinal approach is certainly the more suitable way to control dengue durably. The existence of at least four serotypes that are sufficiently antigenically different necessitates the use of four monovalent vaccines. However, as mentioned above, there is safety concern about a possible increase of virus infectivity *via* antibody dependent enhancement when a vaccinated subject is exposed to a wild virus. Although no vaccine against dengue is presently available, several approaches have been proposed for controlling the spread of disease (for review, see[25,26]). The most advanced solution is a live-attenuated tetravalent vaccine based on chimeric yellow fever dengue virus that is produced by Sanofi and could be commercially-available before the end of this year.

At the vector level, the eradication of susceptible mosquitoes is the more effective way to contain the epidemic. However, the large use of insecticides has shown its limits in terms of toxicity for the environment together with the rapid development of cross-resistances. A vector control program has been launched by the WHO[27]. It is based on actions combining the elimination of containers harbouring larval and adult mosquitoes (plastic cups, broken bottles, used tyres, flowerpots), the use of insect repellents, mosquito traps and mosquito net in the home. Future strategies are in progress to modify the vectors by biological interventions including transgenic mosquitoes or their infection by the intracellular bacterium *Wolbachia* that reduces the replication of arboviruses in susceptible vectors[28,29].

Curative treatment

The curative treatment is mainly symptomatic. No antiviral drug has yet demonstrated any effect against DENV. DF resolves spontaneously within a few days; analgesics containing ibuprofen and aspirin must be avoided to prevent hemorrhagic complications. Cases of DHF must be hospitalised; with replacement of fluid leakage and intensive monitoring; the mortality can be reduced under 1% when adequate cares are given but may reach up to 20% in case of poor medical intake.

DSS and severe forms of dengue involving organ failure constitute a critical medical issue that needs urgent hospitalisation in an emergency unit.

DENGUE IN THE LARGER CONTEXT OF ARBOVIRAL DISEASES ASSOCIATED TO A DEMONSTRATED OR POSSIBLE RISK OF TRANSMISSION *VIA* BLOOD PRODUCTS

A total of approximately 130 arboviruses are known to cause disease in humans. Since they are transmitted *via* arthropod bite, these viruses are present in the bloodstream for a few days, which imply an at least theoretical risk of transmission *via* blood products if the patients are sampled during the viremic stage. The arboviruses known or suspected to be transmitted to recipients *via* blood products are presented in Table 1.

As reviewed by Petersen *et al*[30], the emergence of West Nile virus (WNV) in New York City in 1999 ant its rapid dissemination through Northern America during the following years is a good illustration of the sudden recognition of the role of transfusions in the spread of the virus, a fact that had been completely occulted before, despite many decades of circulation of WNV in the Ancient world. At the early phase of the USA outbreak, it was relatively difficult to establish a relationship between WNV infection and blood products[31,32], mainly due to the limits of contemporary diagnostic tools (IgM serology and NAT) that were insufficiently sensitive to identify infected donors, even retrospectively[30]. Another lesson driven from the WNV outbreak in USA was the decreased sensitivity of NAT when tested on minipools, a measure intended to decrease the costs and delay of WNV screening in blood donors.

The very successful emergence of Chikungunya virus (CHIKV) in the Indian Ocean and notably in the French Reunion Island is another illustration of the recent recognition of a new transfusion-transmitted risk. Even if no positive case was documented by NAT, probably for the same reasons as those evoked just before, it was modelled that, given a mean duration of 7.5 d for viremia and an exposition rate to CHIKV of 38% in inhabitants of the island, the prevention measures taken (eviction of autochthonous donors for red blood cells and systematic treatment of platelets by the Intercept® technology) had prevented the use of approximately 40 infected gifts during the whole epidemic period[33].

Concerning arboviral diseases in general, these examples illustrate that "planning efforts are hindered by the notoriously unpredictable nature of outbreaks and that importations of exotic arboviruses are random events with uncertain consequences"[30]. The rapid extension of dengue suggests that the subsequent transfusion transmission risk can be partly anticipated.

EVIDENCE FOR THE TRANSMISSION OF DENV BY BLOOD PRODUCTS AND ITS IMPACT ON PUBLIC HEALTH

As stated above, the global burden of dengue is considerable: according to the WHO, 2.5 billion people over more than 100 countries are concerned; 50 to 100 million infections occur every year, with a number of fatal cases of approximately 20000. A recent study[34] estimated that these figures could be increased by a factor of 3 to 4 to reflect the real load of dengue.

Despite the fact that dengue is the leading arboviruses in the world, there are only three reported observations of DENV transmission *via* blood products in the literature. The first report concerned a 76 year-old woman who received a blood transfusion in 2002 in a Hong-Kong hospital following a severe anaemia; two days later, she developed low-grade fever that resolved spontaneously (she received antibiotics for a suspicion of urinary infection). The case was secondarily related to dengue because the donor presented a typical dengue infection documented by serology. Molecular testing performed on the donated blood product was positive for DEN-1. Two months after transfusion, the recipient exhibited IgM antibodies confirmed by seroneutralisation assay. The case was published only six years later[35]. The second study, also published in 2008[36], involved a cluster of three cases contaminated in Singapore by the same donor who developed fever and myalgia after blood donation. Two days after transfusion, 2 of the 3 recipients developed a symptomatic infection that resolved spontaneously. The 3 recipients demonstrated serological evidence of acute dengue infection. A PCR assay performed on blood specimens from the donor and the 2 symptomatic recipients was positive for DEN-2.

The third observation, published in 2012[37] was documented from the outbreak of dengue that occurred in Puerto-Rico in 2007. Of 15350 donation samples tested retrospectively, 29 were found positive for DENV genome by TMA assay. Three of the recipients of these contaminated samples could be tested by NAT and one of them, who received red blood cells containing 10^8 copies/mL DEN-2, was found positive. Three days after transfusion, he developed DHF. Both donor and recipient were shown to harbour viruses with the same envelope sequence. This is the first case of severe dengue infection transmitted by blood products.

One may wonder about the gap between the important role played by dengue in Public Health worldwide and the limited number of transfusion-transmitted documented cases reported so far. Different arguments can be advanced for explaining such a paradox: (1) in the absence of documented inquiry between donor and recipient, it is often difficult to differentiate infection

transmitted by mosquitoes and blood products; (2) the disease is frequently asymptomatic or mild in donor, recipient or both, with spontaneous resolution within a few days; (3) most of transfusion-transmitted cases are intended to occur in areas where dengue is endemic, which contributes to minimize the risk, especially in low-income countries where the virological documentation of dengue cases is not available easily, and, last but not least, and (4) most recipients of blood products have been already exposed to mosquito-transmitted DENV early in their life, which prevents them from being infected again *via* infected blood products.

In 2009, the American Association of Blood Banks stratified in four levels (red, orange, yellow and green) the emergent or re-emergent infectious agents that could represent a potential threat to transfusion in North America for the next years[38]. Besides epidemiological considerations and subjective assessment of public perception, the following scientific criteria were taken into consideration: (1) the agent must be present in blood at least for a few hours or days; (2) this blood phase must be at least in part asymptomatic for allowing the blood donor to pass through the filter of clinical selection; (3) the infectious agent must be able to induce, at least in some cases, a severe disease; and (4) finally, the blood pathogen must resist to inactivation by the innate or adaptative immunity of the donor (*i.e.*, bacterical power of serum). According to these criteria, DENV was classified in the upper red level, together with *Babesia sp* and the human variant of Creutzfeldt-Jakob disease. These agents were considered as low to high scientific/epidemiologic evidence of risk regarding blood safety with the potential for severe clinical outcomes.

The arguments that pleaded for the upper-level classification of DENV with regard to blood safety in North America were as follows[38]: (1) the viremia is frequently asymptomatic and usually lasts for 2 to 7 d; (2) the viral load may be relatively high (from 10^4 to 10^8 copies/mL by NAT) in blood with the four serotypes of DENV, as exemplified by retrospective studies conducted in blood donors from Honduras, Brazil[39] and Puerto-Rico[37], with recovery of live virus from PCR-positive products in a few cases; (3) the disease can occur as important outbreaks; (4) the competent mosquitoes have a large distribution in the considered area (here United States); (5) the viral infection has a high seroprevalence in populations boarding the considered area; and (6) infected blood products could be imported from epidemic or endemic areas. At the opposite, the prevalence of positive samples was relatively low in the retrospective studies cited above (0.07% in 16521 blood gifts from Puerto-Rico[40], 0.30% in 2994 blood gifts from Honduras[39] and 0.06% in 4858 blood gifts from Brazil[39]).

The potential threat of dengue to transfusion safety is majored by the rapid spread of the disease worldwide whose incidence has increased 30-fold in the past 50 years[41]. Half of the planet is already exposed (Figure 1A) and the distribution of competent vectors (Figures 1B and C) is progressing very rapidly, notably with the climate changes[42] and the development of transcontinental travels. Regions with temperate climate as Europe or North America[43] can be the target of future outbreaks as illustrated by the recent cases observed in Croatia[5], Nice[6] or Florida[44]. In non-dengue endemic areas, asymptomatic infection is primarily associated with travellers returning from dengue-endemic areas. A few years ago, the recovery of areas endemic for malaria and dengue favoured the selection of blood donors returning from these countries. By now, dengue, as well as other arbovirosis, constitutes a risk that needs to be taken into consideration specifically.

MEASURES AVAILABLE FOR REDUCING THE RISK OF TRANSFUSION-TRANSMITTED DENGUE

Until a vaccine is widely used for preventing the expansion of dengue through the world population, it will be necessary to implement measures able to reduce the risk of transfusion-transmitted dengue. These measures include (1) the clinical selection of donors; (2) the implementation of screening tests specific for dengue; and (3) the non-specific reduction or inactivation of pathogens by the use of physical or chemical treatments applied to blood products. Their indications may differ in endemic and non-endemic areas[45].

Clinical selection of donors

In endemic areas, this measure would consist in excluding donors who may be at higher risk of infection. Given the fact that the exposition to mosquito bite is rather unpredictable, such a measure is not realistic. On the other hand, the presence of fever in donors of blood products is a general contra-indication of blood gift.

In non endemic areas, the clinical selection of donors consists in excluding travellers returning from endemic regions for a period of 4 wk. For instance, the latter measure was adapted in Europe towards tourists returning from Madeira during the recent 2012-2013 outbreak. The main limit of this strategy is the need for continuous adaptation of these exclusion measures to various epidemiological situations, which may lead to complicate the work of personnel in charge of this selection and to discourage donors from coming again for blood gift.

Screening tests specific for dengue

This strategy is useful in endemic areas or during an outbreak. Serology is not adapted for screening purpose because the viremia precedes of a few days the antibody answer. Only NAT could allow detecting the

presence of viral genome in blood from infected donors. Such a strategy was applied in the Puerto-Rico outbreak in 2005[40] and 2007[37]. During the Madeira outbreak, an in-house RT-PCR assay was implemented for screening blood products; 43 of 1948 donations tested positive for DENV genome (further identified as DEN-1) between 9 September 2012 and 11 March 2013[46]. For large-scale screening purpose as in blood donors, Gen-Probe Inc. (San Diego, CA, United States) developed a prototype TMA assay using highly conserved primers; the analytical sensitivity of the test was of approximately 15 copies/mL for each serotype[39]. The low levels of viremia in many donors with dengue justify the individual testing of blood products, which limit this strategy to countries with high-income economy. By contrast to West Nile virus, no automated molecular screening test is currently commercially available.

In the future, the development of fully automated multiplexing assays detecting simultaneously several blood-transmitted pathogens in microarray plates or using nanotechnology would be very useful for areas where multiple infectious agents at risk for blood safety may circulate at the same time (*i.e.*, in the Caribbean or in South-East Asia)[47].

Non specific reduction or inactivation of pathogens

Many systems are now available for treating blood products in order to inactivate some pathogens (for reviews see[38,48-50]). Most of these techniques are able to inactivate bacteria and lipid-enveloped viruses as DENV. Due to technical purposes, they can be applied to plasma, platelets or red blood cells. The main techniques that are efficient on DENV are briefly describer thereafter.

Some techniques are exclusively dedicated to plasma. Solvent-detergent treatment is able to disrupt viral envelopes. Dyes containing phenothiazine like methylene blue, when activated by visible light, are responsible for an oxidation of guanine present in viral genomes. Nanofiltration is able to retain viral particles whose size is over that of the pores of the nanofilter.

Other techniques based on photoactivation by ultra-violet (UV) rays may be applied to both plasma and platelet concentrates. The Intercept® system from Cerus Corporation (Concord, CA, United States) uses a psoralen derivative, amotosalen, as active compound. The Mirasol® system from Terumo BCT (Lakewood, CO, United States) use riboflavin (vitamin B2) as active compound. The Theraflex UV® system from MacoPharma (Tourcoing, France), by combining an exposition to UV light and strong shaking, induces the formation of cyclobutyl rings. Using those different technologies, a small proportion of platelets may be lost but the properties of activation, adhesion and aggregation of the cells resisting to the treatment are sufficiently well conserved to warrant their clinical use.

For red blood concentrates, some processes are in experimentation, including riboflavin (Caridian), Inactine® (PEN110 from the Vitex Company, Prestons, NSW, Australia) and an alkylating agent, Amustaline, from Cerus Corporation, whose activation occurs through exposition to acidic pH.

The main advantage of these strategies is the inactivation or reduction of a wide range of pathogens, including those that are still unidentified. However, the benefit-risk of each treatment needs a careful evaluation.

Economic considerations

The measures listed above regarding the prevention of transfusion-transmitted dengue represent an extra-cost for the Health system, especially those involving screening molecular tests specific for dengue that would be dedicated to the transmission of a single pathogen. No cost-effectiveness study has already been conducted to evaluate the economic burden of the implementation of a molecular screening targeting DENV neither in endemic or non endemic areas.

Lessons can be drawn from the experience acquired with the systematic screening of blood products for the presence of WNV in the United States during the epidemic period. Two studies were published on this topic in 2005[51] and 2006[52]. They demonstrated that the optimal cost-effectiveness strategy for WNV screening in blood products depends on different factors, including mainly the prevalence of the agent in the considered population, but also the ability to pool or not the samples before screening (*i.e.*, mean viral load), the seasonal period concerned by the screening and the consequences for the recipients. Globally, these studies demonstrated that targeted donor screening seems to be more cost-effective than mass donor screening.

It is too early to consider whether these conclusions regarding WNV in a developed country may be applied to DENV in endemic and non endemic area. In dengue non endemic countries that correspond mostly to places with high living standards, it is likely that the emergence of a dengue outbreak will conduct to the set-up of a molecular screening, as it was done in Madeira recently[46]. In the epidemic of DENV that occurred in northern Queensland, Australia, in 2008-2009, the risk for a dengue-infectious blood donation was estimated as 1 in 7146[53]. Although the temporary exclusion of potentially infected donors was chosen to limit transfusion-transmitted dengue during these outbreaks, the authors raised the question of the better cost-effectiveness of a strategy involving the use of a suitable screening test or of a pathogen reduction technology[53].

In dengue-endemic areas, the risk may be higher, as shown during the 2005 outbreak in Singapore through a mathematical modelling, with an estimated risk for a dengue-infectious blood donation of 1 in 1667 to 6154[54]. The implementation of a screening test would be probably cost-effective as compared to the exclusion of blood donors but it is likely that neither of these two strategies could be implemented in low income

countries where the disease is the more prevalent, at least in a near future.

CONCLUSION

Dengue provides an excellent model of transfusion-transmitted disease. Despite the large distribution of the disease worldwide, the risk with blood products from infected donors was only recognized recently. Except for one case of DHF[37], the disease, when transmitted by blood, does not seem to be more severe than after mosquito bite. However, the area of dengue extended considerably during the last 50 years; after having been limited to intertropical regions for a long time, the disease is now reaching temperate areas because of the worldwide distribution of its two main vectors (Figure 1) and of the climate change[42]. Considering these emerging risks, there is an urgent need for mathematical models able to predict the spread of DENV and its consequence on the supply of blood products. While waiting for an efficient prophylactic vaccine that could be able to reduce the burden of the disease, it is important to develop efficient measures for securing blood products in endemic and non endemic areas. The attention paid to DENV as a transfusion-transmitted pathogen could help to prevent the emergence of other more harmful known or unknown viruses.

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研究報告の概要

台湾では、鳥インフルエンザウイルス（AIV）のサブタイプ H5N2、H6N1 および H7N3 は家禽で特定されており、これらのサブタイプの幾つかの株は家禽で流行している。職業的な暴露のために、トリからヒトへの伝播の可能性を評価するために、家禽労働者における AIV 抗体状態の調査分析を行った。我々は 335 人の生きた家禽行商人（LPVs）、335 人の養鶏農家（PFs）、および 577 人の非家禽労働者（NPWs）を含む、670 人の家禽労働者を登録した。ウイルスの種々のサブタイプに対する血清抗体力価を分析し、比較した。LPVs と PFs の全体的な血清陽性率は H5N2 に対して、それぞれ 2.99%（10/335）と 1.79%（6/335）であった；そして H7N3 ウイルスにたいして、それぞれ 0.6%（2/335）と 1.19%（4/335）であった。NPWs の内、0.35%（2/577）と 0.17%（1/577）は、それぞれ H5N2 と H7N3 に対して血清陽性であった。職場が家禽の H5N2 アウトブレイクが報告された近い場所であった家禽労働者が高い H5N2 抗体価に至るウイルスに曝されているより大きな危険に直面することが、地理的分析で分かった。H6N1 抗体はただ 1 人の PF で検出された、そして H7N9 抗体は研究被験者で見つからなかった。H5N2、H6N1 および H7N3 ウイルスに起因する無症候性感染は、台湾の家禽労働者においてこのように特定された。職業的曝露は AIV 感染の高い危険性に関連しており、ヒトにおける特定の鳥インフルエンザ株の血清陽性率は、この地域の家禽での流行株を反映している。

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 IH 静注 250 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV 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。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見

インフルエンザウイルス（influenza virus）は、オルトミクソウイルス科（Orthomyxoviridae）に属する A 型インフルエンザウイルス（influenzavirus A）、B 型インフルエンザウイルス（influenzavirus B）、C 型インフルエンザウイルス（influenzavirus C）の 3 属を指す。A 型と B 型のウイルス粒子表面にはヘマグルチニン（HA）とノイラミニダーゼ（NA）の糖蛋白があり、これらが感染防御免疫の標的抗原となっている。特に A 型では、16 種類の HA と 9 種類の NA の組み合わせにより様々なウイルスが、ヒト以外にもブタやトリなどその他の宿主に広く分布している。ウイルスの大きさは直径 80～120nm の球形粒子で、エンベロープを有する 1 本鎖 RNA ウイルスで、万一原料血漿にインフルエンザウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

今後の対応

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。

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Serological comparison of antibodies to avian influenza viruses, subtypes H5N2, H6N1, H7N3 and H7N9 between poultry workers and non-poultry workers in Taiwan in 2012

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SUMMARY

In Taiwan, avian influenza virus (AIV) subtypes H5N2, H6N1 and H7N3 have been identified in domestic poultry, and several strains of these subtypes have become endemic in poultry. To evaluate the potential of avian-to-human transmission due to occupational exposure, an exploratory analysis of AIV antibody status in poultry workers was conducted. We enrolled 670 poultry workers, including 335 live poultry vendors (LPVs), 335 poultry farmers (PFs), and 577 non-poultry workers (NPWs). Serum antibody titres against various subtypes of viruses were analysed and compared. The overall seropositivity rates in LPVs and PFs were 2·99% (10/335) and 1·79% (6/335), respectively, against H5N2; and 0·6% (2/335) and 1·19% (4/335), respectively, for H7N3 virus. Of NPWs, 0·35% (2/577) and 0·17% (1/577) were seropositive for H5N2 and H7N3, respectively. Geographical analysis revealed that poultry workers whose workplaces were near locations where H5N2 outbreaks in poultry have been reported face greater risks of being exposed to viruses that result in elevated H5N2 antibody titres. H6N1 antibodies were detected in only one PF, and no H7N9 antibodies were found in the study subjects. Subclinical infections caused by H5N2, H6N1 and H7N3 viruses were thus identified in poultry workers in Taiwan. Occupational exposure is associated with a high risk of AIV infection, and the seroprevalence of particular avian influenza strains in humans reflects the endemic strains in poultry in this region.

Key words: Avian influenza virus, poultry worker, seroprevalence.

INTRODUCTION

Influenza A virus is a highly infectious respiratory pathogen that can infect both humans and animals; it poses a public health threat every year. This virus is a member of the family Orthomyxoviridae and is further classified into subtypes based on characteristics of two surface glycoproteins: haemagglutinin (HA) and neuraminidase (NA). Eighteen HA (H1–H18) and 11 NA (N1–N11) subtypes have been identified that circulate in wild birds and bats [1, 2]. Of these subtypes, only H1N1, H2N2 and H3N2 have been known to establish stable lineages in humans. These subtypes have caused sustained epidemics in human populations since 1918 [3]. In addition, the

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H5, H6, H7, H9 and H10 subtypes have caused infections in humans since 1959 [4–7]. Sporadic infections resulting from these subtypes have occurred mainly as a result of direct viral transmission from infected birds to humans through direct and indirect contacts [8, 9]. Human-to-human transmission of these influenza viruses of avian origin has rarely occurred.

Investigation of the relationship between poultry exposure and avian influenza infections in human populations is important for understanding possible transmission of the disease at the poultry–human interface. Previous epidemiological and virological reports have proposed that individuals with intense occupational exposure, especially poultry-farm and live-market workers, may be at an increased risk for avian influenza infection because environmental exposure may promote the transmission of avian influenza viruses (AIVs) [10–12]. To date, it remains unclear whether subclinical infections with regionally predominating AIVs have occurred in these high-risk populations through direct or indirect poultry contact. In Taiwan, several subtypes of AIVs, including H5N2, H6N1 and H7N3, have been identified in domestic poultry [13–15]. During the past decade, the H5N2 virus, which has low pathogenicity, has become the predominant infectious agent in chickens. Outbreaks caused by this virus were reported in 2003–2004 and 2008–2014 [16]. The highly pathogenic avian influenza (HPAI) A(H5N2) virus was first isolated in Taiwan in 2012; since then, this virus has caused subsequent outbreaks in several poultry farms [17]. Avian influenza A (H6N1) virus is frequently isolated from Taiwanese layers and broilers. It usually presents as a low pathogenic virus and continuously circulates as an endemic enzootic agent in animals. In 2013, this virus caused the first known human infection in Taiwan [6]. The low pathogenic H7N3 virus caused two outbreaks in domestic duck farms located in southern Taiwan in 2011 [15].

Few seroepidemiological studies of AIVs in high-risk populations in Taiwan have been performed. Recently, a study conducted by the Taiwan Centers for Disease Control (Taiwan CDC) reported that 1·4% of individuals in contact with H5N2-infected chickens were suspected to have been subclinically infected by the virus [18]. This finding emphasises that occupational exposure to infected poultry may pose a high risk of avian influenza infection in human populations. To better understand potential subclinical avian influenza infections in individuals who have frequent contact with poultry in Taiwan, we conducted an exploratory analysis in poultry workers for the presence of antibodies against H5N2, H6N1 and H7N3 viruses, all of which have caused infections in domestic poultry in Taiwan. Because four imported human cases of infection with the influenza A(H7N9) virus were confirmed in Taiwan between March 2013 and April 2014, this virus was also included in the study.

METHODS

Study subjects

A total of 1247 subjects, including 670 poultry workers and 577 non-poultry workers (NPWs), were enrolled in the study. The poultry workers were further sub-classified into 335 live poultry vendors (LPVs) and 335 poultry farmers (PFs); the LPVs and PFs in this study were randomly selected from 1148 live poultry stalls and 11 296 poultry farms to be representative of the regional distribution of LPVs and PFs in 22 cities and counties in Taiwan. The 577 NPWs without a history of poultry vending or farming were selected as control subjects and were chosen to match the poultry workers by sex, age, and workplace for each farm or stall. Written informed consent was obtained from all subjects, and the study was reviewed and approved by the Institutional Review Board of the Taiwan CDC. During the study period from May 2012 to July 2012, participants were interviewed by staff members at Taiwan CDC and local health agencies. A written questionnaire was completed for each participant by one of these staff members to obtain the personal background information, previous poultry exposure histories, and influenza vaccination histories, among other information. In addition, a single whole blood specimen was collected from each subject for antibody measurements.

Viruses for antibody testing

Four AIVs, subtypes of H5N2, H6N1, H7N3 and H7N9, were used as the antigens for the haemagglutination inhibition (HI) test in this study. The A/Taiwan/2/2013(H6N1) and A/Taiwan/1/2013(H7N9) viruses were human strains isolated from clinical specimens of infected patients. The A/chicken/Taiwan/1209/2003(H5N2) and A/duck/Taiwan/A1741/2011 (H7N3) viruses were provided by the Taiwan Animal Health Research Institute. All four viruses were propagated in the allantoic cavity of 9-day-old

embryonated chicken eggs, according to standard procedures [19]. These viruses were selected for the following reasons. The A/chicken/Taiwan/1209/2003 (H5N2) virus was the prototype and representative isolate of the H5N2 viruses circulating in Taiwanese chickens and was antigenically similar to the descendant chicken H5N2 viruses from 2003 to 2012 in Taiwan based on the results of HI tests conducted with ferret antisera (M. C. Cheng, unpublished data). Furthermore, phylogenetic analysis of A/chicken/Taiwan/1209/2003(H5N2) and other chicken H5N2 isolates in Taiwan has also indicated that these viruses grouped together forming two sub-clades [20]. The A/duck/Taiwan/A1741/2011(H7N3) virus was a representative isolate from the two low pathogenic outbreaks in domestic ducks in southern Taiwan. To determine the risks of human infection with the A/Taiwan/2/2013 (H6N1)-like and A/Taiwan/1/2013(H7N9)-like viruses before these viruses were first identified, the two human isolates were used to test sera collected in 2012.

Serum specimen processing and HI assay

Whole blood samples were centrifuged at 1000 g for 10 min at 4 °C, and serum specimens were then collected and stored in aliquots at −20 °C. Before antibody measurements, serum specimens were incubated with receptor destroying enzyme (RDE, Denka Seiken, Japan) at a ratio of 1:3 at 37 °C overnight to remove non-specific HA and were then heat inactivated at 56 °C for 30 min. RDE-treated sera were further diluted with PBS to a final dilution of 1:10. The resulting sera were used in the HI assay at Taiwan CDC without prior adsorption with erythrocytes.

The HI assay was used to investigate the existence of specific antibodies against various AIVs in human sera and was performed as previously described [21]. Serial twofold dilutions of RDE-treated sera were prepared in 96-well V-bottom microtitre plates for the analysis of H5N2, H6N1 and H7N9 antibodies and in 96-well U-bottom plates for the analysis of H7N3 antibodies; 25 μl/well of the virus antigens (4 haemagglutination units) were added to their respective wells. After a 60-min incubation period at room temperature, 50 μl of 1% horse (for H5N2 subtype), 0·5% turkey (for H6N1 and H7N9) or 0·75% guinea pig (for H7N3) erythrocytes were added and mixed gently. The plates were incubated at room temperature for 60 min. HI titres were expressed as the reciprocal of the highest dilution of serum that inhibited virus-induced haemagglutination. Sera that tested negative at a dilution of 1:10 were indicated to have a titre of <10. Back titrations were also performed, and titres were only accepted when both replicates yielded matching results. When performing HI assays, human sera that had previously been shown to have elevated titres against H5N2 virus, mouse sera raised against H6N1 virus and ferret sera raised against H7N9 virus were used as positive controls to validate the test procedure. Pre-immune sera collected from naive mice were used as negative controls.

Statistical analysis

Questionnaire data were manually entered in duplicate, and data-entry problems, as well as inconsistencies, were verified. Pearson's χ^2 test and Fisher's exact tests were used to compare categorical variables of demographic data. Logistic regression was used to calculate the odds ratio and P value. Statistical significance was considered when a P value of $<0·05$ was obtained. All tests were performed with SPSS v. 14 (SPSS Inc., USA) and were two-tailed. ArcGIS v. 10.0 software (ESRI, USA) was used to demonstrate the locations (districts/towns/villages) of poultry outbreaks and subjects with elevated antibody titres against AIVs.

RESULTS

Demographics

Detailed demographics of the 1247 study subjects are presented in Table 1. Of the poultry workers, 59% were male and more than 60% were aged ⩾50 years (mean age 52·3 years, range 17–83 years) in LPVs and 54·1 years (range 24–89 years) in PFs. Most of the subjects had worked in the poultry industry for more than 10 years (LPVs 86·6%, PFs 79·4%) and had close contact with poultry every day (LPVs 90·5%, PFs 94·0%). The majority of LPVs and PFs had not received H5N1 and/or seasonal influenza vaccines during the 2 years prior to the specimen collection date. For NPWs, the ages ranged from 21 to 88 years (mean age 53·2 years). Their education level was higher ($P<0·05$) than that of poultry workers. More than 50% of the NPWs received seasonal influenza vaccines in 2010 and/or 2011, whereas the H5N1 vaccination coverage was still low. Overall, a higher proportion of PFs did not use personal protective equipment (PPE) compared to LPVs (LPVs: 2·1%, PFs: 12·5%; $\chi^2=27·0$, $P<0·0001$). Of the PPE used, the most common were gloves, boots and masks.

Table 1. *Demographic characteristics of the 1247 study subjects*

	Live poultry vendors ($N = 335$)		Poultry farmers ($N = 335$)		Non-poultry workers ($N = 577$)	
Subjects	*N*	%	*n*	%	*n*	%
Gender						
Male	171	51·04	226	67·46	339	58·75
Female	164	48·96	109	32·54	238	41·25
Age, years						
<20	1	0·30	0	0·00	0	0·00
20–29	7	2·09	7	2·09	12	2·08
30–39	39	11·64	43	12·84	60	10·40
40–49	82	24·48	68	20·30	138	23·92
50–59	112	33·43	99	29·55	191	33·10
⩾60	94	28·06	118	35·22	176	30·50
Mean	52·25	–	54·11	–	53·20	–
Education level						
Illiteracy	24	7·16	35	10·45	13	2·25
Elementary school	100	29·85	99	29·55	65	11·27
Junior high school	82	24·48	66	19·70	49	8·49
Senior high school	112	33·43	100	29·85	168	29·12
College	17	5·07	35	10·45	282	48·87
Years of working						
<1 year	5	1·49	11	3·28	–	–
1–5 years	21	6·27	31	9·25	–	–
6–10 years	19	5·67	27	8·06	–	–
>10 years	290	86·57	266	79·40	–	–
Frequency of working						
Seldom	1	0·30	2	0·60	–	–
Once per several month	1	0·30	1	0·30	–	–
Once per month	0	0·00	4	1·19	–	–
Once per week	30	8·96	13	3·88	–	–
Every day	303	90·45	315	94·03	–	–
Received H5N1 influenza vaccine						
Never	245	73·13	271	80·90	524	90·81
1 dose	34	10·15	28	8·36	24	4·16
2 doses	11	3·28	15	4·48	15	2·60
>3 doses	41	12·24	15	4·48	12	2·08
Uncertain	4	1·19	6	1·79	2	0·35
Received seasonal influenza vaccine						
2011						
Yes	64	19·10	107	31·94	291	50·43
No	270	80·60	225	67·16	285	49·39
Uncertain	1	0·30	3	0·90	1	0·17
2010						
Yes	69	20·60	128	38·21	307	53·21
No	266	79·40	206	61·49	269	46·62
Uncertain	0	0·00	1	0·30	1	0·17
Personal protective equipment use						
None	7	2·09	42	12·54	–	–
Gloves	251	74·93	170	50·75	–	–
Mask	111	33·13	200	59·70	–	–
Hair cover	16	4·78	44	13·13	–	–
Goggle	7	2·09	4	1·19	–	–
Shoe cover	33	9·85	38	11·34	–	–
Boots	301	89·85	249	74·33	–	–
Water-resistant apron	306	91·34	100	29·85	–	–

Table 2. *Distribution of antibody titres against various avian influenza viruses*

		No. of serum samples		
Virus antigen	Antibody titre*	Live poultry vendors ($N = 335$) n (%)	Poultry farmers ($N = 335$) n (%)	Non-poultry workers ($N = 577$) n (%)
H5N2	<1:10	5 (1·49)	10 (2·99)	30 (5·20)
	1:10	23 (6·87)	36 (10·75)	100 (17·33)
	1:20	170 (50·75)	169 (50·45)	296 (51·30)
	1:40	127 (37·91)	114 (34·03)	149 (25·82)
	1:80	**10 (2·99)**	**6 (1·79)**	**2 (0·35)**
H7N3	<1:10	307 (91·64)	318 (94·93)	551 (95·49)
	1:10	16 (4·78)	9 (2·69)	19 (3·29)
	1:20	10 (2·99)	4 (1·19)	6 (1·04)
	1:40	**2 (0·60)**	**4 (1·19)**	**1 (0·17)**
H7N9	<1:10	335 (100·00)	335 (100·00)	577 (100·00)
	1:10	0	0	0
H6N1	<1:10	335 (100·00)	334 (99·70)	577 (100·00)
	1:10	0	0	0
	1:20	0	0	0
	1:40	**0**	**1 (0·30)**	**0**

* The cut-off antibody titre (bold font) of seropositivity was 1:80 for H5N2 and 1:40 for H7N3, H7N9 and H6N1 viruses.

Seroprevalence of HI antibodies to various AIVs

The distribution of HI titres against H5N2, H6N1, H7N3 and H7N9 viruses in all 1247 study subjects is shown in Table 2. Based on the results, poultry workers (LPVs or PFs) have antibody titres against the H5N2 virus (A/chicken/Taiwan/1209/2003) that are significantly higher than those of NPWs ($P < 0·001$). The overall seropositivity rates in LPVs, PFs and NPWs were 2·99% (10/335), 1·79% (6/335) and 0·35% (2/577), respectively, with a cut-off value of 1:80. Furthermore, geographical analysis revealed that poultry workers whose workplaces (districts/towns/villages) were near locations where H5N2 outbreaks in poultry were reported in 2012 had higher risks of virus exposure resulting in elevated H5N2 antibody titres (Fig. 1) [odds ratio (OR) 5·6, 95% confidence interval (CI) 1·5–20·8, $P = 0·028$]. Moreover, higher HI antibody titres to H5N2 virus were observed in LPVs (OR 8·85, 95% CI 1·1–67·5, $P = 0·005$) and PFs (OR 5·24, 95% CI 0·6–45·0, $P = 0·043$), than in NPWs. These results indicate that the persistently regional circulation of H5N2 viruses in poultry may potentially cause occupational exposure-related subclinical infections in humans. The vaccination histories of seasonal influenza vaccines in 2010 and 2011 in LPVs, PFs and NPWs who had H5N2 antibody titres $\geqslant$1:40 were significantly different ($\chi^2 = 20$, $P < 0·0001$ for received 2010 seasonal influenza vaccine; $\chi^2 = 21·4$, $P < 0·0001$ for received 2011 seasonal influenza vaccine); no difference was observed for histories in the three groups with H5N2 antibody titres = 1:80 ($\chi^2 = 0·4$, $P = 0·8$ for received 2010 seasonal influenza vaccine; $\chi^2 = 1·8$, $P = 0·4$ for received 2011 seasonal influenza vaccine). For subtype H7, seropositive rates of antibody against H7N3 virus (A/duck/Taiwan/A1741/2011) in LPVs, PFs and NPWs were 0·6% (2/335), 1·19% (4/335) and 0·17% (1/577), respectively, with a cut-off value of 1:40. Higher rates of seropositivity were observed in LPVs and PFs compared to NPWs. However, the differences observed were not statistically significant ($P = 0·14$). None of the 1247 serum specimens were identified as being positive for antibodies against the H7N9 virus (A/Taiwan/1/2013) because they all had titres $\leqslant$1:10. The seropositivity of H6N1 antibodies was also low in both poultry workers and NPWs. There was only one PF in southern Taiwan with an antibody titre of 1:40, while all the other subjects had titres $\leqslant$1:10. Seasonal influenza vaccination histories of LPVs, PFs and NPWs were summarized based on the serological test results (Table 3). The serological test results, occupations, and vaccination histories of individuals with high HI titres against various AIVs are summarized in Table 4.

DISCUSSION

This study provides evidence that possible subclinical avian influenza infections may have occurred in poultry workers (LPVs or PFs) and that these poultry

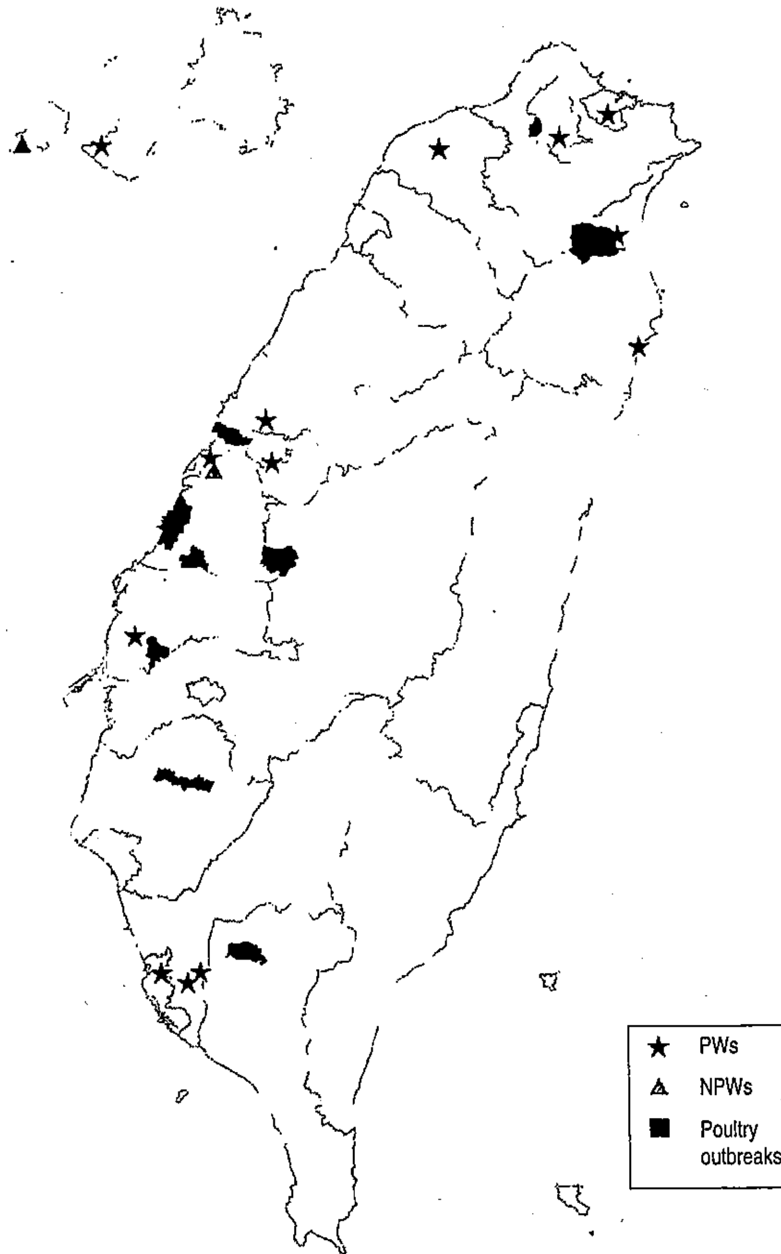

Fig. 1. Locations of workplaces of poultry workers (PWs) with elevated H5N2 antibody titres and poultry farms where H5N2 outbreaks were reported in 2012. Workplaces (districts/towns/villages) of PWs and non-poultry workers (NPWs) with antibody titres against H5N2 virus $\geqslant$1:80 are indicated by black stars and blue triangles, respectively. Locations of H5N2 outbreaks in poultry that occurred in 2012 in Taiwan are indicated in red.

workers have a higher risk of acquiring infections compared to the general public. The study also demonstrates that infected poultry are the principal source of human exposures to AIVs, as evidenced by the elevated HI antibody titres in poultry workers. The endemicity of various subtypes of viruses in poultry in particular countries/regions may contribute to their ability to infect local residents. Close contact, such as consuming uncooked and infected poultry products, or handling or caring for infected avian species, is considered to be a source for avian influenza infection [9]. It has been reported that 10% of poultry workers were seropositive for H5N1 viruses, and 3·1% of government workers who were involved in the

Table 3. *Seasonal influenza vaccination histories of live poultry vendors, poultry farmers and non-poultry workers based on the serological test results*

		Seasonal influenza vaccination history in past 2 years								
		Live poultry vendors ($N = 335$)			Poultry farmers ($N = 335$)			Non-poultry workers ($N = 577$)		
Virus antigen	Antibody titre*	2010	2011	None	2010	2011	None	2010	2011	None
H5N2	<1:10	1	1	4	4	4	6	21	20	8
	1:10	6	5	16	15	12	20	61	60	32
	1:20	40	36	124	72	62	93	152	145	125
	1:40	20	20	102	33	27	76	73	66	70
	1:80	**2**	**2**	**8**	**4**	**2**	**2**	**0**	**0**	**2**
H7N3	<1:10	64	59	231	123	104	185	293	280	225
	1:10	2	2	14	4	2	5	9	6	10
	1:20	2	2	8	0	0	4	5	5	1
	1:40	**1**	**1**	**1**	**1**	**1**	**3**	**0**	**0**	**1**
H7N9	<1:10	69	64	254	128	107	197	307	291	237
	1:10	0	0	0	0	0	0	0	0	0
H6N1	<1:10	69	64	254	128	107	196	307	291	237
	1:10	0	0	0	0	0	0	0	0	0
	1:20	0	0	0	0	0	0	0	0	0
	1:40	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**

* The cut-off antibody titre (bold font) of seropositivity was 1:80 for H5N2 and 1:40 for H7N3, H7N9 and H6N1 viruses.

culling of infected poultry also tested positive during the outbreak in Hong Kong [10]. In the 2003 poultry outbreaks that occurred in The Netherlands, 49% of poultry cullers had serological evidence of H7N7 infection [21]. In the USA, 0·8% and 0·3% of agricultural workers experienced a ⩾fourfold rise in antibodies against avian H5N2 and H9N2 viruses, respectively [22]. Another seroprevalence study conducted in veterinarians exposed to birds demonstrated significantly elevated antibody titres against the H5N2, H6N2, and H7N2 AIVs compared to healthy subjects [23]. In Japan, 5% (13/257) of poultry workers living in Ibaraki, where the H5N2 virus was isolated from chickens, had a ⩾fourfold increase in neutralizing antibodies against avian H5N2 viruses [24]. These data consistently show that occupational exposure to infected poultry may serve as a potential transmission route of avian influenza.

In Taiwan, both avian influenza H5N2 and H6N1 viruses have been co-circulating persistently in poultry and have developed into unique and local lineages [14, 20]. However, based on data from the surveillance of AIVs in Taiwan since 1998, only low pathogenic avian influenza (LPAI) H7N3 virus was detected from the two low pathogenic outbreaks in domestic ducks in southern Taiwan [15]. The novel H7N9-like viruses, which have been identified in China since 2013, have not been detected in poultry in Taiwan. The results of the present study suggest that the H5N2 virus is an important zoonotic agent at the chicken–human interface in Taiwan. However, the lower seropositivity observed in LPVs and PFs against H7N3 virus, compared to that of the H5N2 virus, may be related to the endemic nature of H5N2 compared with the limited detection of H7N3 in Taiwanese domestic ducks in 2011. No seroreactivity for antibodies specific to the novel H7N9 virus currently circulating in China was detected in the subjects, which is consistent with the observation that no H7N9 virus has been reported to date in Taiwanese poultry. We were surprised to find that only one subject (LPV) showed seropositivity to the H6N1 virus in the study, as the H6N1 virus is frequently isolated in Taiwanese chickens and has formed a unique lineage [14]. A previous study showed that only two (18·1%, 2/11) volunteers were experimentally infected even when a high infective dose of duck-derived H6N1 virus was used, and none of the volunteers had a detectable antibody response [25]. Moreover, the first human H6N1 virus-infected case had low HI titres (1:80) in convalescent serum [6]. These observations may indicate that the H6N1 virus exhibits poor immunogenicity in human populations, which may explain the low seroprevalence of H6N1 antibodies detected in the

Table 4. *Serological test results, occupation and vaccination histories of individuals with high haemagglutination inhibition titres against various avian influenza viruses*

	Tested antigens				Vaccination in past 2 years		
Subject no.	H5N2	H7N3	H7N9	H6N1	H5N1	Seasonal flu	Occupation
1	80	<10	<10	<10	–	+	Live poultry vendor
2	80	<10	<10	<10	–	–	Poultry farmer
3	80	<10	<10	<10	–	–	Live poultry vendor
4	80	<10	<10	<10	–	–	Poultry farmer
5	80	<10	<10	<10	–	–	Live poultry vendor
6	80	20	<10	<10	–	–	Live poultry vendor
7	80	<10	<10	<10	–	–	Poultry farmer
8	80	<10	<10	<10	–	–	Poultry farmer
9	80	<10	<10	<10	–	+	Poultry farmer
10	80	<10	<10	<10	+	–	Live poultry vendor
11	80	<10	<10	<10	–	+	Poultry farmer
12	80	<10	<10	<10	–	–	Live poultry vendor
13	80	<10	<10	<10	–	–	Live poultry vendor
14	80	<10	<10	<10	–	+	Live poultry vendor
15	80	<10	<10	<10	–	–	Live poultry vendor
16	80	<10	<10	<10	+	–	Live poultry vendor
17	80	<10	<10	<10	–	–	Non-poultry worker
18	80	<10	<10	<10	–	–	Non-poultry worker
19	40	40	<10	<10	–	–	Live poultry vendor
20	40	40	<10	<10	–	–	Poultry farmer
21	40	40	<10	<10	–	+	Poultry farmer
22	40	40	<10	<10	–	–	Poultry farmer
23	20	40	<10	<10	–	+	Live poultry vendor
24	40	40	<10	<10	–	–	Poultry farmer
25	40	40	<10	<10	–	–	Poultry farmer
26	20	<10	<10	40	–	–	Poultry farmer

present study. However, the responses can be variable as data from another study revealed that HA antibodies were detected in subjects tested against a turkey-origin H6 antigen [26]. Cross-reactive heterosubtypic antibodies elicited from heterologous influenza viruses, such as receiving H5N1 and seasonal influenza vaccines, may confound the interpretation of seropositivity [27, 28]. To evaluate this potential influence, previous influenza vaccination histories, including those of both H5N1 and seasonal influenza vaccines, of all the subjects were reviewed, and a correlation between vaccine administration and serum antibody titres against H5N2 and H7N3 viruses was analysed. No statistical antibody titre differences were observed between vaccinated and non-vaccinated subjects. However, 25·82% of the NPWs had HI titres of 1:40 against H5N2 virus. As these subjects reported no exposure history to poultry in their daily lives, the detected antibody titres were suggested as basal-level titres that may be related to their previous exposure to human seasonal influenza viruses.

HI, neutralization (NT) and the later modified microneutralization (MN) methods are considered to be the most current and commonly used serological assays for antigenic characterization. Due to labour intensity and complex technical requirements, the HI assay has become the most widely used surrogate to screen human sera for antibodies against influenza viruses. Antibody titres obtained from the HI method have been demonstrated to correlate well with those detected by MN in detecting antibodies against human and AIVs [26, 29–32]. Different red blood cells (RBCs) have their own preferences to agglutinate with specific influenza viruses [33]. Turkey, guinea pig and human RBCs were recommended for use in HI tests to detect human antibodies against human influenza viruses. However, for antibodies against avian subtype H5 influenza viruses, several studies have reported that the sensitivity of the HI assay

was elevated when horse RBCs were used compared to those of guinea pigs, turkeys, humans, or chickens [26, 34]. Therefore, we used horse RBCs in our HI assay to increase the sensitivity of detection of antibodies against the H5N2 virus, which is also reported to have high agreement and reproducibility for the detection of H5 antibodies [26].

This study has some limitations when interpreting the serological test results from the field studies. First, there was no available previous history of influenza-like illness in the subjects. Hence, the association of seropositivity with clinical symptoms, as well as the severity of clinical illness caused by specific avian influenza infections, remains unknown. Second, influenza vaccination histories of the study subjects were obtained through questionnaires and, thus, may not be accurate. Third, because there were no reliable or referenced cut-off values of seropositivity for different subtypes in previous studies, cut-off values of seropositivity were set at an HI titre of 40 for H6N1, H7N3 and H7N9 subtypes and at 1:80 for H5N2. In conclusion, this study indicates that poultry workers have a higher risk of exposure to AIVs during occupational activities and consistently supports the results reported previously by other studies [10, 21–24]. Therefore, active surveillance for the early detection and intervention of viral infections in live poultry should be conducted continuously. These screenings can improve the control of measures to prevent AIV-induced human illnesses. For poultry workers, especially LPVs, the use of appropriate PPE during their occupational activities is also suggested to mitigate the risk of exposure to AIVs.

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DECLARATION OF INTEREST

None.

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販売名（企業名）	解凍赤血球液-LR「日赤」（日本赤十字社） 照射解凍赤血球液-LR「日赤」（日本赤十字社）				

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
○致死的ヒト脳炎に関連するvariegated squirrelボルナウイルス（VSBV-1） 2011年から2013年までの期間に、3名のカワリリスのブリーダーが脳炎を発症した。3名は同様の臨床症状を呈し、発症の2～4ヶ月後に死亡した。次世代シーケンシングおよびリアルタイムRT-qPCRによるメタゲノム解析手法により、接触があったリスおよび3名の脳のサンプルから未知のボルナウイルスが検出された。暫定的にvariegated squirrel 1ボルナウイルス（VSBV-1）と命名された当該ウイルスの系統は、系統発生学的解析により既知のボルナウイルス属の系統とは異なるものであることが判明した。 患者3名全員が自営のリスのブリーダーであり、リスのつがいを交換していた。3名中2名については、リスの噛み付き・引っ掻き行動に起因する皮膚損傷が家族により報告された。 しかしながら、このリスから患者へ伝播した人畜共通感染症の経路は依然として不明である。リスの口腔咽頭スワブ中のウイルスRNA量が高値であったことにより、擦過傷または咬傷を介して感染するという仮説が支持される可能性がある。	解凍赤血球液-LR「日赤」 照射解凍赤血球液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
3名のリスのブリーダーが脳炎を発症し、飼育するリス及び3名の脳のサンプルから未知のボルナウイルス（暫定的にvariegated squirrel 1ボルナウイルス（VSBV-1））が検出されたという報告である。	今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

15705

JRC2015T-028

BRIEF REPORT

A Variegated Squirrel Bornavirus Associated with Fatal Human Encephalitis

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SUMMARY

Between 2011 and 2013, three breeders of variegated squirrels (*Sciurus variegatoides*) had encephalitis with similar clinical signs and died 2 to 4 months after onset of the clinical symptoms. With the use of a metagenomic approach that incorporated next-generation sequencing and real-time reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR), the presence of a previously unknown bornavirus was detected in a contact squirrel and in brain samples from the three patients. Phylogenetic analyses showed that this virus, tentatively named variegated squirrel 1 bornavirus (VSBV-1), forms a lineage separate from that of the known bornavirus species. (Funded by the Federal Ministry of Food and Agriculture [Germany] and others.)

BEGINNING IN LATE 2011, THREE MEN IN SUCCESSION (63, 62, AND 72 YEARS of age) from the state of Saxony-Anhalt, Germany, had a progressive encephalitis or meningoencephalitis that led to death within 2 to 4 months after the onset of clinical symptoms. The clinical course was characterized by fever, shivers, or both; progressive psychomotor slowing; confusion; unsteady gait; myoclonus, ocular paresis, or both; and finally, coma. All three patients had preexisting medical conditions (hypertension, diabetes, or obesity). In all three patients, the disease was also accompanied, at some point during the course of the illness, by bilateral crural-vein thrombosis, which led to pulmonary embolism in two patients. An analysis of the cerebrospinal fluid showed pleocytosis, and cranial imaging revealed edematous lesions in the cerebral cortical areas and basal ganglia or meninges that were increasing in size, a finding consistent with a viral infection (Fig. 1A and 1B). While the patients were alive, no infectious agent could be detected by means of microscopic, culture, molecular, or serologic investigations of cerebrospinal fluid samples, biopsy samples, or serum. All three patients were treated in intensive care units, had to undergo mechanical ventilation, and died despite receiving treatment with broad antiinfective chemotherapy. Biopsy and postmortem analysis of the affected brain areas showed tissue edema, necrosis, glial activation, and lymphocyte infiltration, often as perivascular cuffing, but no viral inclusions or any microorganisms. Details of the characteristics of the patients, the clinical symptoms, and the results of laboratory analyses are shown in Table 1, and in Tables S1 and S2 in the Supplementary Appendix, available with the full text of this article at NEJM.org.

From the Institute of Diagnostic Virology (B.H., D.H., M.J., B.A., M.B.), Department of Experimental Animal Facilities and Biorisk Management (J.P.T.), and Institute of Novel and Emerging Infectious Diseases (R.G.U.), Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Bernhard Nocht Institute for Tropical Medicine, World Health Organization Collaborating Center for Arbovirus and Hemorrhagic Fever Reference and Research, Hamburg (D.T., D.C., J.S.-C.), German Center for Infection Research (DZIF), Hamburg-Lübeck-Borstel (D.T., D.C., J.S.-C.), Institute of Veterinary Pathology, Justus-Liebig-University Gießen, Gießen (C.H.), Department of Neurology, Bergmannstrost Hospital (A.B., O.N.), and Department of Neurology, University Hospital Halle (Saale) (T.M.), Halle (Saale), Institute of Neuropathology, Otto-von-Guericke Universität, Magdeburg (C.M.), State Institute for Consumer Protection of Saxony-Anhalt, Department of Veterinary Medicine, Stendal (E.v.d.G.), and Special Service for Veterinarian Affairs and Consumer Protection, Salzlandkreis, Bernburg (Saale) (C.L.) — all in Germany. Address reprint requests to Dr. Beer at the Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, OIE Collaborating Center for Zoonoses in Europe, Südufer 10, 17493 Greifswald-Insel Riems, Germany, or at martin.beer@fli.bund.de.

Drs. Hoffmann and Tappe contributed equally to this article.

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All three patients were breeders of variegated squirrels (*S. variegatoides*). They were friends, had met privately on a regular basis, and had exchanged their squirrel breeding pairs on multiple occasions (a detailed description is provided in the Supplementary Appendix). At least two of the patients had been scratched by their squirrels in the past, and one had been bitten. Because the initial pathogen-specific screening of a squirrel from the breeding population of Patient 3 revealed no evidence of any of the tested pathogens (for details, see Table S3 in the Supplementary Appendix), a panel of samples from the squirrel was analyzed by means of metagenomic sequencing. All further analyses were based on the detection of several short sequence fragments with a strong similarity to *Mammalian 1 bornavirus*.

METHODS

SAMPLES OF TISSUE AND BODY FLUIDS

Organ, blood, oropharyngeal-swab, and chest-cavity fluid samples from a healthy variegated squirrel that had direct contact with Patient 3 were available for the analyses. In addition, archived formalin-fixed, paraffin-embedded (FFPE) brain tissue from Patients 1 and 2, as well as fresh-frozen brain samples, cerebrospinal fluid, and serum from Patient 3, were tested. FFPE brain tissue from 10 unrelated humans (tissue from patients with Alzheimer's disease, human immunodeficiency virus–induced encephalopathy, or herpes simplex virus–induced encephalitis, as well as normal brain tissue), materials from polymerase-chain-reaction (PCR)–negative variegated squirrels, and a Borna disease virus (BoDV)–infected horse brain were used as control material.

METAGENOMICS, WHOLE-GENOME SEQUENCING, AND SEQUENCE ANALYSIS

A metagenomic analysis of squirrel samples was performed in accordance with a standard workflow, as described elsewhere.[1,2] Sequencing was performed with the use of a MiSeq instrument (Illumina). The obtained reads were analyzed with the use of RIEMS[3] for the detection of pathogens and with Genome Sequencer software, version 2.8 (Roche), for sequence assembly. The complete coding sequences, as well as the nucleoprotein (N) nucleotide and amino acid sequences, were used to analyze the evolutionary relationships among this newly discovered bornavirus, previously reported bornavirus species, and endogenous bornavirus-like sequences from

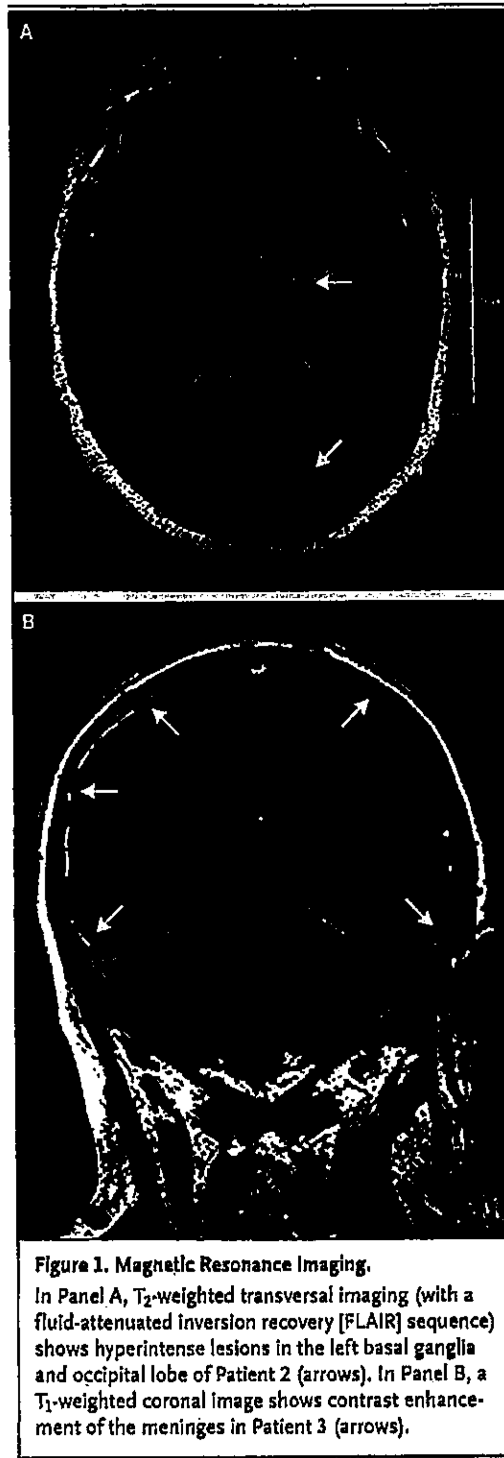

Figure 1. Magnetic Resonance Imaging.
In Panel A, T_2-weighted transversal imaging (with a fluid-attenuated inversion recovery [FLAIR] sequence) shows hyperintense lesions in the left basal ganglia and occipital lobe of Patient 2 (arrows). In Panel B, a T_1-weighted coronal image shows contrast enhancement of the meninges in Patient 3 (arrows).

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Table 1. Characteristics and Symptoms of the Patients and Imaging Results.

Characteristic	Patient 1	Patient 2	Patient 3
Age (yr)	63	62	72
Clinical diagnosis	Encephalitis	Encephalitis	Meningoencephalitis
Month of disease onset	November 2011	June 2013*	November 2013
Estimated time from onset of clinical symptoms until death (mo)	3	2*	4
Preexisting medical conditions	Hypertension	Hypertension, type 2 diabetes, renal insufficiency	Hypertension, obesity
Initial symptoms	Severe fatigue, constipation, anorexia, nocturnal agitation, confusion, psychomotor slowing, shivers	Weakness, vertigo, unsteady gait, headache, abdominal pain, confusion, psychomotor slowing, shivers, fever	Unsteady gait, anogenital numbness, problems passing urine and stool, headache, confusion, psychomotor slowing, mood disturbances, fever
Later symptoms	Myoclonus, grimacing, divergent bulbi, tetraparesis, sopor, coma	Myoclonus, opsoclonus, sopor, coma	Ocular paresis, sopor, coma
Additional diagnoses during illness	Bilateral crural-vein thrombosis, hepatopathy, bronchopulmonary infection due to assumed aspiration	Bilateral crural-vein thrombosis leading to pulmonary embolism; bronchopulmonary infection due to assumed aspiration	Bilateral crural-vein thrombosis leading to pulmonary embolism; bronchopulmonary infection due to assumed aspiration
Magnetic resonance imaging timing and findings			
Initial	December 2011: normal	July 2013: normal	December 2013: dilated ventricles
Follow-up	3 weeks after initial scan: hyperintense (T_2-weighted imaging) lesions in temporal, parietal, and insular cortex and midbrain; spine not affected	10 days after initial scan: hyperintense (T_2-weighted imaging), partly symmetric lesions in temporal, parietal, and frontal cortex, basal ganglia, and brainstem; spine not affected	6 days after initial scan: meningeal contrast enhancement; spine not affected
Electroencephalography timing and findings	December 2011: delta activity with short alpha episodes	August 2013: low-voltage theta and delta activity	December 2013: delta and theta activity
Antiinfective treatment	Ceftriaxone, ciprofloxacin, carbapenems, doxycycline, glucocorticoids†	Ceftriaxone, ampicillin, clindamycin, carbapenems, erythromycin, acyclovir, glucocorticoids‡	Ceftriaxone, ampicillin, acyclovir, doxycycline, ribavirin, tuberculosis treatment§

* This patient had peripheral facial nerve palsy that developed in April 2013; the palsy subsided after glucocorticoid treatment.
† This patient was highly positive for anti-Yo (Purkinje cell) autoantibody in the cerebrospinal fluid (CSF) but not in the serum and was therefore given a single high dose of methylprednisolone; however, a full-body computed tomographic scan did not reveal any neoplasia. Paraneoplastic autoimmune serologic testing of the serum and CSF in all three patients did not reveal any other autoantibodies, such as anti-Yo (except in Patient 1), anti-Hu, anti-Ri, anti-amphiphysin, anti–NMDA receptor, anti–glutamate receptor, and anti-CV2.
‡ Single-dose methylprednisolone was administered, because initially an autoimmune encephalitis was considered on the basis of the patient's clinical presentation.
§ This patient had positive results of a *Mycobacterium tuberculosis* interferon release assay and was treated with ethambutol, isoniazid, rifampin, and pyrazinamide.

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humans and squirrels (Fig. S1 in the Supplementary Appendix).

REAL-TIME RT-QPCR DETECTION

On the basis of sequences obtained from a metagenomic analysis of the squirrel samples, two independent primer–probe RT-qPCR systems were established, corresponding to two different regions within the bornavirus genome (Table S4 in the Supplementary Appendix). Both systems used standard RT-qPCR reagents and cycling conditions and were combined with an internal control system, as described elsewhere.[4] To rule out endogenous DNA sequences as the source of virus sequence amplification, qPCR analysis was also performed without reverse transcription.

IMMUNOHISTOCHEMICAL ANALYSIS

A standard staining protocol was applied with the use of bornavirus-specific polyclonal and monoclonal antibodies that recognized the viral proteins N, X, and phosphoprotein (P), as described previously.[5,6] A BoDV-positive horse sample and the 10 above-mentioned unrelated human brain-tissue samples were used as controls (Fig. S2A, S2B, and S3 in the Supplementary Appendix).

INDIRECT IMMUNOFLUORESCENCE ASSAY

For the detection of bornavirus-specific IgG antibodies in the serum and in the cerebrospinal fluid from Patient 3, a persistently BoDV-infected cell line was used in a standard indirect immunofluorescence procedure (Fig. S4 in the Supplementary Appendix). The specificity of this serologic assay was confirmed through the investigation of 40 serum samples obtained from febrile patients; all of the samples tested negative. For confirmation, the serum was titrated in a validated routine immunofluorescence assay for the detection of bornavirus-specific antibodies.

RESULTS

METAGENOMIC ANALYSIS

The metagenomic analysis revealed five sequence fragments that had 70.3% to 81.2% identity with isolates of the species *Mammalian 1 bornavirus* in samples L00652 (liver, lung, and kidney) and L00651 (chest-cavity fluid) (Table S5 in the Supplementary Appendix). Targeted screening of the sequencing reads from the remaining pools additionally detected 23 reads related to *Mammalian 1 bornavirus* sequences (identities between 67.6% and 81.7%) (Table S5 in the Supplementary Appendix). The fragments were related to both mammalian and avian bornavirus sequences, and the virus was tentatively named variegated squirrel 1 bornavirus (VSBV-1). Gross pathological examination of the squirrel did not reveal any specific changes; histologically, however, the brain was found to have satellitosis and mild glial activation.

REAL-TIME RT-QPCR ANALYSES

With the use of two independent RT-qPCR systems, VSBV-1 RNA was found in various sample materials from the squirrel and in the samples available from all three human patients (Table 2). High VSBV-1 RNA loads were observed in the squirrel brain, heart, lung, kidney, and oropharyngeal-swab samples. In contrast, in EDTA-treated blood and chest-cavity fluid, only low VSBV-1 RNA loads were detected. Intermediate viral genome loads could be ascertained in FFPE brain samples from Patients 1 and 2. An analysis of fresh-frozen material from Patient 3 revealed high VSBV-1 RNA loads in the brain (Table 2). In all the investigations, the VSBV-1 genome could be detected only when assays including reverse transcription were used; results remained negative in assays without reverse transcription. Moreover, all the control materials from patients with unrelated brain diseases and from healthy persons tested negative in both PCR systems.

WHOLE-GENOME SEQUENCING AND PHYLOGENETIC ANALYSIS

RNA from the brain samples obtained from the squirrel and from Patient 3 was deep-sequenced to determine the viral genomes for in-depth analyses. Sequencing yielded two nearly identical complete coding sequences (8798 nucleotides; accession numbers LN713680 and LN713681) with two synonymous exchanges at positions 1857 (amino acid 619) and 3702 (amino acid 1234) within the L gene. Annotation of these sequences revealed a canonical bornavirus genome structure (the genes encoding N, X, P, M, G, and L). Phylogenetic analyses of the complete coding sequence and *N* sequence showed that this novel bornavirus forms a distinct lineage within the bornavirus phylogeny in a sister relationship with the *Mammalian 1 bornavirus* lineage (Fig. 2, and Fig. S1 in the Supplementary Appendix). Further-

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Table 2. Real-Time RT-qPCR Results for Samples Obtained from the Squirrel and the Three Patients.*

Origin and Sample	Quantification Cycle Value†	
	VSBV-1 Assay 6	VSBV-1 Assay 10
Squirrel		
EDTA-treated blood	35.7	33.0
Chest-cavity fluid	32.0	28.4
Brain	13.2	13.1
Heart	12.4	14.1
Lung	13.3	13.8
Liver	18.2	18.9
Spleen	17.2	17.4
Kidney	11.0	11.7
Colon	19.1	18.5
Oropharyngeal swab	14.7	15.5
Patient 1 FFPE brain	25.9	24.0
Patient 2 FFPE brain	20.8	19.0
Patient 3		
Serum	29.1	27.8
CSF	30.5	26.3
Brain	12.7	12.6

* For a description of the reverse-transcriptase quantitative polymerase-chain-reaction (RT-qPCR) assays, see the Supplementary Appendix. FFPE denotes formalin-fixed and paraffin-embedded, and VSBV-1 variegated squirrel 1 bornavirus.

† Quantification cycle values denote the cycle during qPCR in which a positive fluorescence signal can be differentiated from the background. Low values indicate higher initial genome copy numbers of the target, and higher values indicate smaller genome copy numbers. A difference by factor of approximately 3.3 in the value corresponds to a $\log_{10}$ difference in the number of genome copies.

more, previously described endogenous N-derived bornavirus-like sequences from squirrels[8] and humans[9] were distantly related to the previously unknown VSBV-1 sequences (Fig. S1 in the Supplementary Appendix). The relationships in the time-resolved phylogeny (Fig. S5 in the Supplementary Appendix), as well as the fact that the VSBV-1 is most closely related to BoDV of horse origin, imply that VSBV-1 emerged from a mammalian bornavirus rather than from an avian one. In accordance with the latest criteria proposed by the International Committee for Taxonomy of Viruses Bornaviridae Study Group,[7] on the basis of the phylogenetic analyses and the nucleotide sequence identities of less than 75% between this bornavirus and those of the most closely related classic *Mammalian 1 bornavirus* sequences, VSBV-1 can be classified as a new bornavirus species (Table S6 in the Supplementary Appendix).

IMMUNOHISTOCHEMICAL ANALYSES

Brain-tissue sections from the squirrel and from Patient 1 were analyzed and showed positive immunostaining in nuclei, cytoplasm, and neuronal and glial processes of brain cells, as well as in the neuropil, when polyclonal (monospecific) antibodies for the detection of the viral N, P, and X proteins were applied (Fig. 3), but not when the monoclonal anti-N antibody was used. Whereas the unrelated human tissue samples and the control squirrel samples were negative (Fig. S2A and S2B in the Supplementary Appendix), the BoDV-positive horse brain had reaction patterns in the brain cells that were similar to the reaction patterns in the squirrel and Patient 1 with all of the applied antibodies (Fig. S3 in the Supplementary Appendix).

DETECTION OF BORNAVIRUS-SPECIFIC ANTIBODIES

Bornavirus-specific IgG antibodies were detected in serum and cerebrospinal fluid from Patient 3 with the use of an indirect immunofluorescence assay (Fig. S4 in the Supplementary Appendix). In both the cerebrospinal fluid and the serum, bornavirus-specific IgG antibody titers (1:2560 and 1:5120, respectively) were identified in a routine immunofluorescence assay that was validated for the detection of antibodies against BoDV.

DISCUSSION

We describe the detection of a variegated squirrel–derived bornavirus associated with the death of three people. The three patients had similar central nervous system (CNS) symptoms and died of progressive meningoencephalitis or encephalitis; bilateral crural-vein thrombosis also developed in all three during the clinical course of their illness. The reason for the thrombosis remains unclear. The spinal cord was not affected in any of the patients; all the lesions were found in the cortical areas, basal ganglia, or brainstem. All three patients were squirrel breeders and members of the same private squirrel-breeding association. Although these findings do not meet Koch's postulates, the fact that the complete coding sequences generated from the squirrel and the human sample material were almost

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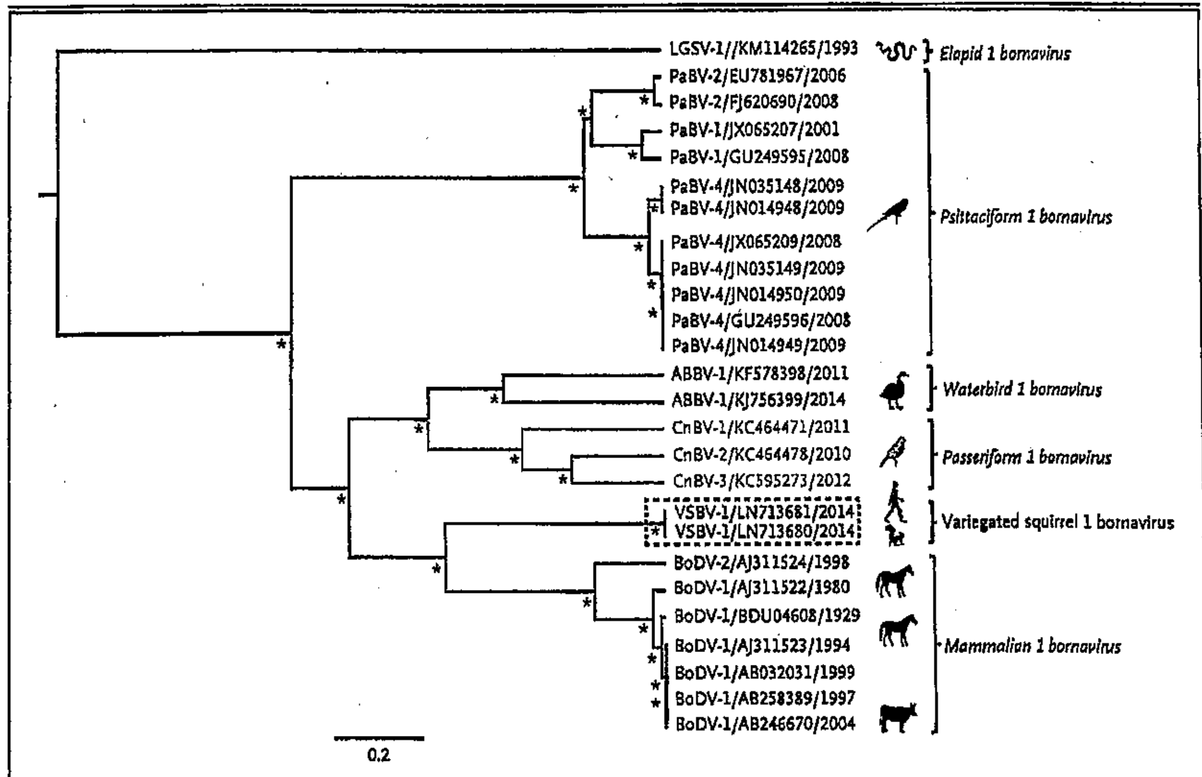

Figure 2. Phylogenetic Analysis of the Members of the Bornavirus Genus, Including the Putative Variegated Squirrel 1 Bornavirus (VSBV-1) Species.

The phylogenetic tree was inferred on the basis of complete coding sequences with the use of the Bayesian Markov chain Monte Carlo method and, in parallel, the neighbor-joining and maximum-likelihood methods. Statistical support of grouping from Bayesian posterior probabilities (clade credibilities ≥90%) and 1000 neighbor-joining and maximum-likelihood bootstrap replicates (≥70%) is indicated with an asterisk. The taxon information includes the virus abbreviation, GenBank accession number, and year of detection. Branches are colored according to lineage (within the bornavirus species classification and nomenclature proposed by the International Committee for Taxonomy of Viruses Bornaviridae Study Group,[7] with the exception of VSBV-1 [tentative, unclassified bornaviruses]). The VSBV-1 sequences generated during this study are highlighted. The scale bar represents nucleotide substitutions per site.

identical, the detection of viral RNA in the brain tissue of all three patients, the results of bornavirus antigen immunostaining, the similarity of the clinical picture among the three patients, the anti-bornavirus IgG titers in the serum as well as in the cerebrospinal fluid of Patient 3, and the epidemiologic link among all three cases support VSBV-1 as the likely causative agent.

Borna disease is described mainly in association with natural infections of horses and sheep, in which BoDV has a main tropism for the CNS, infecting neurons, astrocytes, oligodendrocytes, and ependymal cells.[10-13] The infection is noncytolytic and persistent, and in the natural hosts, mood, sensorium, sensibility, motility, and the autonomous nervous system are simultaneously or successively affected, with a fatal outcome in up to 90% of infected animals.[14] BoDV-like viruses, the so-called *Psittaciform 1 bornavirus* and *Passeriform 1 bornavirus*, have been described in association with fatal proventricular dilatation disease, mainly in psittacine birds.[6,15-17] Moreover, endogenous BoDV-like sequence fragments have already been detected in humans as well as in other species, including squirrels.[8,9] However, on the basis of the PCR data (suggesting high RNA loads) combined with the whole-genome information from the RNA fraction and the antibody response, it is unlikely that the virus sequences detected in this study are endogenous BoDV-like sequences.

In the 1990s, controversy arose regarding whether BoDV was a zoonotic agent responsible for human psychiatric disorders.[14,18] However,

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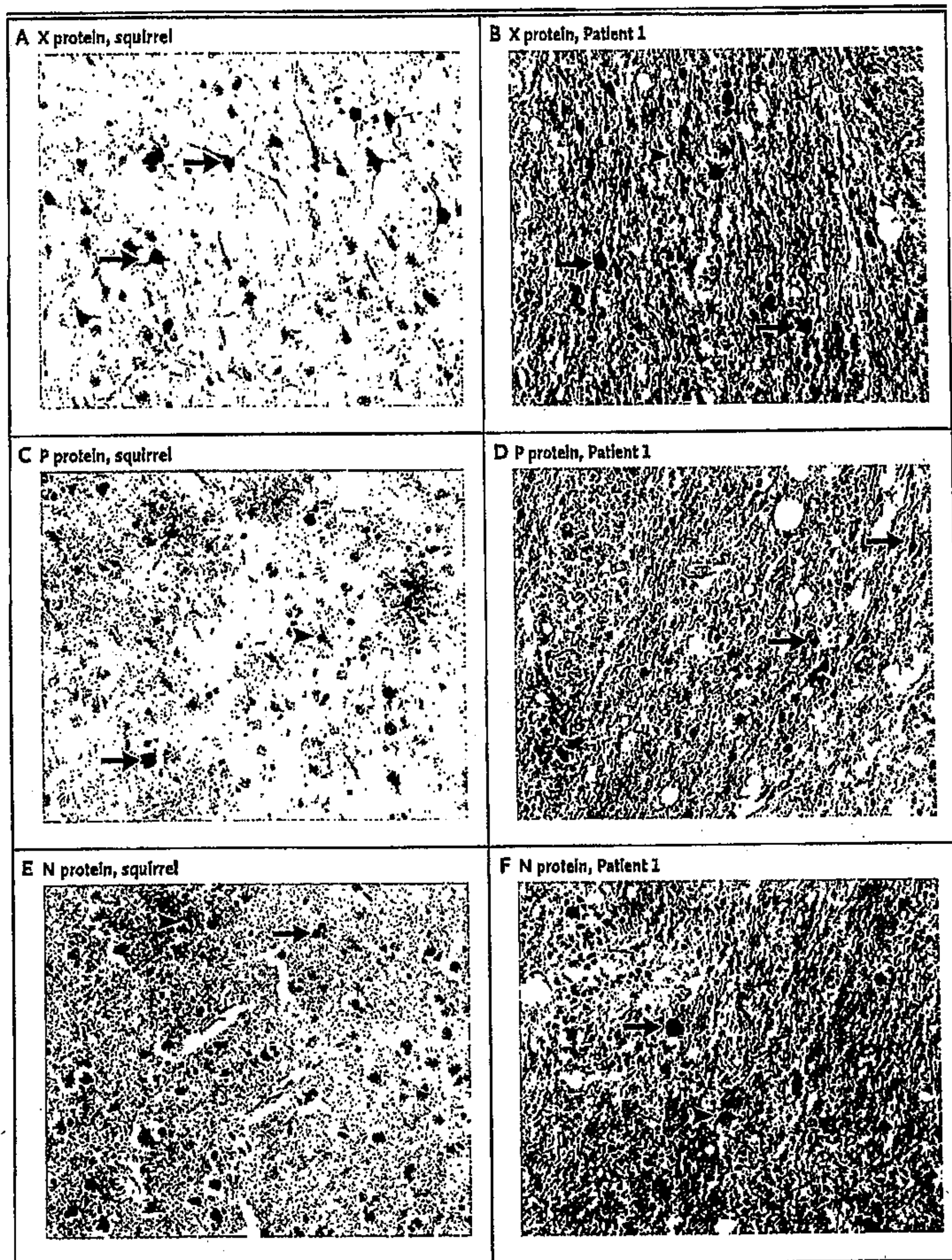

Figure 3. Viral Antigen Detected by Means of Immunohistochemical Analysis.
The presence of viral antigen in the squirrel (Panels A, C, and E) and Patient 1 (Panels B, D, and F) was shown in an immunohistochemical analysis with the use of monospecific polyclonal antibodies against the bornavirus proteins X (Panels A and B), P (Panels C and D), and N (Panels E and F). Viral antigen can be seen in neurons (black arrows), glial cells (black arrowheads), and neuropil. Viral antigen is present in nuclei and cytoplasm.

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several studies have questioned whether BoDV-induced clinical disease actually occurs in humans,[18-21] and the general consensus has been that it does not.[14,22] In contrast, VSBV-1 is different from the classic BoDV, and the RNA loads and antigen loads detected in all three human case patients were high, which allowed for whole-genome sequencing and immunohistochemical detection. Therefore, the human infections described in our study are quite different from those studied in previous investigations or referred to in previous discussions, and VSBV-1 is likely to be a previously unknown zoonotic pathogen transmitted by the variegated squirrel. Rodents, particularly exotic ones, are not uncommon as pets, as is reflected in the high number of such animals imported to Europe and other parts of the world.[23] In our study, all three patients were private squirrel breeders who had close contact with these animals. For two of the patients, skin injuries due to squirrel bites and scratches were reported by family members. However, the route of zoonotic transmission from the squirrels to the patients remains uncertain. The high RNA load in the oropharyngeal-swab sample from the squirrel might support the hypothesis of transmission through scratches or bites. The epidemiologic aspects of BoDV disease are not well understood either,[24] and in natural cases, infection through the olfactory route is suspected.

Of note, all three patients were older than 60 years of age and had preexisting medical conditions, which may have conferred a predisposition to clinical infection with this unusual agent. BoDV has been detected in shrews, which might be also able to transmit the virus to other hosts.[25-27] In addition, it remains to be elucidated whether VSBV-1 was carried by the squirrels when they were imported from Latin America or whether it originated from other small mammals that had contact with the breeding facility.

In conclusion, VSBV-1, a zoonotic bornavirus from a variegated squirrel, was associated with three fatal CNS infections in humans. Further studies, including seroepidemiologic and molecular studies in putative animal reservoirs and human patients, in particular those with unexplained encephalitis or meningoencephalitis, are needed.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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医薬品　研究報告　調査報告書

識別番号・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	－	研究報告の公表状況	http://www.promedmail.org/direct.php?id=3667822		公表国 米国	
販売名（企業名）	－					

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
輸血や血液製剤によって感染する新種のウイルスを科学者のチームが発見した。チームによると，このウイルスはＣ型肝炎ウイルスおよびヒト pegivirus とやや似ており、無害なものと思われる。科学者らはこのウイルスをヒト hepegivirus １型（HHpgV-1）と名付けた。1974 年～1980 年に 46 例のボランティアから採取し、National Institutes of Health に貯蔵されていた血液をチームが調べたところ、2 例のサンプルからウイルスが発見された。いずれの患者においてもウイルスは消失したと思われ、ウイルスにより疾患が発現したとの証拠はなかった。 これらボランティア全例が血友病の治療で血液製剤を使用しており、輸血を受けた後にウイルスが出現した。 別の研究では 106 人から、さらに２人にウイルスを検出したが、１例は５年間にわたり感染していたが２例ともウイルスは消失した。非常に多くのウイルスが存在するが、どのくらいのウイルスが血液を介して伝播しているかは不明であり、輸血の安全性を確実にするために特徴を明らかにする必要がある。	重要な基本的注意 ［患者への説明］ 本剤の投与又は処方にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

報告企業の意見	今後の対応
新しいウイルス（hepegivirus １型（HHpgV-1））が発見されたとの情報である。 ヒトに対して無害と考えられるとのことから、現時点での対応は必要ないと考えるが、情報に留意していきたい。	今後とも新種ウイルスに関する情報に留意していく。

⑥

NIHONSEIYAKU
2008-054

Published Date: 2015-09-24 13:53:23
Subject: PRO/EDR> Human pegivirus type 1: detection by sequence analysis
Archive Number: 20150924.3667822

HUMAN PEGIVIRUS TYPE 1: DETECTION BY SEQUENCE VIRUS
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A ProMED-mail post
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http://www.isid.org

Date: Tue 22 Sep 2015
Source: NBC News [edited]
http://www.nbcnews.com/health/health-news/new-virus-found-blood-supply-n431856

Scientists have found a new virus that can be transmitted by blood transfusions and other blood-based products.

It looks a little like the hepatitis C virus, which can cause permanent liver damage, and a little like the human pegivirus, which appears to be harmless, the team reports in the journal mBio.

They've named it human hepegivirus-1 (HHpgV-1).

"We have been able to find a new virus. It's clearly transmitted as a result of human (blood) transfusion," said infectious disease expert Dr Ian Lipkin, who oversaw the study team at Columbia University [in New York].

"It is the 1st transfusion-associated virus that's been described in a long time. We don't know if it is going to be a significant cause of human hepatitis," Lipkin told NBC News. "But probably not, the researchers said.

"So far there is no need to be concerned," said Amit Kapoor, an assistant professor at Columbia University who led the study. "We really don't know if there is ongoing transmission of this virus. It may be good for you." Lipkin agreed. "I'm not really worried about this particular virus," he said. "This is not SARS. This is not MERS. This is not HIV."

To find it, the team sampled blood banked at the National Institutes of Health from 46 volunteers between 1974 and 1980. They found it in 2 samples. Both patients appear to have "cleared" the virus and there is no evidence it caused any disease.

All the volunteers got blood products to treat hemophilia. The virus only showed up after they got transfusions.

Then they looked at samples from 106 people in another study, and found the virus in 2 more people. One appears to have been infected for as long as 5 years but both cleared the virus, too.

"We just don't know how many viruses are transmitted through the blood supply. There are so many viruses out there, and they need to be characterized in order to ensure that transfusions are safe," Kapoor said.

Hemophilia was once only treated with blood products taken from people. Now it's often treated with genetically engineered products, which do not carry infectious agents.

Now that the genetic sequence of this new virus is published, Lipkin said, other scientists can start looking for it.

"More than 30 million blood components are transfused annually in the United States alone. Surveillance for infectious agents in the blood supply is key to ensuring the safety of this critical resource for medicine and public health," the researchers wrote.

In the same issue of mBio, another team led by Lipkin reports they found a new virus related to hepatitis A virus in seals. It doesn't seem to make the seals sick but it's a possible ancestor of hepatitis A, they wrote.

"This finding suggests that the diversity and evolutionary history of these viruses might be far greater than previously thought and may provide insight into the origin and pathogenicity of hepatitis A virus," they wrote.

They've named it phopivirus.

[Byline: Maggie Fox]

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Communicated by:
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<promed@promedmail.org>

[This report documents the characterisation of an infectious agent from human serum that has been characterised as a hepatitis C, which is not associated with human disease. Another such infectious agent has been detected in the serum of seals, but is not associated with any known virus in seals. Such agent was detected by its sequence characteristics. Sequence analysis alone has not so far proved to be an efficient approach to the identification of potential infectious agents.

The reference of the report is
Kappor A, Kumar A, Simmonds, P, et al: Virome analysis of transfusion recipients reveals a novel human virus that shares genomic features with hepaciviruses and pegiviruses. mBio 6(5): e01466-15. doi:10.1128/mBio.01466-15; available at http://mbio.asm.org/content/6/5/e01466-15.

Abstract

To investigate the transmission of novel infectious agents by blood transfusion, we studied changes in the virome composition of blood transfusion recipients pre- and post-transfusion. Using this approach, we detected and genetically characterized a novel human virus, human hepegivirus 1 (HHpgV-1), that shares features with hepatitis C virus (HCV) and human pegivirus (HPgV; formerly called GB virus C or hepatitis G virus). HCV and HPgV belong to the genera _Hepacivirus_ and _Pegivirus_ of the family _Flaviviridae_. HHpgV-1 was found in serum samples from 2 blood transfusion recipients and 2 hemophilia patients who had received plasma-derived clotting factor concentrates. In the former, the virus was detected only in the post-transfusion samples, indicating blood-borne transmission. Both hemophiliacs were persistently viremic over periods of at least 201 and 1,981 days. The 5' untranslated region (UTR) of HHpgV-1 contained a type IV internal ribosome entry site (IRES), structurally similar to although highly divergent in sequence from that of HCV and other hepaciviruses. However, phylogenetic analysis of nonstructural genes (NS3 and NS5B) showed that HHpgV-1 forms a branch within the pegivirus clade distinct from HPgV and homologs infecting other mammalian species. In common with some pegivirus variants infecting rodents and bats, the HHpgV-1 genome encodes a short, highly basic protein upstream of E1, potentially possessing a core-like function in packaging RNA during assembly. Identification of this new human virus, HHpgV-1, expands our knowledge of the range of genome configurations of these viruses and may lead to a reevaluation of the original criteria by which the genera _Hepacivirus_ and _Pegivirus_ are defined.

Importance

More than 30 million blood components are transfused annually in the United States alone. Surveillance for infectious agents in the blood supply is key to ensuring the safety of this critical resource for medicine and public health. Here, we report the identification of a new and highly diverse HCV/GB virus (GBV)-like virus from human serum samples. This new virus, human hepegivirus 1 (HHpgV-1), was found in serum samples from blood transfusion recipients, indicating its potential for transmission via transfusion products. We also found persistent long-term HHpgV-1 viremia in two hemophilia patients. HHpgV-1 is unique because it shares genetic similarity with both highly pathogenic HCV and the apparently nonpathogenic HPgV (GBV-C). Our results add to the list of human viruses and provide data to develop reagents to study virus transmission and disease association and for interrupting virus transmission and new human infections. - Mod.CP]

....sb/cp/mj/dk

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別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2015年12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	人C1-インアクチベーター		研究報告の公表状況	http://www.promedmail.org/post/3864342	公表国 米国	
販売名（企業名）	ベリナートP静注用500 （CSLベーリング株式会社）					
研究報告の概要	University of California, San Francisco-Abbott Viral Diagnostics and Discovery Centerの研究者主導のチームが，C型肝炎ウイルス（HCV）に感染したヒトにおいて新規のヒトpegivirusを特定した。PLOS Pathogensに報告されたように，この研究者らは慢性肝疾患患者150例以上の血液サンプルについてメタゲノム解析を行い，HCVに感染者で敗血症により死亡した患者1例においてヒトpegivirus 2型（HPgV-2）に相当するものを同定した。研究チームはさらにスクリーニングを行った結果，HPgV-2陽性サンプル11例を見出し，全例がHCV感染患者からのサンプルであった。最初に同定されたHPgV-2は，Columbia Universityなどの研究者によりmBioに報告されている，ヒトhepegivirus 1型と名付けられたヒトpegivirusと約96%相同であった。					使用上の注意記載状況・ その他参考事項等

報告企業の意見	今後の対応
ヒトpegivirus 2型はC型肝炎ウイルスと同じフラビウイルス科に属する新たに発見されたウイルスである。フラビウイルス科はエンベロープを持つ一本鎖RNAウイルスである。製品の製造工程にはウイルス除去・不活化工程があり、C型肝炎ウイルスのモデルウイルス及び各種モデルウイルスを用いたウイルスクリアランス試験で効果が認められていることから、新型ウイルスに対しても安全対策は取られていると考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

Published Date: 2015-12-15 20:12:53
Subject: PRO/EDR> Human pegivirus 2: description
Archive Number: 20151215.3864342

HUMAN PEGIVIRUS 2: DESCRIPTION
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A ProMED-mail post
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http://www.isid.org

Date: Mon 14 Dec 2015
Source: Genome Web [edited]
https://goo.gl/3kCttN

A team led by investigators at the University of California, San Francisco-Abbott Viral Diagnostics and Discovery Center has identified a new humans pegivirus in a subset of individuals infected with hepatitis C virus (HCV). As they reported in PLOS Pathogens, the researchers did metagenomic sequencing on blood samples from more than 150 individuals with chronic liver disease, identifying a handful of reads corresponding to human pegivirus 2, or HPgV-2, in an individual who had been infected with HCV and died from sepsis.

Using new diagnostic assays aimed at the virus, the team screened thousands more blood samples, turning up 11 more HPgV-2-postive samples, all from individuals with HCV. Along with an analysis of complete or partial genome sequences for the viruses, the group characterized the patients' antibody responses to the HPgV-2 to start unraveling its transmission patterns and potential consequences for infected individuals. "Our next step is to explore whether this new virus can cause disease, and if so, work with blood banks to continue to help safeguard the world's blood supply against these types of new viruses," co-author John Hackett, Jr., vice president of applied research and technology at Abbott Laboratories, said in a statement. The HPgV-2 that Hackett and his colleagues identified in the 1st of the HCV patients was roughly 96 percent identical to a human pegivirus described in mBio by researchers from Columbia University and elsewhere. That virus, dubbed human hepegivirus 1 (HHPgV-1), was identified in blood transfusion recipients in the US or Europe. Meanwhile, studies going back a decade hinted at the presence of an apparently non-pathogenic human pegivirus -- known as HPgV-1 or GBV-C -- in blood samples from some hepatitis patients.

With that in mind, Chiu and colleagues did metagenomic sequencing on blood samples from 169 individuals who had been treated for chronic liver disease at the University of Chicago Medical Center. With the help of a bioinformatics pipeline focused on pathogen detection, they narrowed in on HPgV-2 reads in the index patient, a deceased 70-year-old woman with a history of sickle cell disease, HCV infection, and kidney problems. Through deeper Illumina sequencing coupled with Sanger sequencing, the team put together an almost 9900 base draft genome assembly for HPgV-2, which appeared to be distantly related to pegiviruses found in bat and rodent hosts. Additional metagenomic sequencing on another blood sample from the same individual confirmed the HPgV-2 infection and produced reads that the researchers used to assemble an independent HPgV-2 genome assembly that was nearly identical to the original draft sequence. To get a sense of how widespread HPgV-2 infection might be, they used a combination of multiplex RT-PCR and serological assays to screen thousands more blood samples from individuals with or without HCV, HIV, and/or hepatitis B virus infection. In the process, the team narrowed in on 11 samples with HPgV-2 RNA. All of the infected individuals also had HCV. On the other hand, the new pegivirus did not turn up in almost 1500 screened samples from virus-free individuals nor in almost 500 individuals infected with HIV alone.

Based on complete genomes for 8 of the HPgV-2 isolates and partial genome sequences for 4 more, the researchers saw some 93 to 94 percent identity between the HPgV-2 strains identified in the study. Finally, although HPgV-2 infection was only identified in those with HCV, the study's authors did see some HCV-free

individuals with apparent antibodies to the virus, hinting that they may have successfully cleared the virus. Such findings suggest HCV co-infection contributes to HPgV-2 persistence, they speculated, though further research is needed to explore the prevalence and pathogenic potential of HPgV-2.

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[The manuscript citation and abstract is:
Berg MG, Lee D, Coller K, et al: Discovery of a novel human pegivirus in blood associated with hepatitis C virus co-infection. PLOS Pathogens 2015; 10.1371/journal.ppat.1005325

Abstract:
"Hepatitis C virus (HCV) and human pegivirus (HPgV), formerly GBV-C, are the only known human viruses in the Hepacivirus and Pegivirus genera, respectively, of the family Flaviviridae. We present the discovery of a 2nd pegivirus, provisionally designated human pegivirus 2 (HPgV-2), by next-generation sequencing of plasma from an HCV-infected patient with multiple bloodborne exposures who died from sepsis of unknown etiology. HPgV-2 is highly divergent, situated on a deep phylogenetic branch in a clade that includes rodent and bat pegiviruses, with which it shares less than 32 percent amino acid identity. Molecular and serological tools were developed and validated for high-throughput screening of plasma samples, and a panel of 3 independent serological markers strongly correlated antibody responses with viral RNA positivity (99.9 percent negative predictive value). Discovery of 11 additional RNA-positive samples from a total of 2440 screened (0.45 percent) revealed 93-94 percent nucleotide identity between HPgV-2 strains. All 12 HPgV-2 RNA-positive cases were identified in individuals also testing positive for HCV RNA (12 of 983; 1.22 percent), including 2 samples co-infected with HIV, but HPgV-2 RNA was not detected in non-HCV-infected individuals (p less than 0.0001), including those singly infected by HIV (p = 0.0075) or HBV (p = 0.0077), nor in volunteer blood donors (p = 0.0082). 9 of the 12 (75 percent) HPgV-2 RNA positive samples were reactive for antibodies to viral serologic markers, whereas only 28 of 2429 (1.15 percent) HPgV-2 RNA negative samples were seropositive. Longitudinal sampling in 2 individuals revealed that active HPgV-2 infection can persist in blood for at least 7 weeks, despite the presence of virus-specific antibodies. One individual harboring both HPgV-2 and HCV RNA was found to be seronegative for both viruses, suggesting a high likelihood of simultaneous acquisition of HCV and HPgV-2 infection from an acute co-transmission event. Taken together, our results indicate that HPgV-2 is a novel bloodborne infectious virus of humans and likely transmitted via the parenteral route."

This virus is a newly described member of the human pegiviruses. These viruses were 1st described in 1967 when the experimental inoculation of the serum of a surgeon (initials GB) caused acute hepatitis in tamarin monkeys. Eventually, 2 viruses (GBV-A and GBV-B) were characterized. Subsequently, another related human virus named GBV-C was described and has been referred to as hepatitis G virus although this is a misnomer as it does not replicate in the liver or cause liver disease per se although GBV-B does. These 3 viruses were classified into a new genus called Pegivirus within the family Flaviviridae (which contains the hepatitis C virus). A distantly related bat virus has also been classified in the pegivirus genus.

HPgV (formerly GB-C or hepatitis G virus), or HPgV 1, is estimated to actively be infecting about 750 million individuals worldwide with at least that many showing evidence of previous infection. It appears to replicate in lymphocytes (both T and B), monocytes and natural killer (NK) cells and, mostly based on extensive work by my friend and colleague Jack Stapleton at the University of Iowa, appears to modulate host immune activation with a positive effect on HIV infection (1) and Ebola virus infection (2) and perhaps other human virus infections. An excellent review of the host immunomodulation has recently been published (3).

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1. Stapleton JT, Chaloner K, Martenson JA, et al: GB virus C is associated with altered lymphocyte subset distribution and reduced T cell activation and proliferation in HIV-infected individuals. PloS One 7, e50563.
2. Lauck M, Bailey KG, Andersen KG, et al: GB virus C. Co-infections in west Africa Ebola patients. J Virol 2015; 89:2425-2429
3. Chivero ET, Stapleton JT: Troposm of human pegivirus (formerly known as GB virus C/hepatitis G virus) and host immunomodulation: insights into a highly successful viral infection. J Gen Virol. 2015;96:1521-1532 -
Mod.LL]

See Also

Human pegivirus type 1: detection by sequence analysis 20150924.3667822

....sb/ll/msp/dk

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*【組成・性状】

1. 組成

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添加物	グリシン	100mg	
	クエン酸ナトリウム水和物	30mg	
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【効能又は効果】
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【用法及び用量】
1. 用法
本剤を添付の日局注射用水全量で徐々に溶解し、直接静注するか、点滴静注する。直接静注の場合は、緩徐に行う。

2. 用量
通常、成人には1,000～1,500国際単位を投与する。本剤投与後、数時間以内に効果の発現が認められないか、あるいは、不十分な場合には、500～1,000国際単位を追加投与する。また、24時間後でも症状の改善が不十分な場合には、その症状に応じて繰り返し投与する。

【使用上の注意】
1. 慎重投与（次の患者には慎重に投与すること）
(1)溶血性・失血性貧血の患者［ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
(2)免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］

2. 重要な基本的注意
［患者への説明］
本剤の使用にあたっては疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを患者に対して説明し、理解を得るよう努めること。

*(1)本剤の原材料となる血漿については、HBs抗原、抗HCV抗体、抗HIV-1抗体及び抗HIV-2抗体が陰性であることを確認している。さらに、プールした試験血漿については、HIV、HBV、HCV及びHAV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。また、ヒトパルボウイルスB19についてもNATによるスクリーニングを実施し、適合した血漿を用いている。
その後の製造工程である60℃、10時間液状加熱処理及びナノフィルトレーションは、HIVをはじめとする各種ウイルス除去・不活化効果を有することが確認されているが、投与に際しては、次の点に十分注意すること。
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。

(3)肝炎ウイルス等の感染症の危険性を完全に否定できないので、観察を十分に行い、肝障害があらわれた場合には適切な処置を行うこと。

(4)頻回輸注した場合、患者の血清中にC1-インアクチベーターに対するインヒビターの発生を完全に否定できないので、観察を十分に行うこと。

3. 副作用

国内での総投与例7例中、本剤との因果関係が疑われる副作用は認められなかった。また、本剤に起因すると思われる臨床検査値異常も認められなかった。[1,2] なお、国外では過敏症が2例報告されている。(承認時)
市販後には使用成績調査で77例中2例にALT (GPT)、Al-Pの上昇各2件、AST (GOT)、γ-GTPの上昇、好酸球増多各1件が報告された。(再審査終了時)

(1)重大な副作用

*ショック、アナフィラキシー(頻度不明)…ショック、アナフィラキシーがあらわれるおそれがあるので、観察を十分に行い、頻脈、血圧上昇、血圧低下、潮紅、蕁麻疹、呼吸困難、頭痛、めまい、悪心等が認められた場合には直ちに投与を中止し、適切な処置を行うこと。なお、アナフィラキシーは遺伝性血管性浮腫の発作と同様の症状を示すため、観察を十分に行うこと。

(2)その他の副作用

	頻度不明
過敏症	発疹、発熱、発赤等
投与部位	注射部位反応

4. 高齢者への投与

一般に高齢者では生理機能が低下しているので、患者の状態を観察しながら慎重に投与すること。

5. 妊婦、産婦、授乳婦等への投与

妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。[妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害(流産、胎児水腫、胎児死亡)が起こる可能性がある。]

6. 小児等への投与

低出生体重児、新生児、乳児、幼児又は小児に対する安全性は確立していない。

7. 適用上の注意

調製時:

(1)溶解時に著しい沈殿の認められるものは投与しないこと。

(2)他剤との混合注射は避けることが望ましい。

(3)本剤は溶解後ただちに使用すること。

(4)一部を使用した残液は、細菌汚染の恐れがあるので使用しないこと。

8. その他の注意

海外において、体外循環下の心臓外科手術の前後でのCapillary Leak Syndrome (毛細血管漏出症候群) を予防または治療するために本剤を投与した場合において、致死的な血栓症を発現したとの報告がある。(承認外用法・用量)

【 薬物動態 】[3]

遺伝性血管性浮腫患者に本剤を投与したときの最高血中濃度到達時間、血中半減期、回収率、上昇率は以下のとおりである(外国人データ)。

		18歳未満(6例)	18歳以上(19例)
投与量(国際単位/kg)	平均±標準偏差	16.2±4.9	15.2±3.3
最高血中濃度到達時間(hr)	中央値(範囲)	0.6(0.1−2.0)	1.0(0.3−32.0)
血中半減期 (hr)		32.9(7.3−70.5)	39.1(19.4−90.4)
回収率 (%)[注1]		98.2(69.2−106.8)	74.8(57.2−195.9)
上昇率(%/国際単位/kg)[注2]		2.2(1.7−2.6)	2.0(1.5−5.1)

注1) 理論的上昇期待値に対する実測上昇値の百分率
注2) 投与後のC1-インアクチベーター活性が最高に達した時点の上昇率

【 臨床成績 】[1,2]

遺伝性血管性浮腫患者のうち急性発作を発症した患者3例に1,000~2,500国際単位を投与した臨床試験において、全症例とも有効以上であった。

【 薬効薬理 】[4~6]

C1-インアクチベーターの作用
C1-インアクチベーターは分子量105kDaの糖蛋白で、補体成分C1r及びC1sの他、血液凝固・線溶系の第XIIa因子、第XIa因子、プラスミン及びカリクレインに対して阻止作用を有する。C1-インアクチベーターは、1対1のモル比で前述の活性物質と複合体を形成することによって、その活性を阻害する。

【 取扱い上の注意 】

本剤は特定生物由来製品に該当することから、本剤を投与又は処方した場合は、医薬品名(販売名)、その製造番号(ロット番号)、投与又は処方した日、投与又は処方を受けた患者の氏名、住所等を記録し、使用日から少なくとも20年間保存すること。

**【 包 装 】

500国際単位1バイアル
(日局注射用水 10mL×1バイアル、薬液用両刃針添付)

【 主要文献 】

1) 児玉順三 他:診療と新薬, 24(10), 2041, 1987
2) 笹田昌孝 他:産婦人科の進歩, 42(1), 124, 1990
3) Martinez-Saguer, I., et al.: Transfusion, 50(2), 354, 2010
4) Agostoni, A., et al.: J. Allergy Clin. Immunol., 114(3), S51, 2004
5) Cicardi, M., et al.: Springer Semin. Immun., 27(3), 286, 2005
6) Davis III, A.E., et al.: Mol. Immunol., 45(16), 4057, 2008

【 文献請求先 】

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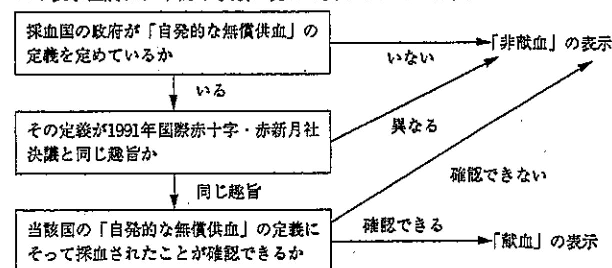
0120-534-587 FAX(03)3534-5861

【 献血又は非献血の区別の考え方 】

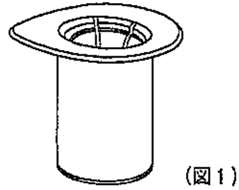
献血又は非献血の区別は製剤の安全性の優劣を示すものではありません。この表示区別は、下記の手順に従って決められています。



[ベリナートP静注用500の使用方法]

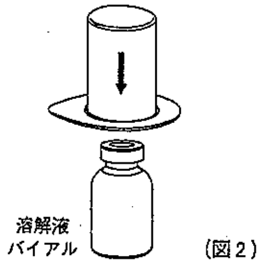
1. 薬剤バイアル及び溶解液バイアルを室温に戻す。両バイアルのプラスチックキャップをはずし、ゴム栓をアルコール綿等で消毒する。

2. 溶解器（薬液用両刃針）のシールを完全にはがして開封する。ブリスター包装から取り出さないこと。（図1）



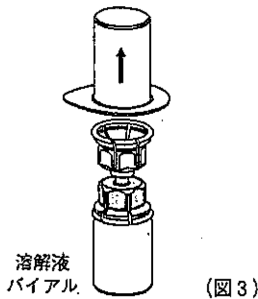
（図1）

3. 溶解液バイアルを水平の台に置き、しっかりと握る。溶解器をブリスター包装に入れたままの状態で取り、青色側アダプターの穿刺部を、溶解液バイアルのゴム栓にまっすぐ下向きに刺しこむ。（図2）



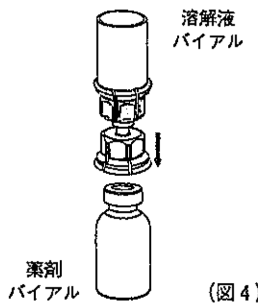
（図2）

4. ブリスター包装の縁をつかみ、ブリスター包装のみを垂直に引き上げ、溶解器から慎重に取り外す。このとき、溶解器を一緒に引き上げないよう注意する。（図3）



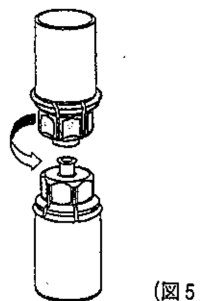
（図3）

5. 薬剤バイアルを水平の台に置き、しっかりと握る。溶解器を付けた溶解液バイアルを逆さまにして、バイアル全体をしっかりと握り、溶解器の透明側アダプターの穿刺部を薬剤バイアルのゴム栓にまっすぐ下向きに刺し込む。このとき溶解液が薬剤バイアル中に移行します。（図4）



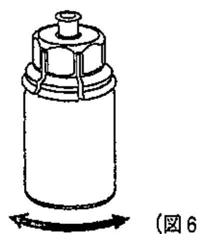
（図4）

6. 片手で青色の部分をつかみ、もう片方の手で透明な部分をつかみ、慎重に回して二つに分ける。（図5）



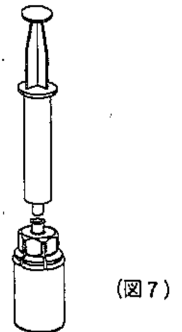
（図5）

7. 透明な部分を付けたまま、薬剤バイアルを泡立てないように緩やかに揺り動かして完全に溶解する。バイアルを振らないこと。（図6）



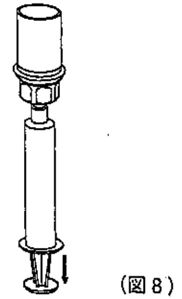
（図6）

8. 空の滅菌済注射器に空気を吸い込む。薬剤バイアルが直立した状態で、注射器を溶解器のルアーロックに接続し、薬剤バイアルの中に空気を注入する。（図7）



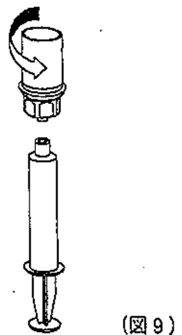
（図7）

9. 注射器のプランジャーを押したまま、薬剤バイアルごと全体を上下逆さまにして、プランジャーをゆっくりと引っ張りながら、薬液を注射器の中に吸引する。（図8）



（図8）

10. 薬液が注射器の中に移行したら、注射器のプランジャーを下向きにしたままの状態で、溶解器を注射器から取り外す。（図9）



（図9）

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医薬品　研究報告　調査報告書

識別番号・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
			2015. 10. 5	該当なし	
一般的名称	人赤血球液	研究報告の公表状況	M Kozakai1, M Matsumoto, C Matsumoto, S Uchida, M Satake, K Tadokoro. AABB Annual Meeting Anaheim, CA, October 24-27, 2015 SP325	公表国 日本	
販売名（企業名）	赤血球液-LR「日赤」（日本赤十字社） 照射赤血球液-LR「日赤」（日本赤十字社）				

研究報告の概要

○血小板製剤から*Lactococcus garvieae* が分離された初めての例

【背景】日本では血小板製剤の使用期限は4日間という短い期間を設定し、厳しい外観検査により汚染のない製品の供給に努めているが、それでも細菌汚染事例は年間数例発生している。2013年4月から、凝集塊形成などの異常により血液センターの供給部門や医療機関から回収された血小板製剤には無菌試験を実施している。今回、血小板製剤から分離された菌株について同定試験を行ったところ、生化学試験法と遺伝子解析法の二法で結果が乖離したことから、この菌株についてさらに検討を行った。【研究計画】BacT/ALERT（自動培養装置）と平板培養法により血小板製剤から菌を分離した。菌種同定は生化学試験法であるVITEK2（自動同定装置）と16S rRNA遺伝子(以下、16S rDNAとする)解析法を用い、最近縁種と判定された菌種とその類近縁種について、16S rDNAから系統樹を作成した。加えて、分離された菌の低温10℃下での増殖性を観察した。【結果】細菌が混入した血小板製剤は凝集塊の形成により供給部門で検出され、採血から11日後に検査室に持ち込まれた。到着した時点での菌数は1.7×10^{8} CFU/mLで、検鏡でグラム陽性球菌が観察された。最近縁種はVITEK2で*Streptococcus alactolyticus* (86%)、16S rDNA解析で*Lactococcus garvieae* (100%)と判定された。16S rDNA解析では当該菌株と*L. garvieae*基準株との相同性が99%である一方、*S. alactolyticus*基準株との相同性が90%で、系統樹において異なるクラスターに分かれた。さらに低温10℃下での増殖が確認され、当該菌株は、*L. garvieae*である可能性が高いと考えられた。【結論】*L. garvieae*は健常人の糞便から分離され、敗血症の原因となり得る細菌である。低温10℃下でも成長し、血小板製剤と同様に赤血球製剤においても深刻な汚染菌となり得ることから、血液の安全性の脅威となる好冷性細菌の一つに掲げるべきである。

使用上の注意記載状況・その他参考事項等

赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
販売部門の出庫前検品により凝集塊が確認された血小板製剤から*Lactococcus garvieae*が16S rDNA解析及び低温培養により初めて同定されたという報告である。	今後も細菌やウイルスの検出や不活化する方策について情報の収集及び安全対策に努める。

MedDRA/J Ver.18.1J

JRC2015T-033

SP325

First Case of *Lactococcus garvieae* Isolation from a Platelet Concentrate

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Background/Case Studies: All platelet concentrates (PCs) issued in Japan have a shelf life of 3.5 days. They are strictly inspected visually, to ensure the supply of contamination-free blood components. Several cases of bacterial contamination, however, occur each year. Since April 2013, we have conducted a sterility test of all PCs that were rejected by the supply division of blood centers or hospitals because of abnormal appearance, such as the formation of aggregates. *Staphylococcus aureus* has been the major species isolated, accounting for >75% of all cases. We have recently experienced a case in which discrepant results were obtained between the methods used in determining the bacterial species isolated from a PC, which required us to conduct further analyses. **Study Design/Methods:** Bacteria were isolated from a PC by using BacT/ALERT (bioMerieux) and by plate culture. Bacterial species were determined by analyzing biochemical properties using VITEK2 (bioMerieux) and sequencing a nearly complete 16S rDNA. A phylogenetic tree was constructed by using the 16S rDNA sequences of our isolate and species that were close to our isolate by the two identification methods. In addition, to observe growth activity at a low temperature, the bacterial isolate was cultured in HI broth at 10 . **Results/Findings:** A contaminated PC was detected at a supply division because of the presence of aggregates, and it was brought to our laboratory 11 days after blood donation. The bacterial concentration was 1.7×10^8 CFU/mL. Gram-positive cocci were observed under an optical microscope. *Streptococcus alactolyticus* and *Lactococcus garvieae* were identified to be the closest species by VITEK2 (86%) and 16S rDNA sequence analysis (100%), respectively. According to the 16S rDNA sequence analysis, the similarities of the isolate to type strains of *S. alactolyticus* and *L. garvieae* were 90% and 99%, respectively. The phylogenetic tree showed that these two species belong to different clusters. Furthermore, we observed that the isolate grew completely at 10°C. Therefore, the isolate was determined to be *L. garvieae*, not *S. alactolyticus*. **Conclusion:** *L. garvieae*, an agent that could cause sepsis in hosts, has been isolated from feces from healthy subjects. As *L. garvieae* can grow at a temperature as low as 10°C, it can seriously contaminate RBCs as well as PCs. Thus, *L. garvieae* should be listed as one of the cryophilic bacteria that could threaten blood safety. Analysis of the 16S rDNA sequence enabled us to identify a bacterial species that was difficult to classify by conventional biochemical tests. Although the major bacterial species frequently contaminating blood components are limited, unexpected strains could contaminate PCs. This fact implies the necessity for blood facilities to prepare multiple techniques, including genetic analysis, to identify the contaminating bacterial species.

Disclosure of Commercial Conflict of Interest

M. Kozakai: Nothing to disclose; C. Matsumoto: Nothing to disclose; M. Matsumoto: Nothing to disclose; M. Satake: Nothing to disclose; K. Tadokoro: No Answer; S. Uchida: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Kozakai: Nothing to disclose; C. Matsumoto: Nothing to disclose; M. Matsumoto: Nothing to disclose; M. Satake: Nothing to disclose; K. Tadokoro: No Answer; S. Uchida: Nothing to disclose

医薬品 研究報告 調査報告書

識別番号・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
				2015. 10. 5	該当なし	
一般的名称	人赤血球液		研究報告の公表状況	Robyn MP, Newman AP, Amato M, et al. MMWR Morb Mortal Wkly Rep. 2015 Oct 2;64(38):1071-3. doi: 10.15585/mmwr.mm6438a3.	公表国 米国	
販売名（企業名）	赤血球液-LR「日赤」（日本赤十字社） 照射赤血球液-LR「日赤」（日本赤十字社）					

研究報告の概要

○ドイツへ渡航してlive cell therapyを受けた人におけるQ熱のアウトブレイク — 米国およびカナダ、2014年。
2014年9月から11月、ニューヨーク州の保健局は、ニューヨーク州の住民5名がQ熱の原因病原体である*Coxiella burnetii*に対する検査で血清反応陽性と判定された通知を受けた。全例がQ熱と一致する症状（発熱、疲労、悪寒、頭痛など）を呈しており、「live cell therapy」(fresh cell therapy)と呼ばれる治療を受けるため2014年5月にドイツへ渡航していた。 ニューヨーク州の患者5例はドイツ人医師によるヒツジの胎児細胞の筋肉注射を受けるため、2014年5月30日に10から15名のグループでドイツのラインラント-プファルツ州へ渡航した。カナダの住民（女性）1例も同じドイツ人医師から2014年5月28日に同様の筋肉注射を受け、2014年6月に発熱、疼痛、注射部位の紅斑のため医師の診察を受け、7月にQ熱と診断された。米国およびカナダの公衆衛生当局では、渡航グループの他のメンバーの身元および国籍は把握しておらず、検査を受けたか、またその結果については不明である。
live cell therapy として知られている処置は、動物からヒトへ生きた細胞を投与する異種移植であり、異種移植の規制は国によりさまざまである。このアウトブレイクは、異種移植のために渡航する異種移植旅行(xenotourism)に関連した公衆衛生上の問題を際立たせている。アメリカ食品医薬品局は、異種移植を受けた患者を登録して定期的に臨床的および検査上のモニタリングを生涯において実施すること、そして患者とその親しい接触者については供血及び臓器提供を控えることを勧告した。しかしながら、自己申告以外には異種移植旅行の渡航者を特定する方法はない。医師は、live cell therapyの治療歴のある症例においてQ熱および人獣共通感染症のような健康リスクについて、認識しておくべきである。

使用上の注意記載状況・その他参考事項等

赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ドイツにて健康および活力の改善のためのヒツジ胎児細胞注射術を受けた米国およびカナダからの渡航者においてQ熱がアウトブレイクしたという報告である。	今後も異種移植による病原性因子伝播に関する情報の収集及び安全対策に努める。

JRC2015T-032

Q Fever Outbreak Among Travelers to Germany Who Received Live Cell Therapy — United States and Canada, 2014

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During September–November 2014, the New York State Department of Health (NYSDOH) was notified of five New York state residents who had tested seropositive for *Coxiella burnetii*, the causative agent of Q fever. All five patients had symptoms compatible with Q fever (e.g., fever, fatigue, chills, and headache) and a history of travel to Germany to receive a medical treatment called "live cell therapy" (sometimes called "fresh cell therapy") in May 2014. Live cell therapy is the practice of injecting processed cells from organs or fetuses of nonhuman animals (e.g., sheep) into human recipients (*1*). It is advertised to treat a variety of health conditions. This practice is unavailable in the United States; however, persons can travel to foreign locations to receive injections. Local health departments interviewed the patients, and NYSDOH notified CDC and posted a report on CDC's Epidemic Information Exchange to solicit additional cases. Clinical and exposure information for each patient was reported to the Robert Koch Institute in Germany, which forwarded the information to local health authorities. A Canada resident who also received live cell therapy in May 2014 was diagnosed with Q fever in July 2014. Clinicians should be aware of health risks, such as Q fever and other zoonotic diseases, among patients with a history of receiving treatment with live cell therapy products.

The five New York patients had traveled in a group of 10–15 persons to the state of Rhineland-Palatinate in Germany to receive intramuscular injections of fetal sheep cells from a German physician on May 30, 2014. A Canada resident, who received intramuscular injections of fetal sheep cells from the same German physician on May 28, 2014, sought medical care in June 2014 for fever, pain, and erythema at the site of the injection. She received a diagnosis of Q fever in July 2014, and public health authorities were notified. Under International Health Regulations, the Public Health Agency of Canada notified German authorities in September 2014. At the time of notification, the ministry of health of the federal state of Rhineland-Palatinate was investigating an outbreak of human Q fever associated with inhalation exposure to a sheep flock that was used for production of fetal sheep cell injections by the German physician.

In September, the German physician notified patients treated during January–July 2014 of their potential Q fever exposure. This prompted Q fever testing of the five patients in New York, three of whom had already sought medical care for symptoms. The other two patients had experienced symptoms but had not sought medical care until notification of their potential Q fever exposure. The test results, with positive Q fever titers, were reported to NYSDOH and prompted investigation by local health departments. No additional U.S. or Canada residents with positive Q fever titers and history of intramuscular injections of fetal sheep cells in Germany have been identified. The identities and nationalities of the other persons in the travel group are unknown to U.S. and Canadian public health authorities. It is not known whether the other persons did not get tested for Q fever, tested negative, or did not report an exposure to fetal sheep cell injections

An outbreak-associated case of Q fever was defined as an illness consisting of clinical signs and symptoms compatible with Q fever, and a single IgG titer ≥1:128 to *C. burnetii* phase II antigen by immunofluorescence assay in a person who received live cell therapy in Germany during May 2014 (*2*). Among the six identified cases, the median patient age was 62 years (range = 59–83 years). Four of the six patients were female. None of the patients reported other potential exposures to Q fever, with the exception of one patient who reported contact with sheep horn or bone. Three patients reported preexisting medical conditions: one patient with atrial fibrillation and kidney stones; one patient with Parkinson disease and osteoarthritis, and one patient with multiple sclerosis.

Signs and symptoms of Q fever began within approximately 1 week of receipt of the intramuscular injections of fetal sheep cells. The majority of symptoms were reported as lasting approximately 10–90 days; however, 9–10 months after exposure, three patients continued to report symptoms of fatigue, chills, sweats, and difficulty sleeping (Table). One patient had initially reported no symptoms during an interview with the local health department after his positive titers were reported in November 2014; however, in February 2015, he informed his physician that symptoms had been occurring since the injections in May.

The patients were tested for Q fever phase I and phase II antibodies at 2–6 months after exposure, using indirect immunofluorescence assay. *C. burnetii* undergoes antigenic phase variation, between a virulent phase I form and an avirulent phase II form. During acute infection, phase II antibodies

TABLE. Signs and symptoms reported by six Q fever patients who underwent live cell therapy — United States and Canada, 2014

Sign/Symptom	Patient 1	Patient 2*	Patient 3	Patient 4	Patient 5	Patient 6
Fever	X		X	X	X	X
Sweats	X		X	X	X	
Fatigue	X	X		X	X	X
Headache	X		X		X	
Chills	X	X	X			
Malaise			X	X	X	
Cellulitis			X		X	X
Confusion	X					
Retrobulbar pain	X					
Injection site abscess			X			
Cough			X			
Dizziness			X			
Shortness of breath			X			X
Sore throat			X			
Dry mouth			X			
Diarrhea			X			
Difficulty sleeping				X		
Joint pain					X	
Myalgia					X	
Duration	10–30 days	9 months (fatigue and chills were ongoing as of February 2015)	2–3 months	14–30 days (fatigue and difficulty sleeping were ongoing as of February 2015)	30 days (fatigue and sweats were ongoing as of February 2015)	10 days (fatigue continued for several months)

* Patient 2 initially reported no symptoms.

appear first and are higher than phase I antibodies. All patients' phase I IgG titers were elevated (1:512–1:2,048), but were lower than phase II IgG titers (1:4,096–1:65,536), suggesting acute disease. Phase I IgM titers were elevated in four patients (1:128–1:8,192) and phase II IgM titers were elevated in all patients (1:64–1:32,768). All patients were treated with doxycycline after receiving a diagnosis of Q fever.

All six patients were initially interviewed by their local health departments; only two of the five New York patients agreed to a follow-up interview by NYSDOH. The two patients reported that a group had traveled to Germany for injections twice each year for the past 5 years. They chose to receive injections of fetal sheep cells to improve their general health and vitality, and had not previously experienced signs or symptoms of illness after injections. They reported that they were not informed of a risk for Q fever infection before injection.

Discussion

The treatment known as live cell therapy was developed in Switzerland during the 1930s by Paul Niehans. Practitioners have used organs, glands, and fetuses of multiple species, including sheep, cows, and sharks* (*1*).

No published clinical evidence supporting therapeutic claims of the treatment known as live cell therapy is available. It is advertised as having anti-aging effects and as a treatment for multiple conditions and diseases (e.g., erectile dysfunction, depression, and joint, neurologic, heart, kidney, lung, endocrine, and liver disease).† Serious adverse events have been reported, including anaphylaxis, vasculitis, encephalitis, polyradiculitis, clostridial infections, paresis, and death (*3–5*).

The treatment known as live cell therapy is a type of xenotransplantation when it involves administration of live cells from a nonhuman animal source into a human recipient (*6*). Xenotransplantation carries a public health risk for transmission of known and unknown infectious agents from the donor organism to the human recipient and possible recombination or reassortment to form new pathogens (*6*). There is a theoretic potential for dissemination of disease from the original recipient to others. For this reason, discussions on safety requirements for xenotransplantation by international and domestic public health agencies continue to occur (*7*).

Regulation of xenotransplantation varies among countries. In the United States, the Food and Drug Administration (FDA) regulates xenotransplantation products as Biologic Drugs under section 351 of the Public Health Service Act§ and the Federal Food, Drug, and Cosmetic Act.¶ FDA approval of a Biologics License Application (BLA) is required to introduce, or deliver for introduction, a biologic product into interstate commerce. FDA has not approved a BLA for a xenotransplantation product known as live cell therapy. If a xenotransplantation product

*Additional information available at http://www.extendlife.com/livecell.php.

†Additional information available at http://www.janson-mueller.de/index.php?id=22&L=2.

§42 U.S.C. 262.

¶21 U.S.C. 321 et seq.

is proposed for use in a clinical investigation in the United States, an Investigational New Drug Application would be required. In Canada, xenotransplantation cell therapy products are regulated as drugs under the Food and Drugs Act** and the Food and Drug Regulations.†† Authorities in Canada have not authorized for sale any xenotransplantation products, nor have any clinical trials that involve xenotransplantation been authorized. In Germany, xenotransplantation products are regulated under the Medicinal Products Act; however, an attempt to ban fresh cell therapy in 1997 was later determined to be null and void because the federal law does not cover drugs manufactured by doctors only for use in their own patients (*8*). According to an assessment supported by the World Health Organization and its partners, during January 1994–September 2009, xenotransplantation procedures were identified in 12 different countries, of which nine had no clear national regulations on xenotransplantation (*9*).

This outbreak highlights one of the public health issues associated with xenotourism, the travel outside a country of residence for the purpose of participating in xenotransplantation programs. FDA recommends that xenotransplantation product recipients enrolled in research studies remain under lifelong surveillance with periodic clinical and laboratory monitoring and that both they and their intimate contacts refrain from blood and tissue donation (*6*). However, other than self-reporting, no method to identify returned xenotourists is available. Clinicians should be aware of xenotourism and consider the potential for zoonotic disease in a patient with a history of xenotransplantation.

** R.S.C., 1985, c. F-27.
†† C.R.C., c. 870.

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Summary

What is already known on this topic?

Q fever is a zoonotic disease caused by *Coxiella burnetii* and is usually transmitted through inhalation of air contaminated with animal excreta. The disease is considered to be underdiagnosed because symptoms are nonspecific and can vary from patient to patient, making diagnosis difficult.

What is added by this report?

During September–October 2014, the New York State Department of Health identified Q fever in five patients with exposure to a treatment known as live cell therapy, an alternative medicine practice involving injections of fetal sheep cells, which is a type of xenotransplantation. Investigation revealed that a group of U.S. residents traveled to Germany twice a year to receive this treatment.

What are the implications for public health practice?

Clinicians should consider zoonotic diseases, such as Q fever, in patients whose history includes receipt of a treatment known as live cell therapy. International travel for xenotransplantation procedures can facilitate transmission of zoonotic disease.

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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2015 年 9 月 19 日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	S D Med. 2015 Jul;68(7):298-9.	公表国 米国	
販売名（企業名）	別紙のとおり。					

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：これまでウサギとブタからしか分離されていなかった *Streptococcus thoraltensis*（レンサ球菌属）が初めてヒトに感染した。 健康であった30歳の妊婦が妊娠24週時に急性上腹部痛で緊急搬送された。搬送24時間以内に悪臭のする羊水とともに破水した。分娩後、児の口からの大量の分泌物を吸引した。気管吸引物の培養液は*S. thoraltensis*陽性であった。児は低出生児合併症により、NICUに3.5ヵ月ほど入院した。 胎盤の検査により患者は急性羊膜炎疑いとされ、胎盤の培養液から*S. thoraltensis*が同定された。分娩6ヵ月後の検診では子宮頚部の培養液は陰性であった。 感染前に患者はウサギやブタとの接触はなく、ペットも飼っていなかった。家族も同様の病気には罹患していなかった。 患者の夫は食肉包装工場に勤めており、毎日ブタと接触していたことから、職業上の暴露で夫に微生物が定着したことにより、患者の生殖管を通じて膣に定着し、最終的に胎盤に感染したと推測された。	記載なし。

報告企業の意見	今後の対応
別紙のとおり。	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

MedDRA/J ver. 18.1

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第XⅢ因子*、㉗フィブリノゲン加第XⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第X因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロン－Ⅰ静注用 500mg、⑧献血ベニロン－Ⅰ静注用 1000mg、⑨献血ベニロン－Ⅰ静注用 2500mg、⑩献血ベニロン－Ⅰ静注用 5000mg、⑪ベニロン*、⑫注射用アナクトC2,500 単位、⑬コンファクトＦ注射用 250、⑭コンファクトＦ注射用 500、⑮コンファクトＦ注射用 1000、⑯ノバクトＭ静注用 400 単位、⑰ノバクトＭ静注用 800 単位、⑱ノバクトＭ静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン "化血研"、㉔献血トロンビン経口・外用５千「化血研」、㉕献血トロンビン経口・外用１万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンＰ500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20%化血研*、㉛アルブミン 5%化血研*、㉜静注グロブリン*、㉝アンスロビンＰ1500 注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用 500 単位、㊱ノバクトM静注用 1000 単位、㊲ノバクトM静注用 2000 単位
報告企業の意見	*Streptococcus thoraltensis*は最近報告された種で、ブタの腸管・生殖管、ウサギの糞便から分離された。ヒトにおける病原性はほとんどわかっていない。 弊所で製造している全ての血漿分画製剤の製造工程には、約0.2μmの無菌ろ過工程およびウイルスの除去を目的としたウイルス除去膜ろ過工程が導入されているので、仮に製造原料に*Streptococcus thoraltensis*が混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに上記製剤によるレンサ球菌感染の報告例は無い。 以上の点から、上記製剤はレンサ球菌感染に対する安全性を確保していると考える。

*：現在製造を行っていない

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Case Report: Chorioamnionitis Attributed to Streptococcus thoraltensis

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Case Report: Chorioamnionitis Attributed to *Streptococcus thoraltensis*

By Mark Vukonich MD; Heidi Moline, MPH, MSIV;
Michael Chaussee, PhD; Brian Pepito, MD; and
Mark K. Huntington, MD, PhD

Abstract

Streptococcus thoraltensis is a recently described species, isolated from the intestinal and genital tracts of swine and from rabbit feces. We describe here a case of chorioamnionitis, with paternal swine exposure, potentially attributable to *S. thoraltensis*. To our knowledge, this is the first reported human infection by this organism.

Introduction

Streptococcus thoraltensis is a relatively recently described species that has been isolated from the intestinal and genital tracts of swine and from rabbit feces.[1,2] Very little is known about the pathogenic potential of this strain to humans; to our knowledge, there has not been a previously reported human infection by this organism.[3] We describe here a case of chorioamnionitis, with paternal exposure to swine, potentially attributable to *S. thoraltensis*.

Case Report

A 30-year-old, previously healthy, immunocompetent G5P4 pregnant female at 24 3/7 weeks gestational age presented to the emergency department of a South Dakota hospital with acute abdominal pain. Pain started 48 hours prior, with paroxysms of lower abdominal cramping coming every five minutes. She was afebrile (36.7° C), mildly tachycardic (115 BMP), had uterine tenderness, and had cervical change consistent with preterm labor. Her admission laboratory studies were significant for a leukocyte count of 13,300. There was no history or symptoms to suggest rupture of membranes, and there were no symptoms of illness prior to onset of the abdominal cramping.

Prenatal course was uncomplicated. She had received routine prenatal care from her family physician; prenatal screening was all normal. Her other four children are healthy. Her first and third pregnancies were term gestations delivered vaginally, the second pregnancy (delivered in Africa) was reported by the patient to be "early."

Less than 24 hours after admission, spontaneous rupture of membranes occurred with foul-smelling amniotic fluid. Upon delivery moments later, the infant had a brief cry followed by no respiratory effort and the mouth was suctioned of copious secretions. Apgar scores[4] were recorded as 1 at one minute, 5 at five minutes, and 7 at 10 minutes. The infant was intubated in the neonatal intensive care unit (NICU) and preemptive ampicillin and gentamicin was administered per protocol for neonatal early-onset sepsis prevention measures in chorioamnionitis.[5] Peripheral blood and tracheal aspirate cultures of the neonate were collected prior to initiation of antibiotics. No samples of the amniotic fluid or maternal blood were collected.

Tracheal aspirate cultures were reported positive for *S. thoraltensis*. Susceptibility testing of the strain showed intermediate resistance to penicillin and erythromycin, and susceptibility to cefotaxime, ceftriaxone, and chloramphenicol. Peripheral blood culture showed no growth. The antibiotics were continued for seven days; follow-up tracheal aspirate cultures showed no growth. The neonate had a prolonged stay in the NICU with complications associated with extreme prematurity and was discharged after 3.5 months.

Prior to delivery, the mother received a single dose of IV ampicillin; no additional antibiotics were administered

following delivery. Her postpartum course was unremarkable; she remained afebrile throughout her hospital stay and was discharged home on hospital day three.

Pathological evaluation of the placenta confirmed the suspicion of acute chorioamnionitis (grade II-III). Placenta culture grew out gram positive cocci identified *S. thoraltensis*. Cervical cultures were obtained at the six-week postpartum visit and were negative.

Discussion

To our knowledge, this is the first reported human case of *S. thoraltensis* infection. A PubMed search conducted using the search term "*Streptococcus thoraltensis*" returned only five citations with no human infections identified. Although isolated from diseased and dead pigs, the pathogenic potential of *S. thoraltensis* is unclear.[6]

After the pathology and microbiology reports were received, an in-depth social and exposure history was obtained. Both parents of this child immigrated to the U.S. in 2008, and are of African descent. Prior to this pregnancy, the mother had given birth to two living children in Africa, and two living children in South Dakota. She stayed at home with her four children and had no direct exposure to either rabbits or swine. The family did not have any pets. No other family members had any signs or symptoms of concurrent illness.

Interestingly, her husband was employed by a local meat packing plant and worked with swine on a daily basis. It is tempting to speculate that occupational exposure resulted in colonization of her husband with subsequent introduction of the organism to her genital tract, leading to vaginal colonization and ultimately placental infection. Clinically apparent rupture of membranes (typically seen in chorioamnionitis due to group B streptococcus and other genital-dwelling organisms) was absent, as were preceding systemic symptoms (typically seen with hematogenously-acquired *Listeria* chorioamnionitis). She had not undergone any invasive procedures (also occasionally associated with chorioamnionitis). Subclinical rupture of membranes, asymptomatic bacteremia, or even direct invasion are all potential avenues by which the organism may have gained access to the placenta.

A significant limitation to this report is the method used to identify the pathogen. Conventional phenotypic bacterial identification methods, rather than the more specific 16s rRNA typing, were employed in our clinical laboratory which uses an automated system employing 43 tests of carbon source, enzymic activities and resistance (VITEK 2 GP).[7] The tests were repeated for confirmation. While identification by the former is 95 percent specific,[7] the lack of 16s rRNA typing represents a potential limitation to the certainty of the organism's identity of this case report. Nevertheless, identification of *S. thoraltensis* from both the maternal placenta and newborn tracheal aspirate cultures presents a strong case to implicate this organism, suggesting a novel emergence in humans resulting in clinically relevant outcomes.

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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
○パーキンソン病様症状を呈する多系統萎縮症患者を引き起こすα-シヌクレインプリオンに関するエビデンス 多系統萎縮症（MSA）がα-シヌクレイン蛋白質から構成されるヒトプリオンに起因することを報告する。MSAの神経病理学的特徴は、α-シヌクレインのフィラメントから成るグリア細胞質内封入体である。ヒトα-シヌクレインがプリオンを形成するか否かを判定するため、我々は14名の患者の脳抽出物を用いて、黄色蛍光タンパク質を融合させた全長変異型ヒトα-シヌクレイン（α-syn140*A53T-YFP）を発現する培養ヒト胎児腎臓（HEK）細胞、並びにα-シヌクレイン（A53T）を発現するTgM83+/-マウスへの伝播について調べた。導入したヒトα-シヌクレイン遺伝子がヘテロ接合であるTgM83+/-マウスには自発的神経機能障害は認められなかったが、ホモ接合体であるTgM83+/+マウスでは認められた。120日以内のインキュベーション期間後、MSA患者14名の脳抽出物は全例がTgM83+/-マウスに神経変性を伝播した。これには神経細胞体と軸索におけるα-シヌクレインの沈着が伴った。また、MSA患者の脳抽出物は全て、α-syn*A53T-YFPの培養細胞における凝集を誘発したが、6名のパーキンソン病（PD）患者の脳抽出物もしくは対照サンプルでは、凝集を誘発したものはなかった。我々の知見は、PDを引き起こすと推測されるプリオンとも、TgM83+/+マウスにおいて神経の自発的変性を引き起こすと推測されるプリオンとも異なる、特異な株のα-シヌクレインプリオンによってMSAが引き起こされることを示す。注目すべきことに、我々の知る限りでは、α-シヌクレインはクロイツフェルト・ヤコブ病が伝播性を有する疾患であるという発見以降、初めて確認される新たなヒトプリオンである。	赤血球液-LR「日赤」 照射赤血球液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
パーキンソン病様症状を呈する多系統萎縮症患者の脳から、伝播性α-シヌクレインプリオンタンパク質の存在が遺伝子改変細胞及びトランスジェニックマウスにおいて確認されたという報告である。	今後も伝播性を有する神経変性疾患に関する情報の収集及び安全対策に努める。

Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism

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Prions are proteins that adopt alternative conformations that become self-propagating; the PrP^{Sc} prion causes the rare human disorder Creutzfeldt–Jakob disease (CJD). We report here that multiple system atrophy (MSA) is caused by a different human prion composed of the α-synuclein protein. MSA is a slowly evolving disorder characterized by progressive loss of autonomic nervous system function and often signs of parkinsonism; the neuropathological hallmark of MSA is glial cytoplasmic inclusions consisting of filaments of α-synuclein. To determine whether human α-synuclein forms prions, we examined 14 human brain homogenates for transmission to cultured human embryonic kidney (HEK) cells expressing full-length, mutant human α-synuclein fused to yellow fluorescent protein (α-syn140*A53T–YFP) and $TgM83^{+/-}$ mice expressing α-synuclein (A53T). The $TgM83^{+/-}$ mice that were hemizygous for the mutant transgene did not develop spontaneous illness; in contrast, the $TgM83^{+/+}$ mice that were homozygous developed neurological dysfunction. Brain extracts from 14 MSA cases all transmitted neurodegeneration to $TgM83^{+/-}$ mice after incubation periods of ~120 d, which was accompanied by deposition of α-synuclein within neuronal cell bodies and axons. All of the MSA extracts also induced aggregation of α-syn*A53T–YFP in cultured cells, whereas none of six Parkinson's disease (PD) extracts or a control sample did so. Our findings argue that MSA is caused by a unique strain of α-synuclein prions, which is different from the putative prions causing PD and from those causing spontaneous neurodegeneration in $TgM83^{+/+}$ mice. Remarkably, α-synuclein is the first new human prion to be identified, to our knowledge, since the discovery a half century ago that CJD was transmissible.

neurodegeneration | Parkinson's disease | synucleinopathies | strains

Looking back almost 50 y ago, kuru was the first human prion disease to be transmitted to an experimental animal (1). Subsequently, Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease, and fatal familial insomnia were transmitted to nonhuman primates or transgenic (Tg) mice; all of these disorders were eventually found to be caused by PrP^{Sc} prions that were initially discovered in hamsters with experimental scrapie. Attempts to transmit other neurodegenerative diseases, including Alzheimer's and Parkinson's, to monkeys were disappointing; none of the animals developed signs of neurological dysfunction, and none showed recognizable neuropathological changes at autopsy (2).

In 1960, Milton Shy and Glenn Drager described two male patients suffering from orthostatic hypotension, additional forms of autonomic insufficiency, and a movement disorder resembling Parkinson's disease (PD). They also found an additional 40 cases of idiopathic hypotension in the literature, which shared many features with their patients. Nine years later, Graham and Oppenheimer suggested that Shy–Drager syndrome should be combined with striatonigral degeneration and olivopontocerebellar atrophy and that these three entities should be called multiple system atrophy (MSA) (3). They presciently argued that all three disorders were likely caused by a similar neurodegenerative process. Two decades passed before support for this hypothesis began to emerge when the brains of 11 MSA patients were reported to contain silver-positive accumulations or glial cytoplasmic inclusions (GCIs) primarily in oligodendrocytes (4). The nature of these GCIs remained elusive for another decade until three groups reported that GCIs exhibited positive immunostaining for α-synuclein (5–7). The discovery that MSA is a synucleinopathy followed a study reported 1 y earlier showing that Lewy bodies in PD contain α-synuclein by immunostaining (8). Such investigations were prompted by molecular genetic studies showing genetic linkage between the A53T point mutation in α-synuclein and inherited PD (9).

MSA is a sporadic, adult-onset, progressive neurodegenerative disorder with an annual incidence of ~3 per 100,000 individuals over the age of 50 (10, 11). The duration of MSA is generally 5–10 y and is substantially shorter than most cases of PD, which

Significance

Prions are proteins that assume alternate shapes that become self-propagating, and while some prions perform normal physiological functions, others cause disease. Prions were discovered while studying the cause of rare neurodegenerative diseases of animals and humans called scrapie and Creutzfeldt–Jakob disease, respectively. We report here the discovery of α-synuclein prions that cause a more common neurodegenerative disease in humans called multiple system atrophy (MSA). In contrast to MSA, brain extracts from Parkinson's disease (PD) patients were not transmissible to genetically engineered cells or mice, although much evidence argues that PD is also caused by α-synuclein, suggesting that this strain (or variant) is different from those that cause MSA.

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The authors declare no conflict of interest.

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leads to death in 10–20 y. MSA has been subdivided based on the predominance of Parkinson's symptoms (MSA-P) or cerebellar dysfunction (MSA-C) (12).

The unanticipated results of an earlier study in 2013 showed that two cases of MSA transmitted CNS dysfunction to transgenic ($TgM83^{+/-}$) mice expressing mutant human α-synuclein*A53T protein (13). In that initial report, brain homogenates prepared from two cases of MSA were intracerebrally (IC) injected into $TgM83^{+/-}$ mice, which resulted in progressive CNS dysfunction after ~120 d. The brains of the Tg mice exhibited extensive phosphorylated α-synuclein deposits in the cytoplasm and axons of neurons.

To determine whether the transmissions of two MSA cases were anomalous, we inoculated $TgM83^{+/-}$ mice with another dozen cases from three different countries: the United Kingdom, Australia, and the United States. We report here that homogenates prepared from each of the additional 12 cases produced an experimental synucleinopathy in all of the IC inoculated $TgM83^{+/-}$ mice with incubation times of ~120 d. The mice developed intraneuronal deposits of aggregated, phosphorylated α-synuclein in their brainstems and some other CNS regions. Using multiple brain regions from some of the MSA cases, a total of 19 homogenates from 14 MSA cases produced CNS dysfunction in $TgM83^{+/-}$ mice and infected human embryonic kidney (HEK) cells expressing α-syn140*A53T–YFP, resulting in cytoplasmic aggregates of the fusion protein that were measured by fluorescence microscopy (14). From these transmission studies in both $TgM83^{+/-}$ mice and cultured cells, we conclude that MSA is a transmissible human neurodegenerative disease caused by α-synuclein prions.

Results

Patient Histories. Brain specimens from 14 deceased patients carrying the clinical and neuropathological diagnosis of MSA, as well as 6 patients with the diagnosis of PD, were obtained from (*i*) the Parkinson's UK Brain Bank at Imperial College London in London, England; (*ii*) the Sydney Brain Bank in Sydney, Australia; and (*iii*) the Massachusetts General Hospital Alzheimer's Disease Research Center in Boston, MA. Clinical descriptions of the 20 synucleinopathy patients are summarized in Table 1 and Table S1.

The MSA patients exhibited autonomic dysfunction manifested as orthostatic hypotension and/or erectile dysfunction with either parkinsonism or cerebellar dysfunction and, thus satisfied the clinical criteria for possible or probable MSA (15). For the majority of the MSA patients, parkinsonism rather than cerebellar symptoms dominated the clinical presentation. Parkinsonism was consistent with the origin of the patient population, as MSA-P is known to be more common in Western countries (16), whereas MSA-C is more frequent in Asian countries (17). The mean age of onset of disease in this MSA patient population was 59 ± 9 y, consistent with previous reports (18). The average duration of disease in this patient population was 7.8 y, consistent with reports of an average duration of disease of ~6–8 y (18, 19). Most of the MSA patients in this study received symptomatic treatment with carbidopa/levodopa or levodopa alone. Although the majority of the patients in this study showed an initial response to levodopa treatment, the MSA patients worsened regardless of this response to therapeutic intervention. The PD patients displayed classical symptoms of the disease, including resting tremor, rigidity, bradykinesia, and postural instability. The mean age of disease onset for the PD patients was 68 ± 5 y, and the average duration of disease was 8.2 y.

Table 1. Demographic, clinical, and neuropathological characteristics of patient samples

Case	Country	Sex	Age at onset (y)	Duration (y)	Cause of death	Clinical diagnosis	Neuropathological diagnosis
C1	USA	M	77	NA	Cardiovascular disease	Nondiseased control brain	NA
PD1	UK	M	65	8.5		Tremulous hemiparkinsonism, REM sleep disorder, MSA questioned	Lewy body disease
PD2	UK	M	65	8	Myocardial infarction, acute renal failure, pneumonia	Hemiparkinsonism with autonomic features	Lewy body disease
PD3*	UK	M	66	9.5		Parkinsonism with drooling	Lewy body disease
PD5	Australia	M	63	9	Myocardial infarction	Parkinson's disease	Parkinson's disease
PD6	Australia	M	73	6	Myocardial infarction	Parkinson's disease	Diffuse Lewy bodies
PD7	Australia	M	74	8	Cerebrovascular accident	Parkinson's disease	Diffuse Lewy bodies
MSA1	UK	M	78	8		Atypical akinetic-rigid syndrome with prominent ataxia, PSP questioned	MSA
MSA2	UK	M	65	5.5		Akinetic-rigid syndrome with autonomic involvement	MSA
MSA3	UK	F	52	6	Bronchopneumonia	MSA-P	MSA
MSA4	UK	M	68	7	Pneumonia	MSA	MSA
MSA5	UK	M	52	8	Respiratory failure, pneumonia	Parkinsonism, MSA questioned	MSA
MSA6	UK	F	48	13	Pneumonia	Akinetic-rigid syndrome with antecolis and camptocormia: MSA vs. PD	MSA
MSA7	UK	M	52	12	Bronchopneumonia	MSA-C	MSA
MSA8	Australia	M	57	4	Aspiration pneumonia	MSA-P	MSA
MSA9	Australia	M	75	7	Cardiorespiratory failure	MSA-C	MSA
MSA10	Australia	M	56	8	Bronchopneumonia	MSA-P with early autonomic dysfunction	MSA
MSA11	Australia	M	59	2	Respiratory failure	MSA-P with early autonomic dysfunction	MSA
MSA12	USA	F	55	11	Acute bronchopneumonia	MSA	MSA
MSA13	USA	M	55	10	Chronic pneumonia	MSA	MSA
MSA14	USA	M	60	8		MSA-C	MSA

NA, not applicable.
*Clinical report for PD3 was incomplete.

Brain Specimens. A definitive diagnosis of MSA requires postmortem neuropathological microscopic examination, the results of which are summarized in Table 1 and Table S1. On removal of the brains, no gross changes in cortical regions were observed, with gyri and sulci appearing to be normal. On cutting the brains, we found depigmentation of the substantia nigra (Fig. 1*A*) and atrophy of the putamen/basal ganglia and cerebellum. Histological sections from all 14 MSA brains exhibited GCIs that stained with antiphosphorylated α-synuclein antibodies, and α-synuclein-positive Lewy bodies were found in the six PD patient samples (Fig. 1*B*). Interestingly, the density of GCIs varied widely among the patients (Table 2). Total α-synuclein was determined from frozen brain samples by ELISA and found to be higher in the nondiseased control brain compared with PD brains and most of the MSA brains (Table 2). Frozen brain samples were fractionated to determine the level of insoluble, aggregated phosphorylated α-synuclein using SDS/PAGE (Fig. 1*C*). Homogenates from MSA patients contained more (although variable) amounts of phosphorylated α-synuclein in the insoluble fraction compared with the nondiseased control brains. Interestingly, PD cases 1 and 2 contained very small amounts of phosphorylated α-synuclein, whereas the other four cases contained similar amounts as the MSA patients (Fig. 1*C*).

Sequencing of the *SNCA* and *COQ2* Genes. Because inherited cases have been identified in all neurodegenerative diseases, we asked if any of the MSA or PD samples contained either a mutant α-synuclein (*SNCA*) or *COQ2* gene. Duplications, triplications, and missense mutations in *SNCA* have been identified in a minority of patients with PD (20); *SNCA* single nucleotide polymorphisms (SNPs) are associated with MSA risk (21); and, recently, novel *SNCA* mutations were reported in cases with mixed MSA and PD pathology (22–24). In addition, an investigation of familial MSA identified coenzyme Q10, specifically the *COQ2* gene, to be associated with MSA in two families (25).

To determine whether any mutations were present in our study population, we sequenced all five coding exons of *SNCA* and all seven exons of *COQ2* using standard Sanger sequencing (primers used are shown in Table S2). We found no missense mutations in any of the samples tested. No *SNCA* SNPs were

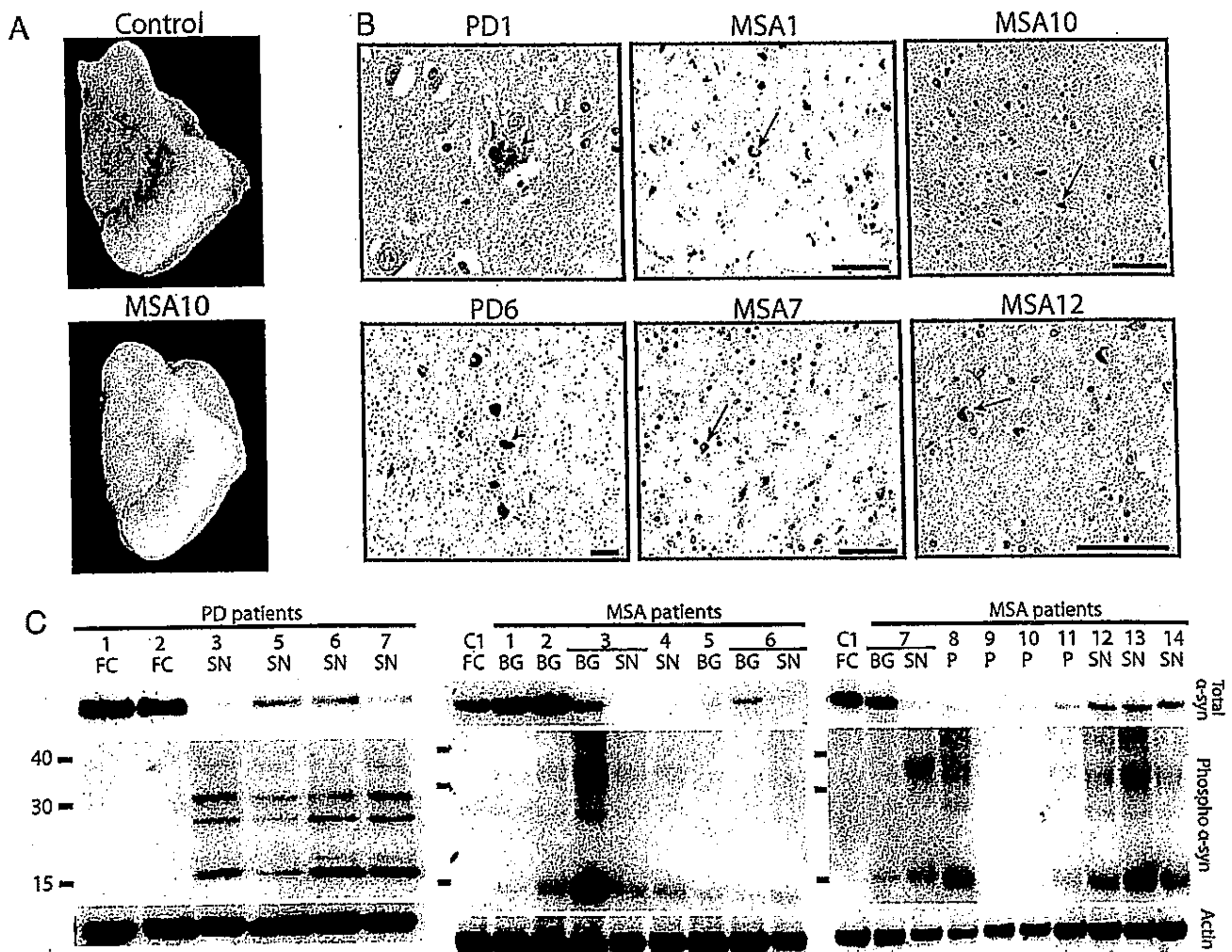

Fig. 1. Neuropathological and biochemical analysis of synucleinopathy cases. (*A*) Gross pathology of one representative sample (MSA10) demonstrating depigmentation of the substantia nigra compared with nondiseased control. (*B*) Immunohistochemical detection of α-synuclein deposits in patient samples. Two representative PD samples and four representative MSA samples were stained for α-synuclein using the antibodies clone 42 (BD Biosciences; PD1, PD6, MSA1, MSA7, MSA10) and LB509 (MSA12). Arrowheads point to Lewy bodies, arrows to GCIs. (Scale bar, 50 µm for all samples.) (*C*) Immunoblots of brain homogenates of human control (C1), PD, and MSA cases show total human α-synuclein (*Top*) and detergent-insoluble phosphorylated α-synuclein (*Middle*). The brain region used for each case is noted: FC (frontal cortex), SN (substantia nigra), BG (basal ganglia), and P (pons). Blots were probed for actin as a loading control (*Bottom*). Total human α-synuclein was probed using the monoclonal antibody Syn211, and S129-specific, phosphorylated α-synuclein was probed with antibody EP1536Y. Molecular weight markers of migrated protein standards are shown in kilodaltons.

Table 2. Transmission of α-synuclein prions to TgM83$^{+/-}$ mice

	Inoculum					Primary transmission		
		α-Synuclein inclusion density (per mm^2)						
Sample	Brain region	GCIs	Lewy bodies	Total α-synuclein (μg/mL)	Mean cell infection ± SEM (%)	Mean incubation time ± SEM (d)	n/n_0	Mean cell infection ± SEM (%)
C1	FC	0	0	3.0	7 ± 1†	>360	0/8	2 ± 0
PD1	FC	0	*	1.9	7 ± 3	>360	0/8	2 ± 1
PD2	FC	0	*	2.3	3 ± 1	>360	0/7	2 ± 0
PD3	SN	0	3.3	1.6	0	> 243‡	0/8	ND
PD5	SN	0	3.0	2.4	6 ± 0	> 208‡	0/7	ND
PD6	SN	0	7.3	0.6	15 ± 1	> 208‡	0/4	ND
PD7	SN	0	6.8	1.5	10 ± 1	> 208‡	0/6	ND
MSA1	BG	69	0	4.2	11 ± 1	143 ± 17§	7/8	29 ± 4
MSA2	BG	82	0	3.6	30 ± 1	109 ± 12§	7/7	31 ± 3
	SN	99	0	ND	ND	119 ± 10	7/7	21 ± 2
MSA3	BG	69	0	2.5	42 ± 2	114 ± 14	5/5	30 ± 2
	SN	20	0	0.3	24 ± 2	119 ± 10	7/7	24 ± 2
MSA4	BG	54	0	0.4	25 ± 4	135 ± 13	8/8	47 ± 3
	SN	25	0	ND	19 ± 5	134 ± 7	8/8	33 ± 2
MSA5	BG	200	0	0.6	47 ± 2	119 ± 12	8/8	45 ± 3
MSA6	BG	50	0	1.6	20 ± 3	108 ± 10	8/8	43 ± 3
	SN	ND	ND	0.1	40 ± 3	106 ± 7	6/6	39 ± 3
MSA7	BG	29	0	4.3	48 ± 1	106 ± 10	8/8	24 ± 1
	SN	21	0	0.2	58 ± 3	122 ± 10	8/8	36 ± 4
MSA8	P	22	0	0.3	31 ± 1	108 ± 15	6/6	33 ± 1
MSA9	P	3.3	0	0.8	24 ± 1	121 ± 8	7/7	14 ± 1
MSA10	P	13	0	0.9	36 ± 1	108 ± 8	6/6	42 ± 2
MSA11	P	12	0	0.8	23 ± 2	144 ± 16	6/6	37 ± 3
MSA12	SN	133	0	0.8	29 ± 1	117 ± 10	8/8	51 ± 3
MSA13	SN	127	0	0.8	40 ± 2	113 ± 9	8/8	41 ± 3
MSA14	SN	17	0	0.9	23 ± 1	130 ± 12¶	6/6	45 ± 2

BG, basal ganglia; FC, frontal cortex; n, number of ill mice; n_0, number of inoculated mice; ND, not determined; P, pons; SN, substantia nigra.
*A single Lewy body was found in the entire section.
†Average value from two alternate age-matched control samples.
‡Experiments ongoing.
§Data previously reported in ref. 13.
¶Data previously reported in ref. 14.

identified in any of the patient cases; however, three *COQ2* SNPs varied among cases (Table S3). In our small sample, there did not seem to be any association between SNP genotype and MSA or PD. Although α-synuclein is the most attractive target for genetic studies of MSA and PD, several other candidate genes have been studied in α-synucleinopathies. These genes have diverse functions, including roles in mitochondrial function, protection against oxidative stress, and inflammatory processes (25–27).

Transmission of MSA Prions to Cultured HEK Cells. We recently described the creation of a cell-based assay for detecting human α-synuclein prions using cultured HEK cells expressing full-length α-synuclein containing the A53T mutation fused to yellow fluorescent protein (α-syn140*A53T–YFP) (14). Using this assay, we selectively precipitated α-synuclein prions from the human patient samples using sodium phosphotungstic acid (PTA). After exposing the cells to the precipitated samples for 4 d in a 384-well plate, we collected four images from each of the six wells for each sample using automated confocal fluorescence microscopy. The images were then analyzed using an algorithm we developed to determine the percentage of cells containing aggregates. On initial analysis of the data, we found 17 of the 18 samples from MSA patients infected HEK cells expressing α-syn140*A53T–YFP fusion protein significantly higher than the control. Conversely, only one of the six PD samples was significantly higher than the control sample (Table 2 and Fig. 2*A*). We reevaluated these two outliers, patients PD6 and MSA1, by manually determining the percentage of cells with aggregates present in a single representative image from each of the six wells plated. We found that sample PD6 was a false positive, infecting only 5 ± 2% of cells with aggregates, whereas MSA1 was a false negative, inducing aggregates in 13 ± 5% of cells (error is reported as SD instead of SEM as presented in Table 2). Notably, the algorithm was developed for high-throughput analysis and cannot consistently distinguish diffuse signals in overlapping cells from small intense aggregates. Importantly, the MSA patient samples were found to induce aggregate formation in the α-syn140*A53T–YFP cells at a significantly higher level than the PD samples (Fig. 2*B*; $P < 0.0001$). Moreover, a concomitant study of 17 brain samples from 11 deceased males and 6 females, all of whom were between the ages 56 and 88 and without evidence of CNS dysfunction, showed that none of these control brain homogenates induced fluorescent aggregates in α-syn140*A53T–YFP cells (14).

MSA Samples Transmit Disease to TgM83$^{+/-}$ Mice. In a preliminary study, we IC inoculated TgM83$^{+/-}$ mice with basal ganglia samples from two MSA patients. Unexpectedly, those MSA samples transmitted CNS dysfunction in ~120 d (13). To determine whether neurological dysfunction could be transmitted with other MSA patient samples, we collected additional brain specimens from a dozen deceased MSA patients and IC inoculated

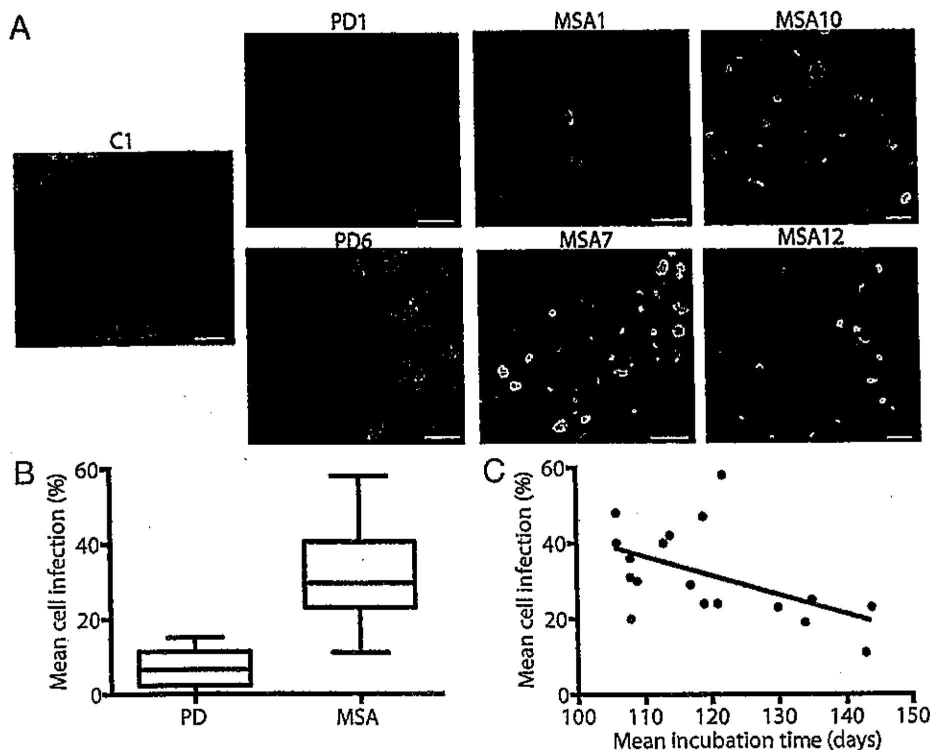

Fig. 2. A cell infectivity assay can quantify infectivity in synucleinopathy tissue samples. (*A*) Representative images of α-syn140*A53T–YFP–expressing cells infected with PTA-precipitated brain homogenate from control (C1), PD, or MSA patients. YFP is shown in green. (Scale bars, 100 μm.) (*B*) Box and whisker plot of cell infectivity from PD and MSA samples shows a significant difference between the two groups ($P < 0.001$). Whiskers indicate maximum and minimum values. (*C*) For each of the MSA samples tested in both cell assay and mouse bioassay, cell infectivity and incubation time were significantly inversely correlated ($R^2 = 0.27$, $P = 0.026$).

them into TgM83$^{+/-}$ mice. Inoculation with all of the MSA samples caused CNS dysfunction with mean incubation periods of 100–150 d postinoculation (dpi) (Table 2). The most common clinical signs were dysmetria and circling behavior. In contrast, brain homogenates from six PD patients or a control inoculated into TgM83$^{+/-}$ mice failed to produce signs of neurological dysfunction in >360 dpi (Table 2), analogous to our findings when these samples were bioassayed in HEK cells expressing α-syn140*A53T–YFP fusion protein.

Comparing the level of cell infectivity from each of the MSA samples with the incubation times observed from inoculation into TgM83$^{+/-}$ mice, we found a significant inverse correlation (Fig. 2*C*; $R^2 = 0.27$, $P = 0.026$): the greater the level of infectivity in the cell assay, the shorter the time to disease onset in mice (Fig. 2*C*).

Neuropathological examination revealed large aggregates of phosphorylated α-synuclein, as well as widespread astrocytic gliosis, in the brains of TgM83$^{+/-}$ mice inoculated with MSA brain homogenates (Fig. 3*A*). Aggregated α-synuclein was primarily observed as neuronal cytoplasmic inclusions (NCIs) and in neurites. Although some of these NCIs resemble Lewy bodies (Fig. 3*A*, MSA5, *Inset*), the majority featured a thin rim around the nucleus with α-synuclein–positive immunostaining extending to the proximal part of the neuronal processes. The predominance of neuronal over oligodendroglial inclusions may reflect the transgene expression that is driven by the prion protein promoter. In comparison, the brains of TgM83$^{+/-}$ mice inoculated with PD brain exhibited low and unspecific background signal of phosphorylated α-synuclein after >360 dpi, similar to that seen in the control. The distribution of the phosphorylated α-synuclein throughout multiple brain regions in the mice was also assessed (Fig. S1). Inoculation with either control or PD brain homogenate did not lead to deposition of appreciable phosphorylated α-synuclein in any brain region. In contrast, mice inoculated with MSA brain homogenate developed phosphorylated α-synuclein deposits in several brain regions. These neuropathological changes were most apparent in the brainstem, especially in the reticular formation, but notably absent from cortical regions. The contralateral hemispheres of TgM83$^{+/-}$ mice inoculated with MSA homogenates contained phosphorylated α-synuclein in the detergent-insoluble fraction, whereas similar brain fractions from mice inoculated with control or PD samples contained low levels of phosphorylated α-synuclein (Fig. 3 *B* and *C*). TgM83$^{+/-}$ mice inoculated with MSA brain homogenates displayed slowly migrating phosphorylated α-synuclein on a Western blot, whereas those inoculated with control brain or PD brain samples did not. Additionally, we tested the mouse brain homogenates in the HEK cell assay and found that the PTA-precipitated homogenates from mice inoculated with MSA infected the α-syn140*A53T–YFP cells, but the homogenates from TgM83$^{+/-}$ mice inoculated with PD or control patient brain did not infect the cells (Table 2). Our results argue that transmission of MSA to TgM83$^{+/-}$ mice results in the de novo formation of prions in mouse brain.

Propagation of α-Synuclein Prions in TgM83 Mice. Because serial propagation is a characteristic of authentic prions, we prepared brain homogenates from four ill TgM83$^{+/-}$ mice inoculated with either MSA or spontaneously ill TgM83$^{+/+}$ mouse brain. Serial passage in TgM83$^{+/-}$ mice was then compared with passage of an aged TgM83$^{+/-}$ brain, which did not transmit disease (Table 3). Incubation periods for serial passaged MSA prions were slightly shorter than those observed for primary transmissions and ~40% shorter than those for serial transmission of spontaneous TgM83$^{+/+}$ prions, indicating that these represent two different

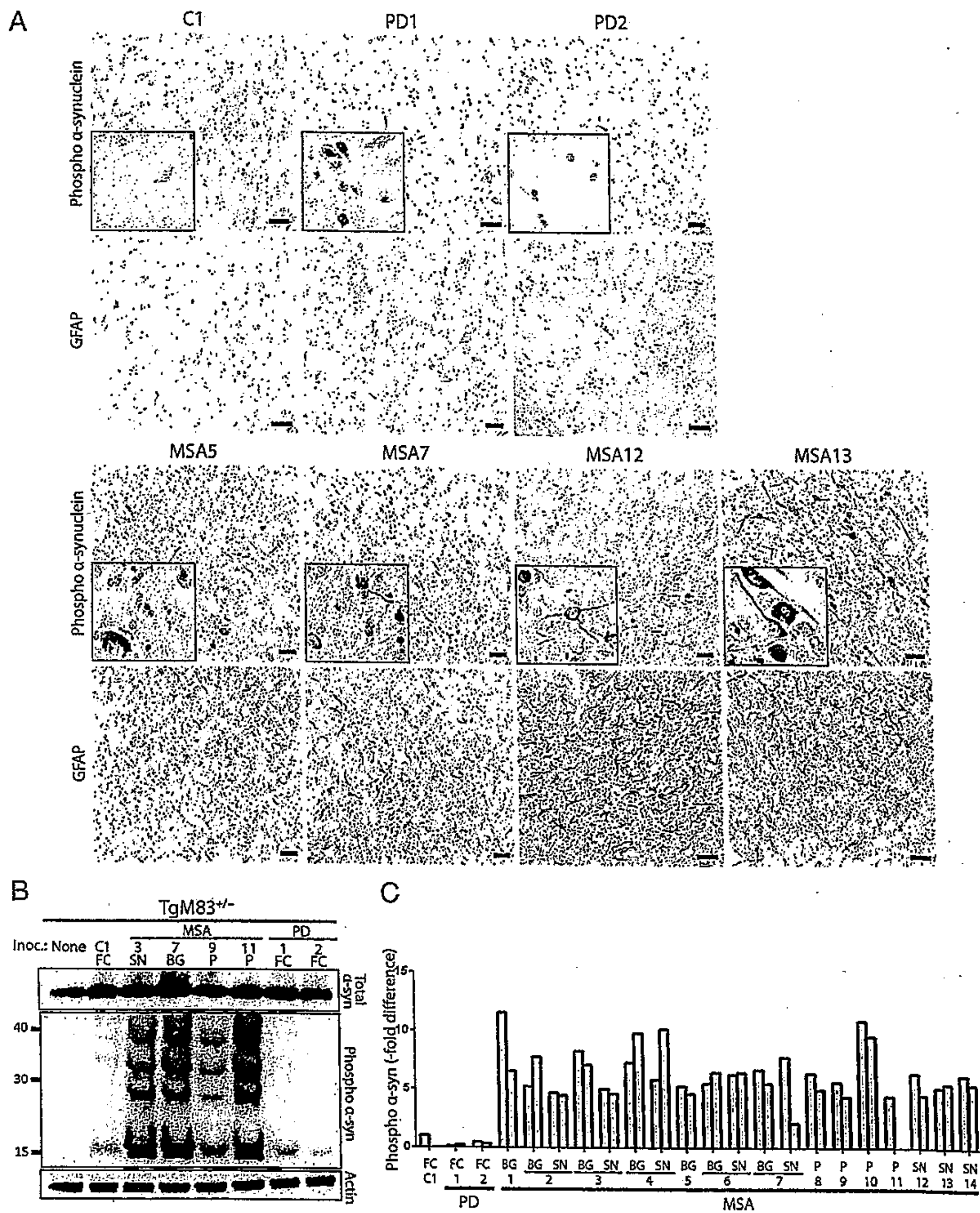

Fig. 3. Inoculation of α-synuclein aggregates from MSA but not PD cases induced deposition of phosphorylated α-synuclein and reactive astrogliosis. (*A*) Brain homogenates were prepared from control (C1), PD, and MSA patients and IC inoculated into TgM83$^{+/-}$ mice. Mice inoculated with MSA homogenates, but not PD or control homogenates, showed deposition of phosphorylated α-synuclein in the brainstem (top panels, staining with EP1536Y antibody); *Insets* show a 4× magnification relative to the main image. These mice also showed prominent reactive astrogliosis, as indicated by GFAP staining (bottom panels). (Scale bars, 50 μm.) (*B*) Representative immunoblot shows total α-synuclein (*Top*) and detergent-insoluble phosphorylated α-synuclein (*Middle*) in the brains of TgM83$^{+/-}$ mice inoculated with homogenates from C1, PD, or MSA patients. The brain region for each inoculum is noted: FC (frontal cortex), P (pons), and SN (substantia nigra). Total α-synuclein was detected from the crude brain homogenates using the Syn211 antibody; phosphorylated α-synuclein was probed with the EP1536Y antibody. Actin is shown as a loading control (*Bottom*). Molecular weight markers of migrated protein standards are shown in kilodaltons. (*C*) Phosphorylated α-synuclein in the brains of TgM83$^{+/-}$ mice inoculated with PD or MSA was quantified by densitometry and expressed as an x-fold difference compared with mice inoculated with nondiseased control brain (C1). The brain-region origin for each inoculum is noted: BG (basal ganglia), FC (frontal cortex), P (pons), and SN (substantia nigra). For each inoculum, data from two animals are shown, except for the MSA11 inoculum, for which only one animal was available for biochemical analysis.

strains of α-synuclein prions. We IC inoculated 30 μL of a 1% (wt/vol) brain homogenate, equivalent to 0.3 mg of the original brain weight, which caused CNS dysfunction in ~120 d, at which time the mice were killed. For samples from MSA1 and MSA2

Table 3. Effect of transgene and serial transmission on incubation period

Inoculum (brain region)	Mouse line	Primary transmission: Mean incubation time ± SEM (d)	Primary transmission: n/n_0	Incubation time of mouse brain inoculated (d)	Secondary transmission in M83$^{+/-}$: Mean incubation time ± SEM (d)	Secondary transmission in M83$^{+/-}$: n/n_0
No inoculum	TgM83$^{+/-}$	>412	0/6	259	>360	0/6
	Tg(*SNCA*) *Snca*$^{0/0}$	>580	0/9		ND	
MSA1 (basal ganglia)	TgM83$^{+/-}$	143 ± 17*	7/8	105	113 ± 13†	5/5
	WT	>360	0/7		ND	
	Tg(*SNCA*) *Snca*$^{0/0}$	>360	0/6		ND	
MSA2 (basal ganglia)	TgM83$^{+/-}$	109 ± 12*	7/7	91	92 ± 5†	6/6
	WT	>360	0/2		ND	
	Tg(*SNCA*) *Snca*$^{0/0}$	>360	0/5		ND	
9 m.o. spont. TgM83$^{+/+}$ (whole brain)	TgM83$^{+/-}$	222 ± 15*	6/6	205	193 ± 19	8/8
11 m.o. spont. TgM83$^{+/+}$ (whole brain)	TgM83$^{+/-}$	216 ± 18*	8/8	162	175 ± 8	8/8

n, number of ill mice; n_0, number of inoculated mice; ND, not determined.
*Data previously reported in ref. 13.
†Data previously reported in ref. 14.

patients (Table 3), the brains were harvested and passaged a second time, representing more than a 1,000-fold dilution of brain homogenate (0.3 mg from an ~0.5-g brain). The second passage had a similar incubation period, suggesting the MSA prions had replicated to the same level that were present in the human brain before dilution for transmission studies. The more than 1,000-fold amplification per passage implies that in two passages, the MSA prions had multiplied more than 1×10^6-fold, underscoring the infectivity of the α-synuclein prions described here.

To determine whether MSA prions could transmit to Tg mice expressing WT mouse or human α-synuclein, we inoculated MSA1 and MSA2 into additional lines of WT and Tg mice. Neither WT mice nor Tg mice expressing WT human α-synuclein developed CNS dysfunction on inoculation with the MSA samples (Table 3). Presumably, the A53T point mutation facilitated prion replication, as has been observed analogously with some PrP mutations for PrP^{Sc} prions. In support of this posit, MSA-inoculated TgM83$^{+/+}$ mice, which were homozygous for the α-synuclein*A53T transgene array, developed CNS dysfunction in ~90 d (Table S4) compared with the TgM83$^{+/-}$ mice that required ~120 d (Table 2). Because expression of endogenous mouse PrP can interfere with the propagation of human PrP prions (28), we decided to test whether endogenous mouse α-synuclein impacts the propagation of MSA prions. We crossed the TgM83 mice onto an α-synuclein knockout background but found no difference in incubation periods among the Tg mice on the *Snca*$^{0/0}$, *Snca*$^{0/+}$, and *Snca*$^{+/+}$ backgrounds (Table S4).

We also investigated alternate routes of inoculation for MSA prions. Recently, it was reported that hind limb intramuscular (IM) inoculations with recombinant human α-synuclein polymerized into fibrils could be as or more efficient than IC inoculation in TgM83$^{+/+}$ mice (29). In other investigations, inoculation of PrP^{Sc} prions into the lingual muscles has been shown to be an effective means of PrP^{Sc} prion transmission (30). We compared MSA inoculations that were performed IC, IM, and intraglossally. Preliminary results suggest that IC and IM inoculations of 5 μL 1% MSA2 brain homogenate produced similar incubation times: IC, 133 ± 6 d (7/8 mice), and IM, 136 ± 10 d (6/8 mice). Both routes were more efficient than intraglossal inoculation, with no transmissions to date, >220 d.

Discussion

The posit that α-synuclein prions cause PD began with speculation that PD, like kuru and CJD, might be caused by slow viruses (31). Although subsequent studies demonstrated that PrP^{Sc} prions, not viruses, cause kuru and CJD, the hypothesis that some CNS diseases, including AD and PD, are also caused by prions has gained increasing support (32–34). Similar to PrP^{Sc}, α-synuclein was found to assemble into β-sheet–rich amyloid fibrils (35, 36). In 2008, two groups reported Lewy bodies in fetal grafts of substantia nigra tissue, which had been implanted more than 10 y earlier in patients with advanced PD (37, 38). These findings argued that PD is a prion disease and that α-synuclein prions spread to the grafts. As the α-synuclein prions multiplied, they were sequestered into Lewy bodies in the fetal implants (39). Subsequently, α-synuclein prions were shown to arise spontaneously in TgM83$^{+/+}$ mice expressing mutant human α-synuclein by three separate groups (13, 40, 41). Concurrent with those studies, other investigations showed that recombinant WT human α-synuclein could assemble into amyloid fibrils in vitro and initiate CNS lesions after IC or IM injection (29, 41–44).

In 2013, we reported our unexpected finding suggesting that the human synucleinopathy MSA is a prion disease (13). Here, we report that brain homogenates prepared from 14 MSA cases (Table 1 and Table S1) were inoculated into the thalamus of TgM83$^{+/-}$ mice, nearly all of which subsequently developed progressive CNS dysfunction at ~120 dpi (Table 2). Homogenates prepared from the brains of selected MSA-inoculated mice were then inoculated into the thalamus of additional TgM83$^{+/-}$ mice, after which the second group of mice exhibited progressive CNS dysfunction with incubation times similar to those found on initial passage (Table 3) (14). Critical to the interpretation of our results is that TgM83$^{+/-}$ mice that were hemizygous for the human α-synuclein*A53T transgene did not develop CNS dysfunction spontaneously (13); in addition, the brains of these mice did not infect cultured HEK cells expressing α-syn140*A53T fused to YFP (14). In contrast, mouse brain homogenates from both the primary and secondary MSA prion transmissions did infect the cultured HEK cells.

The findings reported here demonstrate that α-synuclein prions exist in at least two different strains: MSA and TgM83$^{+/+}$. The incubation time for the TgM83$^{+/+}$ strain of α-synuclein prions in TgM83$^{+/-}$ mice was ~80% longer than that for the MSA strain as shown on both primary and secondary passage (Table 3). In previous studies using cultured HEK cells expressing α-syn140*A53T–YFP, we found evidence for three distinct MSA strains based on the quantity of MSA prions in different regions of three human brains (14).

Although there is no evidence that MSA is a naturally occurring transmissible disease among humans, the unequivocal experimental transmission studies reported here clearly warrant classification of MSA as a novel, bona fide α-synuclein prion

disorder. Like CJD, the vast majority of MSA cases are sporadic, with MSA arising spontaneously. All 14 of our MSA patients appear to be sporadic cases, as their *SNCA* and *COQ2* genes showed no missense mutations (Table S3). Additionally, like PrP^{Sc} prions, MSA prions are capable of spreading from cell to cell along the entire neuraxis (Fig. S1) (13). Importantly, the ability of MSA to induce progressive neurological disease in $TgM83^{+/-}$ mice represents the only other human prion disease apart from that caused by PrP^{Sc} to induce a lethal phenotype in an animal model.

Attempts to transmit PD to $TgM83^{+/-}$ mice were unsuccessful (Table 2). Notably, inoculation of brain fractions enriched for Lewy bodies from PD patients into WT mice and macaque monkeys induced limited Lewy body-like pathology, but neither species developed neurological disease (45). Using a similar approach, the insoluble protein fraction isolated from DLB patients induced phosphorylated α-synuclein pathology 15 mo after inoculation into WT mice, but the inoculations did not induce neurological deficits (46). Importantly, these human DLB transmission studies did demonstrate the spread of α-synuclein throughout the CNS and the phosphorylation of mouse α-synuclein. From our findings and those of others, we conclude that the putative α-synuclein prions causing PD represent one or more strains that differ from those causing MSA and from the one arising spontaneously in $TgM83^{+/+}$ mice. Alternatively, posttranslational chemical modifications might explain the difference between MSA and PD inocula. Ubiquitination, phosphorylation, nitrosylation, and sumoylation of α-synuclein have all been reported to play a role in α-synuclein toxicity (47–50).

Our hypothesis arguing that the MSA and PD prion strains are different is supported by the unique clinical presentations of these disorders and the distinct CNS locations of α-synuclein deposition: in MSA, within oligodendrocytes throughout the neuraxis, and in PD, within neuronal parikarya of the substantia nigra and the striatum, as well as surrounding axons. Our data and those of others contend that transmission of α-synuclein prions from MSA and PD patients to both an animal host and cultured cells requires different conditions to demonstrate infectivity.

Importantly, the transmission of MSA prions requires Tg mice expressing the A53T mutation found in familial PD, as mice expressing WT mouse or human α-synuclein were not capable of supporting MSA prion propagation (Table S4). Although the A53T mutation is likely to accelerate α-synuclein prion propagation by lowering the free energy barrier for replicating MSA prions, it is unclear if this amino acid substitution fundamentally altered the prions themselves. That said, single amino acid substitutions in PrP transgenes dramatically changed the susceptibility to PrP prion infection (51, 52).

Our discovery that MSA is caused by α-synuclein prions seems likely to force a revision in thinking about several important health care issues. First, deep brain stimulation (DBS) has become a widely used adjunct therapeutic intervention in PD, and many MSA cases may be initially misdiagnosed as PD. Our findings argue that the DBS electrodes, together with any associated equipment such as guide tubes and positioning microelectrodes that come in contact with CNS tissue, should not be reused. The accidental transmission of CJD prions from depth electrodes that were reused demands similar precautions for DBS equipment (53, 54). Our findings also contend that the same increased vigilance used in brain biopsies on suspected CJD cases should be applied to all synucleinopathy patients. Increased biocontainment should be considered for PD, DLB, and MSA patients undergoing DBS electrode implantation or other neurosurgical procedures. Even after PrP^{Sc} prions bound to stainless steel wires were subjected to routine decontamination procedures, they retained their ability to infect mice on brain implantation, as well as in cultures of susceptible cells (55, 56). The resistance of MSA prions to standard decontamination and sterilization procedures remains to be determined.

In conclusion, all 14 human brain samples collected from people who died of MSA could be transmitted to both Tg mice expressing α-syn140*A53T and to cultured HEK cells expressing the fusion protein α-syn140*A53T–YFP. Notably, neither normal control brain nor PD brain samples transmitted prions to $TgM83^{+/-}$ mice or HEK cells. Furthermore, we found that the brains from mice infected with MSA, but neither control brain nor PD, were also infectious in the $TgM83^{+/-}$ mouse and HEK cell assay. Based on these findings, we conclude that MSA is a prion disorder and that α-synuclein is the first new bona fide prion to be discovered, to our knowledge, in the last 50 y. Moreover, establishing that MSA is an α-synuclein prion disorder sets the stage for a new therapeutic campaign; disappointingly, past efforts have failed to produce a single drug that slows or halts the ravages of the synucleinopathies since the introduction of levodopa that ameliorates PD but not MSA symptoms (57).

Materials and Methods

All animal procedures were approved by the University of California, San Francisco, Institutional Animal Care and Use Committee, and all procedures are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication, *Guide for the Care and Use of Laboratory Animals* (58).

Human Tissue Samples. Frozen tissue samples were obtained from the Parkinson's UK Brain Bank at Imperial College London, the Sydney Brain Bank, the neuropathology core of the Massachusetts Alzheimer's Disease Research Center (ADRC), and the San Francisco VA Medical Center. Clinical reports were provided and are summarized in Table 1 and Table S1.

Patient Neuropathology. MSA and PD patient samples obtained from the Parkinson's UK Brain Bank were bisected, with one hemisphere fixed in 10% (vol/vol) buffered formalin for diagnostic workup and the other coronally sliced, photographed on a grid, and then rapidly frozen. Blocks of tissue from 20 key anatomical areas were sampled from the fixed hemisphere. Sections from each area were stained with H&E and Luxol fast blue (LFB). For assessment and staging of neurodegenerative pathology, appropriate sections were stained with antibodies against α-synuclein, β-amyloid, tau, and p62. MSA was diagnosed based on the presence of oligodendroglial α-synuclein inclusions. PD cases were staged according to Braak criteria (59).

MSA patient samples obtained from the Massachusetts ADRC were bisected longitudinally. One half was coronally sectioned and rapidly frozen, and the other half was fixed in 10% (vol/vol) neutral buffered formalin and then sectioned. Histological evaluation was performed on a set of blocked regions representative for a variety of neurodegenerative diseases. All blocks were stained with LFB and H&E. On selected blocks, immunohistochemical analysis, including α-synuclein (mouse monoclonal antibody LB509; Life Technologies 18-0215), β-amyloid, and phosphorylated tau, was performed. The neuropathological diagnosis of MSA required the presence of GCIs (15).

Human brain tissue acquired from the Sydney Brain Bank was bisected: one hemisphere was randomly designated for fresh dissection and the other fixed for at least 2 wk in 15% (vol/vol) buffered formalin [39% (vol/vol) aqueous formaldehyde solution] and then sectioned. Standard neuropathological assessment was performed on H&E-stained sections, and a modified Bielschowsky silver stain was used to identify Alzheimer-type pathologies. Immunohistochemical detection for phosphorylated α-synuclein (BD Biosciences USA; 1:7,000), phosphorylated tau (AT8, Thermo Scientific USA; 1:1,000), and β-amyloid (Dako Denmark; 1:500) was also carried out.

Quantification of GCIs and Lewy Bodies. Brain samples from each of the patients were stained for α-synuclein with clone 42 (BD Biosciences; 1:300), counterstained with hematoxylin, and scanned using an AxioScan.Z1 microscope (Zeiss). Within the AxioScan image analysis software, a grid was placed over each image to define 500-μm square fields, starting in a corner and moving in a serpentine fashion, lesions were counted in every 10th (substantia nigra) or 50th (pons and basal ganglia) field with at least 90% coverage and averaged for the whole section.

Quantification of Total α-Synuclein. Brain tissue was homogenized in calcium- and magnesium-free PBS to 10% (wt/vol) and processed with an ELISA kit (Anaspec #AS-55550) according to the manufacturer's instructions. Briefly, brain homogenates were diluted in the buffer provided and incubated in precoated wells overnight at 4 °C. Wells were washed seven times with wash

buffer provided. After a final wash, color was developed using the TMB-ELISA substrate provided. Plates were read at 450 nm optical density with a SpectraMax Plus microplate reader (Molecular Devices).

Sequencing of *SNCA* and *COQ2* Genes. DNA was extracted from the brain homogenates used for cell infection and mouse bioassay studies. The exons of the α-synuclein (*SNCA*) and *COQ2* genes from all 20 cases (14 MSA and 6 PD), as well as a single nondiseased control, were sequenced. Briefly, all five coding exons of *SNCA* and seven exons of *COQ2* were amplified by PCR using primers validated for specificity; primers used in this study are outlined in Table S2. The PCR products, ranging from 340 to 940 bp, were sequenced by Retrogen using standard Sanger sequencing and analyzed using Sequencher (www.genecodes.com) DNA sequence analysis software. Sequences from primer sets C (*SNCA* exon 4) and L (*COQ2* exon 1), which failed quality control, were resequenced using primer sets M and N, respectively (Table 2). A high-quality sequence was obtained in all samples for *SNCA* exons 2–5 and *COQ2* exons 3–5 and 7. For all other exons, a high-quality sequence was obtained for >75% of samples, except *COQ2* exon 1, in which only 8 of 21 sequences were reliable. Identified SNPs are shown in Table S3.

Mice. Homozygous TgM83$^{+/+}$ mice (60) expressing human α-synuclein with the A53T mutation maintained on a B6;C3 background were purchased from the Jackson Laboratory. Hemizygous TgM83$^{+/-}$ mice were generated either by backcrossing to FVB-Tg(*Gfap*-luc)$^{+/+}$ mice, as previously described (13), or to B6C3F1 mice. Because strain background and the presence of the *Gfap*-luc transgene did not appear to affect the incubation period, they are not distinguished further.

To determine the role of endogenous α-synuclein on the effect of MSA transmission, *Snca*$^{0/0}$ mice (61), a gift from Robert L. Nussbaum, University of California, San Francisco, were crossed with TgM83$^{+/+}$ mice. The resulting TgM83$^{+/-}$ *Snca*$^{0/+}$ mice were then intercrossed to generate TgM83$^{+/-}$ *Snca*$^{+/+}$, TgM83$^{+/-}$ *Snca*$^{0/+}$, and TgM83$^{+/-}$ *Snca*$^{0/0}$ mice on a matched genetic background. Mice expressing WT human α-synuclein driven by a P1 artificial chromosome, Tg(*SNCA*) *Snca*$^{0/0}$ (62), were a gift from Robert L. Nussbaum.

Inoculations. Human and mouse brain tissues were homogenized in calcium- and magnesium-free PBS to 10% (wt/vol) and were then diluted to 1% for inoculation using 5% (wt/vol) BSA in PBS. Approximately 2-mo-old mice were anesthetized with isoflurane and inoculated in the right parietal lobe with 30 µL 1% homogenate, unless otherwise stated. Mice were assessed for signs of neurological illness, based on standard diagnostic criteria for prion disease (63), twice a week, and euthanized once signs of progressive neurological dysfunction were apparent. In preliminary studies, mice died within a few days of clinical onset; therefore, to ensure analysis of fresh tissue in the studies reported here, mice were euthanized within 2 d of showing progressive CNS dysfunction. Brains were bisected, and the left hemisphere was frozen for biochemical analysis, whereas the right was fixed in formalin for neuropathology.

Immunohistochemistry of Mouse Brains. Mouse brains fixed in 10% (vol/vol) formalin were embedded in paraffin, and sections were cut at 8 µm. Sections were deparaffinized and treated with 3% (vol/vol) hydrogen peroxide in methanol for 30 min. Slides were then blocked with 10% (vol/vol) normal goat serum and incubated with primary antibody overnight. Sections were stained with anti-phosphorylated synuclein EP1536Y (Abcam #ab51253; 1:1,000 dilution), as well as anti-GFAP (Dako #Z0334; 1:500 dilution). Bound antibody was detected using a Vectastain ABC peroxidase kit (Vector Laboratories) and visualized using 3,3′-diaminobenzidine. Slides were counterstained with hematoxylin and imaged using an AxioScan.Z1 microscope (Zeiss).

Biochemical Analysis of Synuclein Aggregates. Brains were homogenized in calcium- and magnesium-free PBS and then diluted to 10% (wt/vol). Total protein was quantified using the bicinchoninic acid assay kit (Pierce), and 5 µg total mouse brain tissue homogenate or 25 µg human brain tissue homogenate was run on a 4–12% gradient polyacrylamide gel (Invitrogen). Proteins were transferred to PVDF membranes, blocked with 5% (vol/vol) milk, and probed with a monoclonal α-synuclein antibody, Syn211 (ThermoScientific; 1:4,000 dilution), to assess for total synuclein. To assess the presence of phosphorylated synuclein aggregates, 250 µg total protein was incubated with buffer (0.01 M Tris HCl, pH 8.0; 0.15 M NaCl; 0.5% Nonidet P-40; 0.5% deoxycholic acid) for 30 min at room temperature with shaking. Samples were then centrifuged at 100,000 × *g* for 1 h at 4 °C. The pellet was resuspended in 1× NuPAGE loading buffer. Samples were separated and transferred to PVDF as described above, and blots were probed with the antiphosphorylated α-synuclein antibody EP1536Y (Abcam #ab51253; 1:4,000 dilution).

α-Synuclein Prion Cell Assay. The prion infectivity assay was carried out as previously described (14). Briefly, 10% (wt/vol) brain homogenate was combined with benzonase and sarkosyl to a final concentration of 2% (vol/vol) and incubated at 37 °C for 2 h on a shaking incubator. To this, PTA, pH 7.0, was added to a final concentration of 2% (vol/vol) and incubated overnight. The insoluble fraction was isolated by spinning at 16,000 × *g* for 30 min; the pellet was resuspended in 2% (vol/vol) sarkosyl and PTA and incubated at 37 °C for 1 h. Samples were centrifuged for 30 min at 16,000 × *g*, and the pellet was resuspended in PBS. Samples were diluted 1:4 and incubated with 1,000 HEK cells stably expressing the α-syn140*A53T–YFP fusion protein in a 384-well black-walled plate (Greiner) for 4 d before imaging with an IN-Cell Analyzer 6000 (GE). Four images were collected from each well of the plate, and six replicate wells per sample were tested. Images were analyzed for aggregate formation using an algorithm we developed to detect YFP-positive aggregates among living cells, which was determined by nuclear stain. The four images from each well were averaged to determine a value for each well, and the six replicate wells were averaged to determine mean ± SEM for each sample.

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研究報告の概要

世界中で 200 人以上が、プリオンで汚染されたヒトの死後脳下垂体由来成長ホルモンで、一般的に小児期に治療を受けた結果、クロイツフェルト・ヤコブ病 (CJD) を発症した。このような治療は 1985 年に中止されたが、ヒトプリオン感染における長い潜伏期間のため、医原性 CJD の発生は続いている。意外なことに、36～51 歳の 8 人の医原性 CJD 患者の剖検研究では、そのうちの 4 人に中程度から重度の灰白質病変および血管のアミロイドβ (Aβ) 病変が見られた。灰白質のアミロイドβ沈着はアルツハイマー病に一般的に見られるものであり、血管壁のアミロイドβ沈着は脳アミロイド血管症の特徴だが、これらのアミロイドβ沈着はプリオンたん白質の沈着とは共存していなかった。これらの患者に早期発症型アルツハイマー病に関連する病原性変異や、APOE ε4 などのハイリスク対立遺伝子を持つ人はいなかった。ある前向き観察コホート研究に参加した他のプリオン病の患者 116 人を調べたところ、同様の年齢幅、或いは 10 歳年齢が高い症例では、APOE ε4 リスク対立遺伝子を持たない場合、アミロイドβ病変は全く見られないか、あってもごくわずかであることが分かっている。我々は、アミロイドβ病変の見られる患者の脳下垂体を解析し、複数の症例で顕著なアミロイドβ沈着を見つけた。アミロイドβ病変の実験的なシード形成は、以前に霊長類およびトランスジェニックマウスでアルツハイマー病の脳ホモジェネートを中枢神経系、或いは末梢神経に接種することにより実証されている。今回、比較的若い医原性 CJD 患者で、実質や血管に顕著なアミロイドβ沈着が見られたことは、他のプリオン病患者や集団対照とは対照的に、CJD に加えてアミロイドβ病変の医原性伝播もあることと一致しており、曝露された健康な人も医原性のアルツハイマー病や脳アミロイド血管症を発症するリスクがあり得ることを示唆している。これらの知見によって、プリオン伝播の他の既知の医原性感染ルートも、神経変性疾患やその他のヒト疾患に関連するアミロイドβなどのタンパク質病の病原体に関係しているかどうかについての研究が促されるだろう。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV 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。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビンⅢを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
アルツハイマー病 (Alzheimer's disease : AD) は、認知機能低下，人格の変化を主な症状とする認知症の一種であり、アミロイド-β (amyloid-β : Aβ) が脳組織に貯まることによって起こると考えられている。Aβは脳の血管壁にも蓄積し、この状態を脳アミロイド血管症と言い、アルツハイマー型認知症の 90%以上が合併していると言われている。今般、人脳下垂体由来成長ホルモンで治療を受け、その後にクロイツフェルトヤコブ病 (Creutzfeldt-Jakob disease : CJD) を発症した比較的若い 4 名の患者の実質や血管に顕著な Aβ沈着が認められたことから、医原性 CJD と同様に、Aβ病変も医原的に伝播しうる可能性が示唆されたとするものである、との報告がなされた。輸血等による Aβ伝播の可能性を示唆しているものではないが、その可能性を否定できるものでもないと考える。万一、原料血漿に Aβが混入したとしても、製造工程中のウイルス除去膜処理等により、除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるが、今後、関連情報の収集に努める。

(2)

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Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy

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More than two hundred individuals developed Creutzfeldt-Jakob disease (CJD) worldwide as a result of treatment, typically in childhood, with human cadaveric pituitary-derived growth hormone contaminated with prions[1,2]. Although such treatment ceased in 1985, iatrogenic CJD (iCJD) continues to emerge because of the prolonged incubation periods seen in human prion infections. Unexpectedly, in an autopsy study of eight individuals with iCJD, aged 36–51 years, in four we found moderate to severe grey matter and vascular amyloid-β (Aβ) pathology. The Aβ deposition in the grey matter was typical of that seen in Alzheimer's disease and Aβ in the blood vessel walls was characteristic of cerebral amyloid angiopathy[3] and did not co-localize with prion protein deposition. None of these patients had pathogenic mutations, *APOE* ε4 or other high-risk alleles[4] associated with early-onset Alzheimer's disease. Examination of a series of 116 patients with other prion diseases from a prospective observational cohort study[5] showed minimal or no Aβ pathology in cases of similar age range, or a decade older, without *APOE* ε4 risk alleles. We also analysed pituitary glands from individuals with Aβ pathology and found marked Aβ deposition in multiple cases. Experimental seeding of Aβ pathology has been previously demonstrated in primates and transgenic mice by central nervous system or peripheral inoculation with Alzheimer's disease brain homogenate[6–11]. The marked deposition of parenchymal and vascular Aβ in these relatively young patients with iCJD, in contrast with other prion disease patients and population controls, is consistent with iatrogenic transmission of Aβ pathology in addition to CJD and suggests that healthy exposed individuals may also be at risk of iatrogenic Alzheimer's disease and cerebral amyloid angiopathy. These findings should also prompt investigation of whether other known iatrogenic routes of prion transmission may also be relevant to Aβ and other proteopathic seeds associated with neurodegenerative and other human diseases.

Human transmission of prion disease has occurred as a result of a range of medical and surgical procedures worldwide as well as by endocannibalism in Papua New Guinea, with incubation periods that can exceed five decades[12,13]. A well-recognized iatrogenic route of transmission was by treatment of persons of short stature with preparations of human growth hormone, extracted from large pools of cadaver-sourced pituitary glands, some of which were inadvertently prion-contaminated. Such treatments commenced in 1958 and ceased in 1985 following the reports of the occurrence of CJD amongst recipients. A review of all 1,848 patients who were treated with cadaveric-derived human growth hormone (c-hGH) in the United Kingdom from 1959 through 1985 found that 38 had developed CJD by the year 2000 with a peak incubation period of 20 years[1]. Multiple preparations using different extraction methods were used over this period and patients received batches from several preparations. One preparation (Wilhelmi) was common to all patients who developed iCJD and size-exclusion chromatography, used in non-Wilhelmi preparation methods, may have reduced prion contamination[1]. As of 2012, a total of 450 cases of iatrogenic CJD have been recognized worldwide after treatment with c-hGH or gonadotropin (226 cases), transplantation of dura mater (228) or cornea (2), and neurosurgery (4) or electroencephalography recording using invasive medical devices (2)[2]. In France, 119/1,880 (6.3%) recipients developed iCJD, in the UK 65/1,800 (3.6%) and in the USA 29/7,700 (0.4%)[2,14].

Since 2008, most UK patients with prion disease have been recruited into the National Prion Monitoring Cohort study[5], including 22 of 24 recent patients with iatrogenic CJD (iCJD) related to treatment with c-hGH over this period, all of whom necessarily have very long incubation periods. Of this group of patients with iCJD, eight patients (referenced no.s 1–8, Supplementary Information) aged 36–51 years, with an incubation period from first treatment to onset of 27.9–38.9 years (mean 33 years) and from last treatment to onset of 18.8–30.8 years (mean 25.5 years), underwent autopsy with extensive brain tissue sampling at our hospital. In all eight brain samples we confirmed prion disease with abnormal prion protein labelling of the neuropil, perineuronal network and in most cases microplaques as described previously[15–17]. However, four (no.s 4, 5, 6, 8) of the eight patients with iCJD also showed substantial amyloid-β (Aβ) deposition in the central nervous system parenchyma by histology (Fig. 1) and immunoblotting (Fig. 2). A further two brain samples (no.s 1, 3) had focal Aβ pathology in one of the brain regions; one showed Aβ entrapment in PrP plaques and only one was entirely negative for Aβ. Furthermore, there was widespread cortical and leptomeningeal cerebral Aβ angiopathy (CAA)[3] in three patients (no.s 4, 6, 8) and focal CAA in one patient (no. 5) (Fig. 1). Such pathology is extremely rare in this age range, 10/290 in the equivalent 36–50 year age strata without CJD[18], $P = 0.0002$, Fisher's test. None of our patients with iCJD had pathogenic mutations in the prion protein gene (*PRNP*). We used a custom next generation sequencing panel[4] to exclude mutation in any of 16 other genes associated with early-onset Alzheimer's disease, CAA, or other neurodegenerative disorders, and none carried *APOE* ε4 or *TREM2* R47H alleles (Supplementary Table 2). Although such observations are unprecedented in our wide experience of human prion diseases, we nevertheless considered whether prion disease itself might predispose to, or accelerate, Aβ pathology, for example by cross-seeding of protein aggregation or overload of clearance mechanisms for misfolded proteins. We therefore compared the Aβ pathology in the iCJD cohort with that of a cohort of 116 patients with other prion diseases who had undergone autopsy: sporadic CJD (sCJD) ($n = 85$, age 42–83), variant CJD ($n = 2$, age 25 and 36) and inherited prion diseases (IPD) ($n = 29$, age 29–86). None of the patients in the control cohorts had comparable Aβ pathology (Consortium to Establish a Registry for Alzheimer's disease (CERAD) score, $P = 0.001$, CAA,

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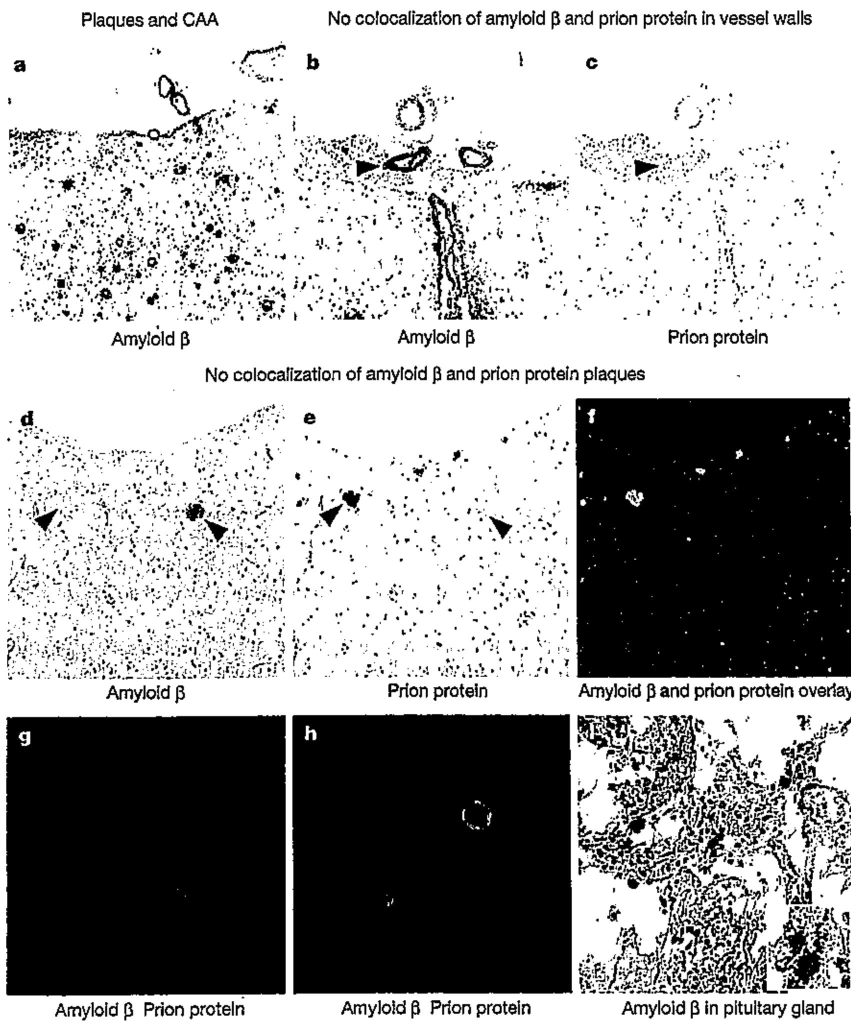

Figure 1 | Aβ accumulation in central nervous system parenchyma and blood vessels (CAA) in iCJD. **a**, Frontal cortex with widespread diffuse Aβ deposition, formation of plaques, and widespread parenchymal and leptomeningeal CAA (patient no. 4). **b**, **c**, Non-colocalized deposition of Aβ and prion protein. Vessels with CAA do not entrap or co-seed prion protein. **d**, **e**, Adjacent histological sections stained for Aβ or prion protein show clearly separated plaques of both proteins (no. 5). **f**, An overlay with colour inversion of prion protein plaques highlights the separation. **g**, **h**, Dual labelling, confocal laser microscopy shows no co-localization of parenchymal Aβ plaques (no.s 5, 6) or CAA (no. 6). **i**, Aβ is detected in pituitary glands in patients with a high Aβ load in the brain. Scale bar corresponds to 200 μm in **a**, 100 μm in **b**–**h**, and 50 μm in **i**.

$P = 0.005$, topographical Aβ score $P = 0.02$, and cumulative Aβ score $P = 0.02$ (rank sum test) and digital Aβ quantification $P = 0.04$ (t-test); all restricted to the strata aged 36–51 years ($n = 19$)) (Fig. 3, and Extended Data Figs 1 and 2 show similar results in adjusted analyses in the full cohort). Indeed none of 35 prion cases aged 52–60 had significant Aβ pathology, with the exception of two cases at ages 57 and 58 positive for *APOE* ε4 alleles. Instead, the sCJD cohort shows Aβ pathology in parenchyma and blood vessels to a similar extent/severity as seen in iCJD, only in a much older age group (Extended Data Figs 1 and 2), in keeping with the chance coincidence of late-onset Aβ pathology and sCJD as previously documented in a large study of 110 sCJD patients and 110 age-matched controls aged 27–84 (ref. 19) and a study

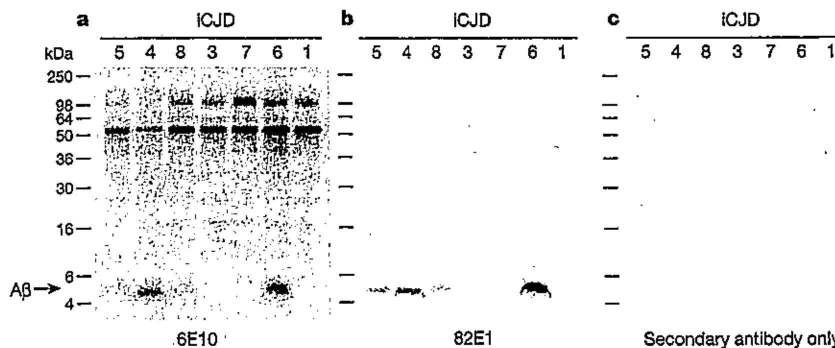

Figure 2 | Immunoblots of Aβ in iCJD patient brains. **a**–**c**, 10% (w/v) brain homogenates from patients with iCJD were analysed by enhanced chemiluminescence using anti-human Aβ monoclonal antibodies 6E10 that recognizes full-length APP and fragments that contain the epitope including Aβ (**a**) or 82E1 that specifically recognizes Aβ (**b**) or secondary antibody only (**c**). The identity of the patient brain sample is designated above each lane and the position of molecular mass markers is shown to the left. The equivalent of 5 μl 10% (w/v) brain homogenate was loaded per lane. The migration position of Aβ is indicated by the arrow. For gel source data, see Supplementary Fig. 1.

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of 2,661 individuals aged 26–95 (ref. 18). Further, we investigated whether prion and Aβ pathology co-localize in the iCJD cases. In our series there was a distinct absence of overlap of Aβ plaques and PrP (Fig. 1d, e) or Aβ CAA and vascular PrP (Fig. 1b, c), consistent with these pathologies developing independently.

We then went on to examine pituitary glands for the presence of Aβ deposits. Pathological species of tau, Aβ and α-synuclein have been reported in the pituitary gland of patients with neurodegenerative disease and controls[20]. We examined 55 pituitary glands, 6 from patients without, and 49 from patients with cerebral Aβ pathology, and found in the latter group seven samples containing Aβ, confirming frequent Aβ in pituitaries of patients with Alzheimer's disease-like pathology[20] (Fig. 1i and Extended Data Fig. 3), consistent with the hypothesis that Aβ seeds have been iatrogenically transmitted to these patients with iCJD.

There has been longstanding interest as to whether other neurodegenerative diseases associated with the accumulation of aggregates of misfolded host proteins or amyloids might be transmissible in a 'prion-like' fashion[21,22]. Experimental seeding of Aβ pathology has previously been demonstrated in primates and transgenic mice by central nervous system inoculation with Alzheimer's disease brain homogenate[6–10]. Of particular interest with respect to our findings is that peripheral (intraperitoneal) inoculation with Alzheimer's disease brain extract into APP23 (ref. 11) transgenic mice has been demonstrated. While ageing APP23 mice show mostly parenchymal deposits, the intraperitoneally-seeded mice showed predominantly CAA, a feature seen in patients with iCJD who had significant Aβ pathology. This experimental study and our findings suggest that there are mechanisms to allow the transport of Aβ seeds as well as prions (and possibly other proteopathic seeds such as tau[23]) from the periphery to the brain[24,25]. While less than 4% of UK c-hGH treated individuals have developed iCJD, one out of eight patients with iCJD had focal, and three had widespread, moderate or severe CAA. Four patients had widespread parenchymal Aβ pathology and two further patients had focal cortical Aβ deposits. This might suggest that healthy individuals exposed to c-hGH are at high risk of developing early-onset Aβ pathology as this cohort ages.

Although none of the iCJD cases with Aβ pathology had hyperphosphorylated tau neurofibrillary tangle pathology characteristic of Alzheimer's disease, it is possible that the full neuropathology of Alzheimer's disease would have developed had these individuals not succumbed to prion disease at these relatively young ages. An earlier study concluded that c-hGH recipients did not seem to be at increased risk of Alzheimer's disease, but this was based on death certificates only without autopsy data[20]. However, the severe CAA seen in the patients with iCJD in our study is unquestionably concerning and individuals with such pathology would be at increasing risk of cerebral haemorrhages had they lived longer. At-risk individuals, including patients who had received dura mater grafts[26] could be screened by magnetic resonance imaging (MRI) for CAA-related pathologies (such as microbleeds) and by positron emission tomography (PET) for Aβ deposition[27].

It is possible, however, that prions and Aβ seeds co-purify in the extraction methods used to prepare c-hGH, which might mean that there would be a relatively higher occurrence of Aβ pathology in those with iatrogenic prion infection. Analysis of any residual archival batches of c-hGH for both prions and Aβ seeds might be informative in this regard[2]. While our data argue against cross seeding, we cannot formally exclude the possibility that prions somehow seed Aβ deposition but do not co-localize with Aβ deposits. While there is no suggestion that Alzheimer's disease is a contagious disease and no supportive evidence from epidemiological studies that Alzheimer's disease is transmissible, notably by blood transfusion[28,29], our findings should prompt consideration of whether other known iatrogenic routes of prion transmission, including surgical instruments and blood products, may also be relevant to Aβ and other proteopathic seeds seen in neurodegenerative diseases. Aβ seeds are known, like prions, to adhere to metal surfaces and to resist formaldehyde inactivation and conventional hospital sterilisation[30].

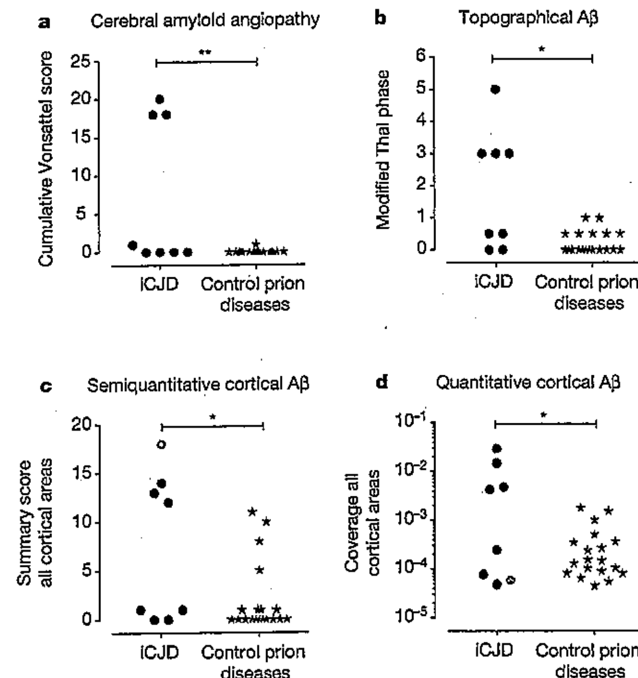

Figure 3 | Early Aβ accumulation in the parenchyma and blood vessels in a subset of eight patients with iCJD aged 36–51 years, but not in controls (stratum aged 36–51 years) of 19 prion diseases of other aetiologies, suggests human transmission. a, Widespread, moderate-to-severe early-onset CAA in three, and focal, mild CAA in one iCJD patient but only one focal, mild CAA in 19 controls. **b,** Significant differences of parenchymal Aβ accumulation (all central nervous system regions, see supplementary material). **c, d,** Cortical Aβ load was assessed semiquantitatively and quantitatively and again was significantly different between the iCJD and age-matched control cohort. For methods of quantification and calculations of significance levels see Supplementary Information.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions Z.J. and S.B. performed autopsies and neuropathological diagnosis and analysis of cases. J.K., S.M., P.R. and J.C. diagnosed and recruited patients to the study and analysed clinical and genetic data. A.S.W. performed statistical analysis and modelling. J.D.F.W. and A.J.N. performed western blots, M.E., F.L., A.R.-L. and J.L. performed immunohistochemistry and analysed data, J.C. and S.B. oversaw the study and drafted the manuscript with contributions from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.B. (s.brandner@ucl.ac.uk) or J.C. (j.collinge@prion.ucl.ac.uk).

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METHODS

No statistical methods were used to predetermine sample size, the experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Patient recruitment and genotyping. A national referral system for prion diseases was established by the Chief Medical Officer in the UK in 2004. UK neurologists were asked to refer all patients with suspected prion disease jointly to the National CJD Research and Surveillance Unit in Edinburgh and the NHS National Prion Clinic (NPC) in London. All patients with possible CJD who had received cadaver-derived growth hormone were referred to the NHS National Prion Clinic (London, UK) and since 2008 were recruited into the National Prion Monitoring Cohort study.

Next-generation sequencing to exclude mutations known to be causal of Aβ pathology. Deep next-generation sequencing using a custom panel was performed as described previously[31]. Analysis was done using NextGENe and Geneticist Assistant software (Softgenetics, USA). Variants were assessed for pathogenicity by reference to the published literature, control population allele frequencies (our primary database for allele frequency was the Broad Institute's ExAC browser (http://exac.broadinstitute.org/)) and *in silico* predictive tools. The analysis methodology has been validated for the detection of *APP* duplication[31], which was important to exclude. No causal mutations for dementia or Aβ pathology were detected, see Supplementary Table 2. As expected, several rare variants were detected which may modify the risk of various neurodegenerative diseases, see Supplementary Table 2.

Autopsies and tissue preparation. Autopsies were carried out in a post mortem room designated for high risk autopsies. Informed consent to use the tissue for research was obtained in all cases. Ethical approval for these studies was obtained from the Local Research Ethics Committee of the UCL Institute of Neurology/National Hospital for Neurology and Neurosurgery. The anterior frontal, temporal, parietal and occipital cortex and the cerebellum (at the level of dentate nucleus) were dissected during the post mortem procedure and frozen. Samples of the following areas were taken and analysed: frontal, temporal, parietal, occipital, posterior frontal cortex including motor strip, basal ganglia, thalamus, hippocampus, brain stem including midbrain, and cerebellar hemisphere and vermis. Pituitary glands were taken in all cases.

Tissue samples were immersed in 10% buffered formalin and prion infectivity was inactivated by immersion into 98% formic acid for one hour. Tissue samples were processed to paraffin wax and tissue sections were routinely stained with haematoxylin and eosin.

Antibodies and immunohistochemistry. The following antibodies were used: Anti-PrP ICSM35 (D-Gen Ltd, London, UK[32,33] 1:1,000), Anti-phospho-Tau (AT-8, Innogenetics, 1:100) and anti-βA4 (DAKO 6F3D, 1:50). ICSM35 was stained on a Ventana Benchmark or Discovery automated immunohistochemical staining machine (ROCHE Burgess Hill, UK); βA4 and Tau were stained on a LEICA BondMax (LEICA Microsystems) or a Ventana automated staining instrument following the manufacturer's guidelines, using biotinylated secondary antibodies and a horseradish-peroxidase-conjugated streptavidin complex and diaminobenzidine as a chromogen.

Immunoblot detection of Aβ in iCJD brain. Biochemical studies were carried out in a microbiological containment level 3 facility with strict adherence to safety protocols. Frozen brain tissue was available from seven of eight patients with growth hormone iCJD (cases 1 and 3–8). 10% (w/v) brain homogenates (grey matter; frontal cortex) were prepared in Dulbecco's PBS lacking Ca^{2+} or Mg^{2+} ions using tissue grinders as described previously[34]. 20-μl aliquots were treated with 1 μl benzonase nuclease (purity >99%; 25 U ml^{-1}; Novagen) for 15 min at 20 °C. Samples were then mixed with an equal volume of 2× SDS sample buffer (125 mM Tris-HCl, 20% (v/v) glycerol pH 6.8 containing 4% (w/v) SDS, 4% (v/v) 2-mercaptoethanol and 0.02% (w/v) bromophenol blue) and immediately transferred to a 100 °C heating block for 10 min. Electrophoresis was performed on 16% Tris-glycine gels (Invitrogen), run for 70 min at 200 V, before electroblotting to Immobilon P membrane (Millipore) for 16 h at 15 V as described previously[34]. Membranes were blocked in phosphate buffered saline (PBS) containing 0.05% (v/v) Tween 20 (PBST) and 5% (w/v) non-fat dried skimmed milk powder. Blots were then probed with anti-human Aβ monoclonal antibodies 6E10 (Covance) and 82E1 (IBL international, Hamburg, Germany) at final concentrations of 0.2 μg ml^{-1} in PBST for at least 1 h. After washing for 1 h with PBST the membranes were probed with a 1:10,000 dilution of alkaline-phosphatase-conjugated goat anti-mouse IgG secondary antibody (Sigma-Aldrich no. A2179) in PBST. After washing (90 min with PBST and 5 min with 20 mM Tris pH 9.8 containing 1 mM $MgCl_2$) blots were incubated for 5 min in chemiluminescent substrate (CDP-Star; Tropix Inc.) and visualized on Biomax MR film (Carestream Health Inc.). Anti-human Aβ monoclonal antibody 82E1 recognizes an epitope specific to the amino terminus of Aβ while 6E10 recognizes an epitope spanning residues 3–8 of Aβ and cross-reacts with full-length APP or APP fragments that contain the epitope.

Examination of prion pathology. In all iCJD cases there was variably prominent microvacuolar change in the neocortices, deep grey nuclei and cerebellar cortex. Immunostaining for the abnormal prion protein revealed synaptic labelling in all grey matter areas examined. In all but one case, there were also microplaques in all grey matter structures. Variability in the intensity of the immunoreactivity for the abnormal prion protein was evident but detailed comparison between the cases and separately within each case was not feasible as prolonged formalin fixation in some cases significantly attenuated the immunoreactivity. It was apparent that more prominent microvacuolar change and synaptic labelling for abnormal prion protein was more intense in the pre-central gyrus and parietal lobe when compared to the anterior frontal and occipital cortices. Deep cortical layers showed more severe changes. In all cases the microvacuolar degeneration and prion protein deposits in the deep grey nuclei and hippocampal formation was prominent. It was most severe in the caudate nucleus and putamen, and appeared less severe in thalamus and it was least prominent in the globus pallidus. In the cerebellar vermis there was marked granule cell atrophy and often widespread loss of Purkinje cells accompanied by severe Bergmann gliosis, while cerebellar hemispherical cortex showed only patchy loss of Purkinje cells and no significant granule cell loss. Microvacuolar degeneration in the molecular layer was more prominent in the vermis than in the cerebellar hemisphere. No apparent difference in prion protein deposition was seen in vermis and hemisphere. In the dentate nucleus variably intense synaptic prion protein immunoreactivity was present, while the cytoarchitecture of the nucleus was well preserved.

Examination, classification and quantification of Aβ pathology. All brains were examined according to the ABC classification[35], which assesses the topographic progression of Aβ pathology in the brain (Thal phases[36]), topographic progression of Tau neurofibrillary tangle pathology (Braak and Braak[37]) and the density of mature (senile), neuritic plaques in the neocortex (Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria[38,39]). To allow a more detailed assessment of neocortical Aβ the original Thal phases were modified as follows. Phase 0, no cortical Aβ; phase 0.5, 1–2 neocortical regions affected; phase 1, 3–4 neocortical regions involved; phases 2–5 were scored as published[36]. In addition we have carried out a semiquantitative assessment of neocortical Aβ load on a standardised region within frontal, temporal, parietal and occipital lobes, and scored as follows. 0, entirely negative; 1, a single small deposit; 2, multiple small deposits, disseminated; 3, multiple small deposits, plus an area with a larger patch; 4, diffuse moderate numbers of deposits; 5, diffuse, frequent numbers of deposits. For each case a cumulative score (0–20) of total semiquantitatively assessed Aβ load in the neocortex was calculated. Cerebral amyloid angiopathy (CAA) was graded (0–3) according to the Vonsattel criteria[3]. CAA was assessed in leptomeninges and parenchyma of all hemispheric lobes and cerebellum with summary score (0–30) calculated for each case.

Image acquisition and processing. Histological slides were digitised on a LEICA SCN400F scanner (LEICA Milton Keynes, UK) at ×40 magnification and 65% image compression setting during export. Slides were archived and managed on LEICA Slidepath (LEICA Milton Keynes, UK). For the preparation of light microscopy images, 1,024 × 1,024 pixel sized image captures were taken, after matching paired images (Aβ and prion staining) in Slidepath, and overlays in Fig. 1f were prepared using the colour conversion function in conjunction with the image overlay in Slidepath. Laser scanning microscopy of double immunofluorescent tissue preparations was on a ZEISS LSM710 confocal microscope (ZEISS Cambridge, UK). Publication figures were assembled in Adobe Photoshop. Data plots were generated using Prism 5 (GraphPad Software, Inc., La Jolla, USA).

Digital image analysis for cortical Aβ quantification. From all cases Aβ immunostained slides from frontal, temporal, parietal and occipital lobes were digitised as described above. Digital image analysis on 496 whole slides was performed using Definiens Developer 2.3 (Definiens, Munich, Germany). Initial tissue identification was performed at a resolution corresponding to 5× image magnification and stain detection was performed at ×10 resolution. Tissue detection and initial segmentation was done to identify all tissue within the image, separating the sample from background and non-tissue regions for further analysis. This separation was based on identification of the highly homologous relatively bright/white region of background present at the perimeter of each image. A composite raster image produced by selecting the lowest pixel value from the three comprising colour layers (RGB colour model) provided a greyscale representation of brightness. The mean brightness of this background region was used to exclude all background regions from further analysis.

Stain detection (brown) is based on the transformation of the RGB colour model to a HSD representation[40]. This provides a raster image of the intensity of each colour of interest (brown and blue). A series of dynamic thresholds (T_x) are then used to identify areas of interest (A_x). Initially, following exclusion of intensely stained areas with values greater than 1 arbitrary unit (au) (values range from 0 au to 3 au in HSD images), the 5th centile ($\overline{C5}$) of brown stain intensity was calculated

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as a baseline. This represents the $T_{\text{brown stain}}$ separating the top 5% of A_{tissue}. The standard deviation ($C5\delta$) within the lower 95% of A_{tissue} was used to update the $T_{\text{brown stain}}$ as $\overline{C5} + (6 \times C5\delta)$ with all pixels above this threshold classed as 'stain' (A_{stain}) and those below as 'unstained' ($A_{\text{unstained}}$). A_{stain} was excluded if the intensity of blue staining was not significantly lower than the level of brown stain (difference less than 0.1au). to remove generically dark areas. The remaining A_{stain} were further categorised using thresholds based on the mean ($\bar{B}$) and standard deviation (Bδ) of brown staining within the $A_{\text{unstained}}$: $T_{\text{brown}} = \bar{B} + (3 \times B\delta)$ (lower threshold); $T_{\text{dark brown}} = \bar{B} + (6 \times B\delta)$ (upper threshold), to give $A_{\text{unstained}} \leq T_{\text{brown}} > A_{\text{light brown}} \leq T_{\text{dark brown}} > A_{\text{A}\beta\text{ deposit}}$. Artefacts were then identified as A_{stain} with area greater than 1 mm^2, or, an area greater than 0.1 mm^2 with a standard deviation of brown staining below 0.2au. These $A_{\text{artefacts}}$ were then expanded to include surrounding pixels with brown staining greater than $\overline{C5}$. This excludes large areas of homogenous staining and areas of more diffuse, non-specific chromogen deposit.

The white matter region within the tissue was then manually selected by an expert neuropathologist (Z.J., S.B.). This white matter was excluded from calculation of proportional coverage of $A_{\text{A}\beta\text{ deposit}}$ within A_{tissue}.

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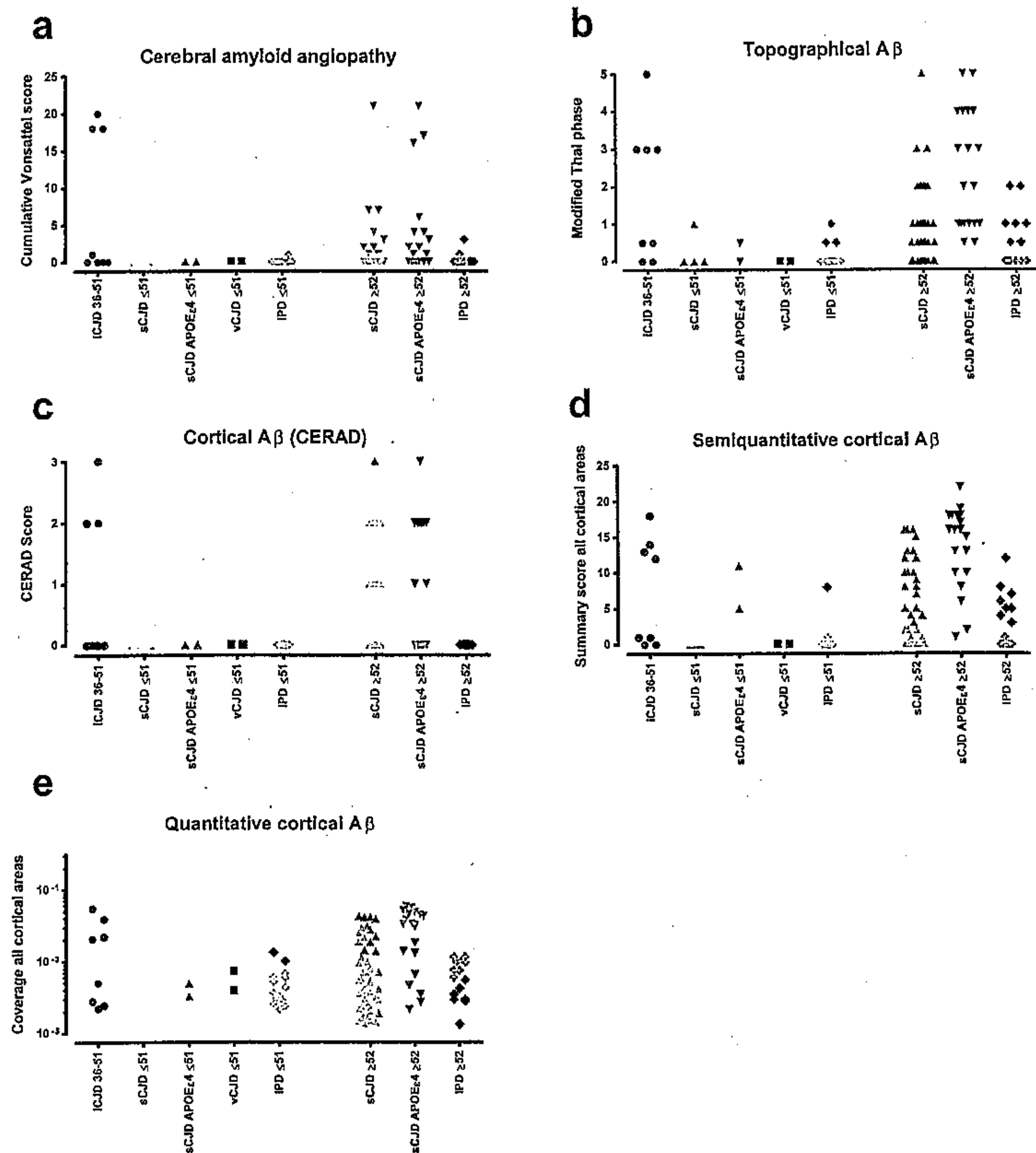

Extended Data Figure 1 | Vertical scatter plot of Aβ pathology in iCJD brain samples, age-matched and older controls. **a**, CAA summary score as described in Methods. iCJD (age range 36–51) with three highly scoring brain samples with CAA. In the age-matched control groups, no comparable pathology was found. Significant CAA was only seen in a cohort including older individuals carrying the *APOE* ε4 allele. The outlier in the sCJD group (pink triangle in the sCJD > 52 year group) had a surgical intervention 40 years before death, and in addition to CJD also had severe CAA. **b**, Topographical Aβ deposition, assessed according to a scheme modified from the Thal classification as described in the Methods section. In the group of individuals of 51 years and below, significant Aβ deposition is seen in the iCJD group, but not in age-matched prion diseases of different aetiology. **c**, Cortical Aβ deposition, assessed according to CERAD. In the group of individuals of 51 years and below, mature (neuritic) plaques are seen in the iCJD group, but not in age-matched prion diseases of different aetiology. Only in the cohort comprising much older individuals is there an increase of cortical mature plaques. **d**, **e**, Semiquantitative assessment and quantification of neocortical Aβ using Definiens Developer image analysis shows a separation that is similar to that shown in **a** and **b**. *APOE* ε4 genotype was unavailable in nine of the 85 patients with sCJD and these were not included in the graphs. Note the logarithmic scale in **e**.

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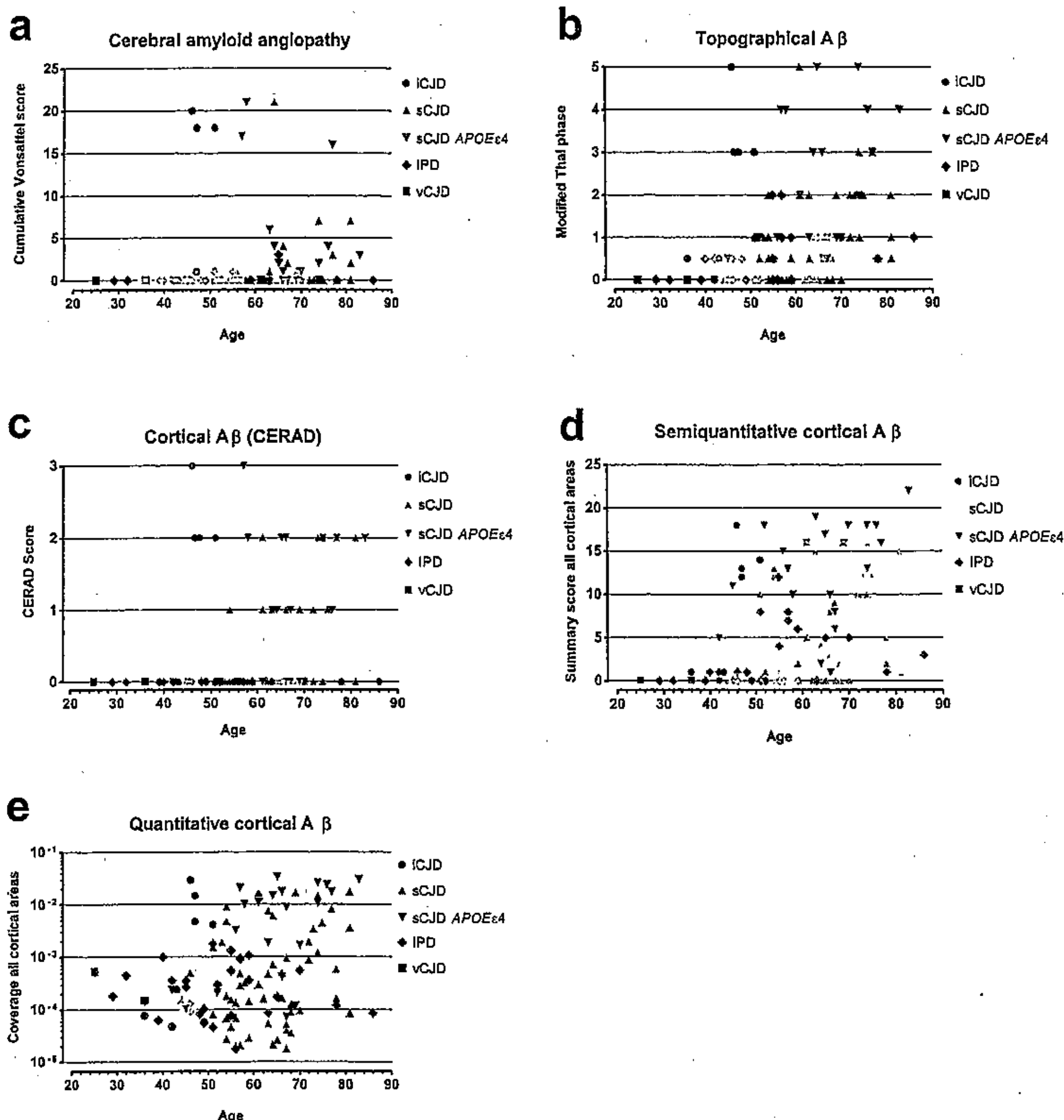

Extended Data Figure 2 | Scattergram of Aβ pathology in iCJD brain samples compared with other prion diseases. Plot of severity scores of CAA or parenchymal Aβ against the age of individuals in the cohorts, demonstrating early-onset of CAA and grey matter Aβ pathology in the iCJD cohort. **a**, Early-onset of CAA. The outlier in the sCJD group (pink triangle) had a surgical intervention 40 years before death, and in addition to CJD also had severe CAA. **b**, **c**, Early detection of grey matter Aβ by a topographical assessment (Thal phase) and using CERAD Criteria. **d**, **e**, Semiquantitative assessment and quantification of neocortical Aβ. *APOE* ε4 genotype was unavailable in nine of the 85 patients with sCJD and these were not included in the graph. Note the logarithmic scale in e.

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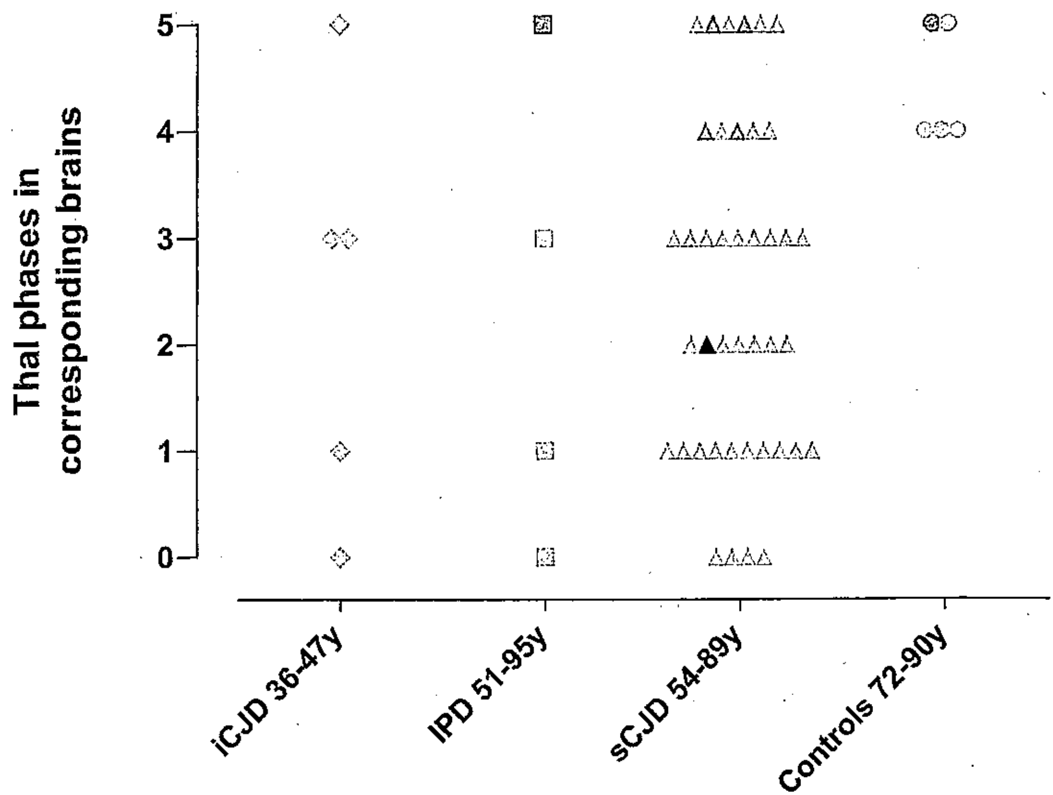

Extended Data Figure 3 | Topographical Aβ of the brain samples corresponding to the pituitaries analysed for Aβ. Plot of the Thal phases (topographical Aβ deposition) of the brain samples corresponding to the 55 pituitary glands examined for the presence of Aβ. Aβ was assessed in 55 pituitary glands from patients with iCJD ($n = 5$, age range 36–47), IPD ($n = 4$, age range 51–95), sCJD ($n = 41$, age range 54–89) and non-CJD controls ($n = 5$, age range 72–90) (groups shown on x axis). In six patients from iCJD, IPD and sCJD groups no Aβ deposits were found in the brain or pituitary gland. In 49 patients from all groups (iCJD, IPD, sCJD and non-CJD) there were variably frequent Aβ deposits in the brain parenchyma, corresponding to Thal phases 1–5 (distribution shown on y axis). Of these 49 cases, six cases (IPD $n = 1$, sCJD $n = 4$, and non-CJD $n = 1$) showed Aβ deposits also in the pituitary glands (positive cases highlighted in red) and in one patient from sCJD group Aβ deposits were seen in the brain tissue attached to the pituitary gland (highlighted in blue).

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CORRECTIONS & AMENDMENTS

ERRATUM

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Erratum: Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy

Zane Jaunmuktane, Simon Mead, Matthew Ellis, Jonathan D. F. Wadsworth, Andrew J. Nicoll, Joanna Kenny, Francesca Launchbury, Jacqueline Linehan, Angela Richard-Loendt, A. Sarah Walker, Peter Rudge, John Collinge & Sebastian Brandner

Nature **525**, 247–250 (2015); doi:10.1038/nature15369

In this Letter, an administrative error led to the publication of an incorrect version of the Competing Financial Interests (CFI) statement. Although the published CFI statement did reference the authors' affiliation with D-Gen, it did not contain all of the information provided by the authors about the interests of the company. The CFI statement for this paper as originally published was "J.C. is a Director and J.C. and J.D.F.W. are shareholders of D-Gen Limited, which supplies antibody ICSM35." The updated CFI statement is "J.C. is a Director and J.C. and J.D.F.W. are shareholders of D-Gen Limited, an academic spin-out company working in the field of prion disease diagnosis, decontamination and therapeutics. D-Gen supplied antibody ICSM35."

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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
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一般的名称	人ハプトグロビン	研究報告の公表状況	Transfusion (Malden) 2015; 55(10): 2390-1397	公表国 イギリス
販売名（企業名）	ハプトグロビン静注 2000 単位「JB」（日本血液製剤機構）			

研究報告の概要

背景：P-Capt プリオン除去フィルター（MacoPharma）は、モデル系においてプリオンの感染性を除去する。この独立した評価は、CE マークが付いた P-Capt フィルターを使用して、内因的に感染した動物の血液からプリオン除去を評価し、英国血液サービス内のフィルターの使用の提案を再現する。

研究デザインと方法：263K スクレイピー感染ハムスターから生成した 2 単位の血液は、白血球除去フィルター（LXT-quadruple, MacoPharma）を用いて処理した。除去血漿の約 100 mL は赤血球（RBCs）に加えられ、血液は P-Capt フィルターを通してろ過した。濾過していない全血、プリオンフィルターを通した（赤血球＋血漿と SAGM[RBCPS]）、およびプリオンろ過白血球除去血（PFB）のサンプルは、ハムスターに頭蓋内に注射した。臨床症状は 500±1 日の間モニターし、脳は海綿症およびプリオンたん白質沈着物について評価した。

結果：ろ過実験 1 では、50 匹のチャレンジした動物の何れも、RBCPS 画分を接種した後、スクレイピーと診断されなかった、一方 PFB を注射した 190 匹のハムスターの内 2 匹が感染していた。ろ過実験 2 では、RBCPS を注射した 49 匹の動物の内 1 匹と、PFB を注射した 193 匹のハムスターの内 2 匹が感染していた。実験 1 は、1.467log まで（白血球除去およびプリオンろ過用で、それぞれ＞1.187log と＜0.280log）感染量(ID)を減少した。実験 2 は、1.424log まで（それぞれ 1.127log と 0.297log）プリオン感染力を減少した。残存感染力は、0.212±0.149IDs/mL（実験 1）および 0.208±0.147IDs/mL（実験 2）と推定された。

結論：白血球除去は、263K スクレイピーハムスターの血液から感染性の大部分を除去した。P-Capt フィルターは残りの感染性の一部を除去するが、感染性の残存が 2 つの独立した工程で観察された。

使用上の注意記載状況・その他参考事項等

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。

報告企業の意見

血漿分画製剤は理論的な vCJD 伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を 2003 年 5 月から添付文書に記載している。2009 年 2 月 17 日、英国健康保護庁(HPA)は vCJD に感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD 異常プリオン蛋白が検出されたと発表したが、日本血液製剤機構の原料血漿採取国である日本及び米国（今回の報告品目は日本のみ）では、欧州滞在歴のある献（供）血希望者を一定の基準で除外している。また、国際獣疫事務局（OIE）により、日本及び米国は「無視できる BSE リスク」の国に認定されたことからも、原料血漿中に異常型プリオン蛋白が混入するリスクは 1999 年以前の英国に比べて極めて低いと考える。

今後の対応

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。

ハプトグロビン

(13)

Evaluation of efficacy of prion reduction filters using blood from an endogenously infected 263K scrapie hamster model

Neil P. McLeod,[1] Philip Nugent,[1] Douglas Dixon,[1] Mike Dennis,[1] Mark Cornwall,[1] Gary Mallinson,[2] Nicholas Watkins,[3] Stephen Thomas,[4] and J. Mark Sutton[1]

BACKGROUND: The P-Capt prion reduction filter (MacoPharma) removes prion infectivity in model systems. This independent evaluation assesses prion removal from endogenously infected animal blood, using CE-marked P-Capt filters, and replicates the proposed use of the filter within the UK Blood Services.

STUDY DESIGN AND METHODS: Two units of blood, generated from 263K scrapie–infected hamsters, were processed using leukoreduction filters (LXT-quadruple, MacoPharma). Approximately 100 mL of the removed plasma was added back to the red blood cells (RBCs) and the blood was filtered through a P-Capt filter. Samples of unfiltered whole blood, the prion filter input (RBCs plus plasma and SAGM [RBCPS]), and prion-filtered leukoreduced blood (PFB) were injected intracranially into hamsters. Clinical symptoms were monitored for 500 ± 1 day, and brains were assessed for spongiosis and prion protein deposit.

RESULTS: In Filtration Run 1, none of the 50 challenged animals were diagnosed with scrapie after inoculation with the RBCPS fraction, while two of 190 hamsters injected with PFB were infected. In Filtration Run 2, one of 49 animals injected with RBCPS and two of 193 hamsters injected with PFB were infected. Run 1 reduced the infectious dose (ID) by 1.467 log (>1.187 log and <0.280 log for leukoreduction and prion filtration, respectively). Run 2 reduced prion infectivity by 1.424 log (1.127 and 0.297 log, respectively). Residual infectivity was estimated at 0.212 ± 0.149 IDs/mL (Run 1) and 0.208 ± 0.147 IDs/mL (Run 2).

CONCLUSION: Leukoreduction removed the majority of infectivity from 263K scrapie hamster blood. The P-Capt filter removed a proportion of the remaining infectivity, but residual infectivity was observed in two independent processes.

A number of reports have described the transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood transfusion and blood products. To date, these transmissions have resulted in three deaths from vCJD[1-4] plus evidence of misfolded prion protein in lymphoreticular tissue in a fourth transmission where the cause of death was not vCJD.[5] There are also reports of a case with a possible link to transfusion in infancy[6] and a probable case of transmission by contaminated blood products.[7] These cases confirm the risk of transmission of vCJD by blood transfusion in the United Kingdom and other countries where there have been reported clinical cases. In the United Kingdom, there remains considerable uncertainty about the carriage of the disease in the population. Recently reported studies, based on the analysis of appendixes removed in 41 UK hospitals between 2000 and 2012, showed the presence of abnormal prion protein accumulation in 16 appendixes

ABBREVIATIONS: ic = intracranially; ID(s) = infectious dose(s); PFB = prion-filtered leukoreduced blood; RBCP = red blood cells plus plasma; RBCPS = red blood cells plus plasma and SAGM; TSE = transmissible spongiform encephalopathy; UFWB = unfiltered whole blood; vCJD = variant Creutzfeldt-Jakob disease.

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out of 32,441 samples.[8] This study estimated the prevalence at 493 per million (95% confidence interval, 282-801 per million), equivalent to approximately 1:2000 of the UK population. All clinical cases of vCJD recorded to date have been PRNP-129 methionine homozygotes (MM), but both valine homozygotes (VV) and methionine/valine heterozygotes (MV) have been identified in the appendix study.[8,9] The blood transmission cases linked to asymptomatic carriage[5] and potentially to contaminated blood products[7] were also MV heterozygotes. The relationship between abnormal prion protein accumulation in the appendix and progression to clinical disease remains uncertain, particularly in genotypes that have not been identified in the clinical vCJD population. As there is no validated antemortem diagnostic test for the disease in blood or other clinical samples, there remains the potential for vCJD-infected blood to be donated and used in transfusion. Control measures that address this risk merit further investigation.

Leukoreduction was introduced as a control measure for donated blood in the United Kingdom in 1999, ahead of clinical evidence of the transmission of vCJD by blood transfusion or blood products. To date, this appears to have been effective with all known cases of blood transfusion–mediated transmission involving blood collected from asymptomatic vCJD patients before this date.[3] Bioassay studies, in rodents, have suggested that leukoreduction removes approximately 40% to 72% of the infectivity in whole blood,[10-12] with the residual infectivity likely to be found in the remaining plasma fraction of blood components. A recent publication has tried to estimate the levels of infectivity in human blood, suggesting a titer of less than 1 infectious dose (ID) per unit of nonleukoreduced blood.[13]

Prion reduction filters, aimed at removing the residual infectivity from blood components, have been described in the literature. These studies have led to the registration ("CE marking") of two prion reduction filters.[11,14] This study is designed to assess the efficacy of one of these filters, the P-Capt filter (MacoPharma, Tourcoing, France), while other studies have already reported on blood safety aspects of this product.[15-18]

The study used a well-characterized rodent model to assess the ability of the P-Capt prion reduction filter to remove endogenous infectivity from blood. The model using 263K scrapie in hamsters is described in various studies,[11,14] and the study aimed to provide independent evaluation of the commercial MacoPharma P-Capt filter in a study designed to replicate proposed usage of the filters by blood services in the United Kingdom. A key feature of the study was the use of blood from a sufficient number of hamsters to create two human-scale units of red blood cell concentrate (RBC) for filtration. All protocols were reviewed with NHSBT, the project sponsor, to ensure that the methods followed similar processes to the intended use of the filters in routine operational use. Data from two independent filtration procedures are presented, based on determination of infectivity using bioassay in 263K scrapie in hamsters.

MATERIALS AND METHODS

All studies were conducted under an animal project license approved by the UK Home Office. Before submission for approval the license was reviewed by the Microbiological Services Porton Ethical Review Committee and signed off by the Establishment Certificate Holder. Project License 30/2700 was granted by the UK Home Office under the Animals (Scientific Procedures) Act, 1986.

Generation of endogenous infected blood

Two separate batches of 252 Syrian golden hamsters, one for each independent filtration experiment, were injected intracranially (ic) with 50 μL of 1% (wt/vol) 263K scrapie brain homogenate (scrapie brain homogenate in phosphate-buffered saline [PBS]), prepared essentially as described previously for mouse brain homogenate.[19] In brief, hamster brains were collected from animals culled with symptoms of terminal scrapie (mean incubation period, 75.2 ± 2 days). Brains were prepared, as a 10% (wt/vol) suspension in PBS, using a circulator (Stomacher, Seward Limited, West Sussex, UK; three cycles, 1 min/cycle) until free flowing. The homogenate was clarified by centrifugation at 1248 × *g* for 3 minutes at room temperature, and the supernatant was removed, pooled, and stored at −80°C. The estimated titer of this material was 7.5 $\log ID_{50}$/mL, based on serial dilution and ic inoculation into groups of 12 hamsters (results not shown).

Specific criteria were defined for the cull of the animals used to generate endogenously infected blood, aiming to ensure a high titer of prion infectivity in blood, while avoiding the loss of animals to culling on welfare grounds. In both groups, hamsters were maintained for 71 days postinoculation before blood was taken. At this time, more than 70% of hamsters showed one or more clinical symptom of infection with transmissible spongiform encephalopathy (TSE), that is, tremor of head, unsteady gait, and/or back rolling.

Each batch of scrapie-infected hamsters was bled separately. Animals were asphyxiated with carbon dioxide and whole blood was removed by cardiac puncture into tubes preloaded with CPD, from blood bags (FQE 6280LB, MacoPharma). The final concentration of anticoagulant was adjusted to 11.3% to 13.5% (vol/vol), in accordance with the manufacturer's protocols, and the contents of each tube were thoroughly mixed. After overnight incubation at room temperature, any clotted samples were discarded, and unclotted blood from each batch of hamsters was pooled to provide units of 448 and 495 mL of whole blood, respectively. A 10-mL sample of this unfiltered

TABLE 1. Details of filtration run performance*

Sample	Filtration Run 1	Filtration Run 2 Pass 1	Filtration Run 2 Pass 2	Acceptance criteria
Volume UFWB plus CPD (mL)	438.6†	485.5	431.2†	468-558
Volume leukoreduced whole blood (mL)	396.9	442.2	389.1	NA
Volume plasma removed (mL)	189.6	215.3	190.7	NA
Leukoreduction filtration flow rate (mL/min)	19.8	25	24.3	NA
Volume RBCs (mL)	208.8	225.1‡	173.3	NA
Volume RBCPS filtered (mL)	302		284.4	250-340
Distance from RBCPS blood bag to P-Capt (cm)	15.2		15.0	15±2
Volume PFB (mL)	283.3		256.4	NA
Laboratory temperature	22°C		21°C	16-24°C
Filtration time (min/sec)	<22/17		25/40	<180
P-Capt filtration flow rate (mL/min)	>12.7		10.0	<20

* All volumes were estimated at each stage of the process, by weighing the blood filtration set and subtracting the weight of an empty set. Discrepancies in volumes reflect either samples taken for bioassays or small volumes of liquid remaining in the filtration sets.
† The volume of blood plus CPD fell below the acceptance criteria for the leukoreduction filter. In both cases, in discussion with the project sponsor, this was accepted as not having a significant impact on the performance of the filter. The volume of blood loaded onto the P-Capt filter was within the manufacturer's specification.
‡ At this point in Filtration Run 2, RBCs and plasma from Pass 1 were recombined and the sample processed again (Pass 2).
NA = not applicable, no criteria set.

whole blood (UFWB) was removed from each for titration of infectivity and was then flash frozen and stored at −80°C. The remaining blood was introduced into separate whole blood filter blood bag sets (LXT quadruple, MacoPharma).

During both batches of blood collection, the brains and spleens were removed from approximately 10% of animals for analysis of the presence of disease-associated prion protein (PrP^{Sc}). Blood from two uninfected hamsters (4 mL total volume) was also collected in CPD, flash frozen, and stored at −80°C to provide a negative control for titration.

Processing

For each of the two batches of infected blood, the pooled blood was leukoreduced using a whole blood filter blood bag Set (LXT quadruple, MacoPharma) according to the manufacturer's instructions. The leukoreduced whole blood was centrifuged (Model RC3BPx, H6000A rotor, Sorvall, Cambridge, UK; 15 min, 2600 × g, 22°C), and most of the plasma was removed using an automated component extractor (Optipress II, Baxter, Newbury, UK). Approximately 100 mL of the removed plasma was reintroduced into the blood bag and mixed back into the RBCs (RBCP). A P-Capt prion removal set (MacoPharma) was connected to the bag containing the RBCP. The P-Capt filter contains approximately 40 mL of saline, adenine, glucose, and mannitol (SAGM), which was allowed to drain into and mix with the RBCP, and a sample of this was taken for bioassay (RBC plus plasma and SAGM [RBCPS]). The RBCPS was then allowed to pass through the P-Capt filter. Details of the processing of blood are provided in Table 1. A sample of prion-filtered blood (PFB) was taken for bioassay. All samples collected were flash-frozen on dry ice and stored at −80°C.

Western blotting

Each brain and spleen taken from the endogenously infected animals was homogenized to 10% (wt/vol) in PBS using a bead beater (Mini-beadbeater-8, Stratech, Suffolk, UK). The homogenate was incubated with 4% phosphotungstic acid (Sigma, Dorset, UK; final concentration, 0.4% [vol/vol]) at 37°C with agitation followed by centrifugation at 19,000 × g for 30 minutes (Biofuge Primo, Sorvall; 24 × 1.5-mL tube rotor, Heraeus, Hanau, Germany). The precipitate was resuspended in PBS and treated with proteinase K (Roche, West Sussex, UK) at 48°C for 60 minutes followed by 95°C for 5 minutes with digestion stop (Roche) and mixed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis loading buffer. Proteins were separated on a 4% to 12% Bis-Tris gel (Invitrogen, Paisley, UK) and Western blotted onto polyvinylidene fluoride membrane (Invitrogen), and the presence of PrP^{Sc} was detected using 3F4 anti-prion antibody (Covance, Leeds, UK), anti-mouse IgG (whole molecule)–peroxidase antibody produced in rabbit (Sigma-Aldrich; UK), and extended duration substrate (Supersignal West Dura, Thermo Scientific, Loughborough, UK). Imaging was carried out using an analyzer (G:BOX, Syngene, Cambridge, UK).

Bioassay of blood fractions

For each filtration run, samples were thawed at room temperature, hamsters were inoculated via the ic route with 50-μL aliquots of UFWB, leukoreduced blood (RBCPS), or prion-filtered leukoreduced blood (PFB), to determine the amount of TSE infectious agent present. Based on titer estimates from previous studies, 30 hamsters were

inoculated ic with UFWB, 50 with RBCPS, and 200 with PFB. Naïve, undiluted whole blood in CPD was injected into 10 hamsters in the same manner. No significant toxicity was observed in pilot studies using undiluted blood in CPD, when injected ic into hamsters, these challenged animals being used as age-matched controls for histology, and as such undiluted blood and blood fractions were used throughout. A small number of hamsters died soon after inoculation with UFWB, RBCPS, or PFB, and these were excluded from subsequent infectivity calculations.

Hamsters were monitored for signs of TSE infection from 50 days postinoculation to 500 ± 1 days postinoculation. Hamsters were routinely monitored three times a week and, upon appearance of clinical symptoms, were monitored daily before being culled at the defined clinical endpoint. A small number of hamsters were also culled, due to non–TSE-related health issues. The remaining animals were all culled at the end of study time point (500 ± 1 days postinoculation). The brains of all animals were removed and stored in neutral buffered formalin (Leica Microsystems, Newcastle Upon Tyne, UK) before histologic examination. The titer (ID/mL) and total measured infectivity for the selected process samples were calculated based on the method of Gregori and colleagues[11].

Histologic examination of brain material

To confirm the presence or absence of scrapie disease, brain samples were examined for vacuolation at defined neuroanatomical sites, after staining with hematoxylin and eosin.[20] Immunohistochemistry was also carried out on all brain sample sections using monoclonal antibody 3F4 against abnormal prion.[21] Samples were compared with brains from 10 age-matched negative control animals, because of the extended incubation period used in this bioassay. The screening of all slides (with the exception of the negative controls) was carried out "blind"; the pathologists had no prior knowledge of the clinical outcome of the samples or the nature of the study. A second antibody, Rb486,[22] was used for a small number of slides that showed elevated background staining, and results concurred with the original analysis (results not shown). All positive slides and a selection of control (known positive and negative reference slides from the pathology laboratory) and negative control slides from the study were reviewed independently and confirmed the original assessment.

RESULTS

Filtration process

Material for the filtration study was generated from hamsters challenged with a relatively high dose of infectious brain material via the ic route. The ID was estimated as 6.20 log (1.58×10^6) ID per animal (50 μL) based on previous titration of the starting material (results not shown). This enabled the challenged hamsters to proceed sufficiently and uniformly through the clinical progression of disease to have a representative titer of infectivity in the blood. Brain and spleen were collected from 10% of the culled animals as an internal check to ensure that disease progression was taking place across the group and also to provide evidence, before filtration, of the peripheral spread of the disease. Disease-associated prion protein (PrP^{Sc}) was detected by Western blot in the brain and spleen of all animals tested.

The filtration processes were carried out with minor deviations from the standard protocol. In the first filtration run, the final end time of the prion filtration process was not recorded and we were therefore unable to calculate an exact flow rate, but the filtration time was not more than 22 minutes 17 seconds (which would be equivalent to 12.7 mL/min) compared to a maximum flow rate recommended by the manufacturer of 20 mL/min. The filter filled up correctly, was flowing at the expected rate for the first 10 minutes, and at the end of the process had an appearance consistent with the expected performance. Observations rule out a catastrophic failure of the filter and it is very likely that the performance was within specification. In the second filtration run, after leukoreduction filtration and centrifugation, a thin layer of buffy was noted on the top of the RBCs. Given that this would invalidate the prion filtration process, the decision was taken to remix the RBCs and plasma to reprocess through the leukoreduction stage using a new filter to ensure that there was no risk of white blood cells (WBCs) being carried over into the prion filtration stage. The subsequent filtration and bioassays (for RBCPS and PFB groups) were performed on these fractions providing an accurate assessment of the material loaded onto the P-Capt filter and the clearance of infectivity by this filter. We were unable to quantify the levels of WBCs at the time of the processing as the presence of infectious prion material in the samples precluded the use of standard measurement methods at the time of the study. However, subsequent evaluation of the fractions, isolating WBC gDNA and using a dsDNA fluorescent stain to quantify the amount of DNA in the leukoreduced blood, showed similar levels in the two RBCPS pools (Table S2, available as supporting information in the online version of this paper), and these were consistent with the levels of leukoreduction seen in normal human blood. Neither deviation is believed to have any impact on the results obtained. Details of the processing runs are provided in Table 1.

Bioassay

The results of the bioassay studies are summarized in Fig. 1 and Table 2. The amount of infectivity in the two independently collected units of UFWB was very similar, causing eight animals to be infected out of 30 (8/30) in Process Run 1 and seven of 29 in Process Run 2. No animals were

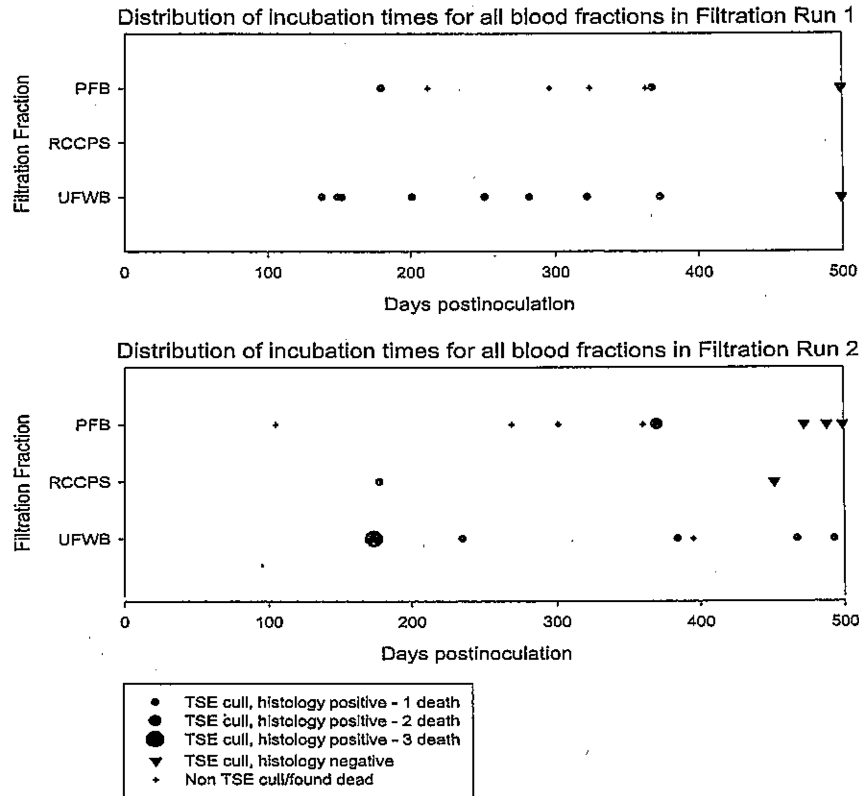

Fig. 1. For each filtration experiment, 30, 50, and 200 Golden Syrian hamsters were inoculated with UFWB, RBCPS, and PFB, respectively, and incubated for up to 499 days. Animals that died during inoculation or before 10 days were excluded from the calculations (<5%). Animals that were culled for displaying TSE symptoms between 10 and 499 days are shown with small red circles (subsequently found to be TSE positive by immunohistochemistry) or blue downward triangles (subsequently found to be TSE negative by immunohistochemistry); where multiple culls were carried out on the same day the red circle is larger to represent the number of culls. Animals that were culled for non-TSE health issues or found dead (without prior TSE symptoms) are shown with a small black cross (+); these animals were excluded from the final calculation if they were culled or found dead earlier than a TSE-positive animal in the same group (as the likely progression to TSE disease cannot be ascertained).

infected on inoculation with material from the leukoreduction step in Process Run 1, while one of 49 was infected from Process Run 2. After completion of the prion reduction process, two animals (out of 190 in Process 1 and two animals out of 193 in Process 2) were infected with TSE.

TABLE 2. Summary of bioassay clinical and histologic data

Filtration Run	Group	Animals inoculated	Animals included in calculation	Histology-confirmed TSE-positive animals	Incubation period for histology confirmed TSE-positive animals (days postchallenge)	Mean incubation (days postchallenge)	SD (days postchallenge)
1	UFWB	30	30	8	138, 149, 152, 201, 251, 282, 322, 373	234	88
	RBCPS	50	50	0	NA		
	PFB*	200	190	2	180, 368	274	NA
2	UFWB*	30	29	7	174, 174, 174, 235, 384, 467, 493	300	134
	RBCPS*	50	49	1	178	178	NA
	PFB*	200	193	2	370, 370	370	NA

* Animals that were scored with clinical TSE symptoms but found to be histology negative. Further details are shown below in Table S1 (available as supporting information in the online version of this paper).

TABLE 3. Calculation of infectivity before and after filtration of blood

						Total measured infectivity (ID) in 9.5 mL†			Reduction factor (log)†	
Fraction	Volume inoculated (mL)*	Total animals assayed	Total animals infected	Titer (ID/mL)	SD (ID/mL)	Before filter	After filter	Removed	Step	Total
A. Process Run 1										
Whole blood	1.5	30	8	6.212	2.035					
RBCPS	2.5	50	0‡	<0.404	0.402	59.014	<3.838	>55.176	>1.187	
PFB	9.5†	190§	2	0.212	0.149	<3.838	2.014	<1.824	<0.280	1.467
B. Process Run 2										
Whole blood	1.45	29‖	7	5.525	1.952					
RBCPS	2.45	49¶	1	0.412	0.410	53.316	3.976	49.34	1.127	
PFB	9.65†	193**	2	0.208	0.147	3.976	2.007	1.969	0.297	1.424

*Volume inoculated (mL) is calculated based on the "Total number of animals assayed (after exclusions)" multiplied by the inoculation volume for each animal (50 µL). Total animals infected based on TSE-positive immunohistochemistry. Titer (ID/mL) = [−ln([total animals assayed − total animals infected]/[total animals assayed])] × [(1 ID/50 µL) × (1000 µL/mL)]. Titer SD (ID/mL) = [(Titer (ID/mL)/(total volume inoculated (mL)]$^{0.5}$. Total measured infectivity at each filtration step is expressed as total IDs in the "Challenge," "Unbound," and "Removed" fractions. Challenge (ID) = Titer (ID/mL) of Fraction 1 × Normalized total volume inoculated. Unbound (ID) = Titer (ID/mL) of Fraction 2 × Normalized total volume inoculated. Removed (ID) = Challenge (ID) − Unbound (ID).

†Normalized total volume = the total ID in each fraction was "normalized" to the inoculation volume used in the PFB fraction (i.e., 9.5 mL in Filtration Run 1 and 9.65 mL in Filtration Run 2); this allows direct comparison of the titer of each fraction. Reduction factors are based on normalized total volume, calculated as Log (challenge titer) − Log (titer after filtration). The reduction factors are expressed for RBCPS (effect of leukoreduction and removal of approximately 50% plasma) and PFB (effect of prion filtration of RBCPS) fractions. The cumulative reduction factor for the whole process is also expressed. When there is no remaining infectivity, the titers and reduction are expressed to take into account the limit of detection of the assay. The limit of detection in a limiting dilution titration is a single infection in the cohort challenged. For example, if one infection is observed in the RBCPS cohort of 50 animals (1 ID/50 animals challenged/2.5 mL of inoculum) this would equate to an average of 0.02 ID per challenge dose (1/50). The limit of detection per mL would be −ln(49/50) × (1000 µL/50) = 0.404.

‡Calculation based on one animal infected as the lower limit of detection.

§Ten animals excluded in PFB Group: four died at inoculation, six were histology-negative culled before the bioassay endpoint (found dead or culled for non–TSE-related health issues) and before TSE-related deaths occurring at a later date in the same group. Their likely progression to TSE disease cannot be ascertained.

‖One animal excluded in UFWB. Likely progression to TSE disease cannot be ascertained.

¶One animal excluded in RBCPS: died at inoculation.

**Seven animals excluded in PFB: two excluded (died at inoculation) and five excluded (likely progression to TSE disease cannot be ascertained).

The infectivity titer of each fraction from the two prion-reduction procedures was based on the calculation used previously by Gregori and coworkers[11] and is shown in Table 3. To allow an estimation of the effectiveness of prion removal by each of the filtration stages in Run 1, the calculation for the amount of infectious material was based on a single animal becoming infected after challenge with the RBCPS fraction. As no animal was infected, by definition, the amount of infectious material was less than this value. Based on this assumption the two filtration processes were similar with the leukoreduction filter removing more than 1.187 and 1.127 log ID, respectively, and the P-Capt filtration process removing less than 0.280 and 0.297 log ID, respectively. The overall clearances of the entire filtration process were 1.467 and 1.424 log ID. However, not all infectivity was removed and the remaining titer in each of the PFB fractions was 0.212 and 0.208 ID/mL, respectively.

DISCUSSION

The study was designed to provide an evaluation of the efficacy of P-Capt filters, in removing endogenous TSE infectivity from hamster blood. As such the study is relevant to evaluating whether the devices would be useful in processing of blood from donors with asymptomatic vCJD infection. The procedure was designed to follow, as closely as is possible, the processing stages used within the UK Blood Services for the generation of RBCs. The prion reduction filtration stage for the P-Capt product is designed to follow leukoreduction filtration, which was implemented in 1999 to reduce the risk of vCJD transmission. This study, therefore, provides efficacy data to complement a broader evaluation of the safety of blood processed through the P-Capt filter.[15-18] This study represents the first published evaluation of the removal of endogenous TSE-infectivity from blood by the CE-marked P-Capt filter.

This study used two independent processing runs to provide robust information on the inherent variability of the process. The hamster blood units were collected from independent groups of animals, challenged at different times and with blood collected on different days. The blood from the two collections was not pooled, both to preserve the independence of the two experimental studies and also due to the need to collect and process each unit of blood within 24 hours. The results from the two

studies were consistent, both in terms of the log reduction values obtained and with the key observation that there was breakthrough infectivity in both samples of PFB. This was despite the relatively low total PFB sample volume (9.5 and 9.65 mL, derived from 190 and 193 animals challenged with 50 μL of PFB each, respectively), which was used for challenge of hamsters. The group sizes used for UFWB, RBCPS, and PFB were based on the expected titer from previous studies on endogenously infected blood in the 263K scrapie hamster model.[10,11] Although there is a limited number of studies that have looked at prion infectivity levels in hamster blood, most suggest titers of approximately 10 ID/mL of whole blood, being reduced to approximately 3 ID/mL for leukoreduced blood with all plasma added back.[10,11] Although the estimated titers in this study were lower than this, at 6.212 ± 2.055 and 5.525 ± 1.952 ID/mL for the 2 units of UFWB, it is not clear whether this is statistically different from the titers described previously by other groups or within the normal range of infectious titers in blood from hamsters challenged with 263K scrapie. In this study, only half of the removed plasma was added back after the leukoreduction filtration step, rather than all of the plasma, as in some previous studies.[11] Nevertheless a significant challenge was still applied to the P-Capt filter with an estimated 755 and 734 ID applied in the two processing runs. Given that plasma was added back to the RBCs before prion filtration, this suggests that this is still a greater challenge than would be encountered in actual usage, where the residual plasma content of RBC would be approximately 20 mL (S. Thomas, personal communication, 2014). Nevertheless, the presence of infectivity after prion filtration must remain a concern even when accounting for the elevated challenge dose.

Two deviations from the standard procedure for leukoreduction of blood occurred and are detailed under Results. Both deviations were unrelated and given the observations made at the time, subsequent analysis, and the high level of consistency between the estimated infectious titers and log-clearance values of the two independent processes, it is unlikely that the two deviations influenced the final results.

A study on the efficacy of two prototype combined leukoreduction and prion removal filters has been reported, using units of blood from scrapie-infected sheep.[23] In that study, LD was shown to have a significant effect in reducing transmission of scrapie by blood transfusion, and although no clinical cases were observed in a small number of sheep transfused with leukoreduction and prion removal–filtered RBCs, one apparently healthy recipient was found to be infected after culling. The authors suggest that this indicates exposure to low-titer infectious material; this may indicate that infectivity cannot be completely removed by prion filtration and is consistent with the results of our study.

In conclusion, despite the low infectivity titers in leukoreduced blood, this study demonstrates that the P-Capt prion reduction filter did not completely remove endogenous hamster scrapie 263K from blood in either of the independent filtration processes. In light of this result, the effectiveness of the filter for removal of prions from human donor blood, to prevent transmission of vCJD through blood transfusion, cannot be assumed.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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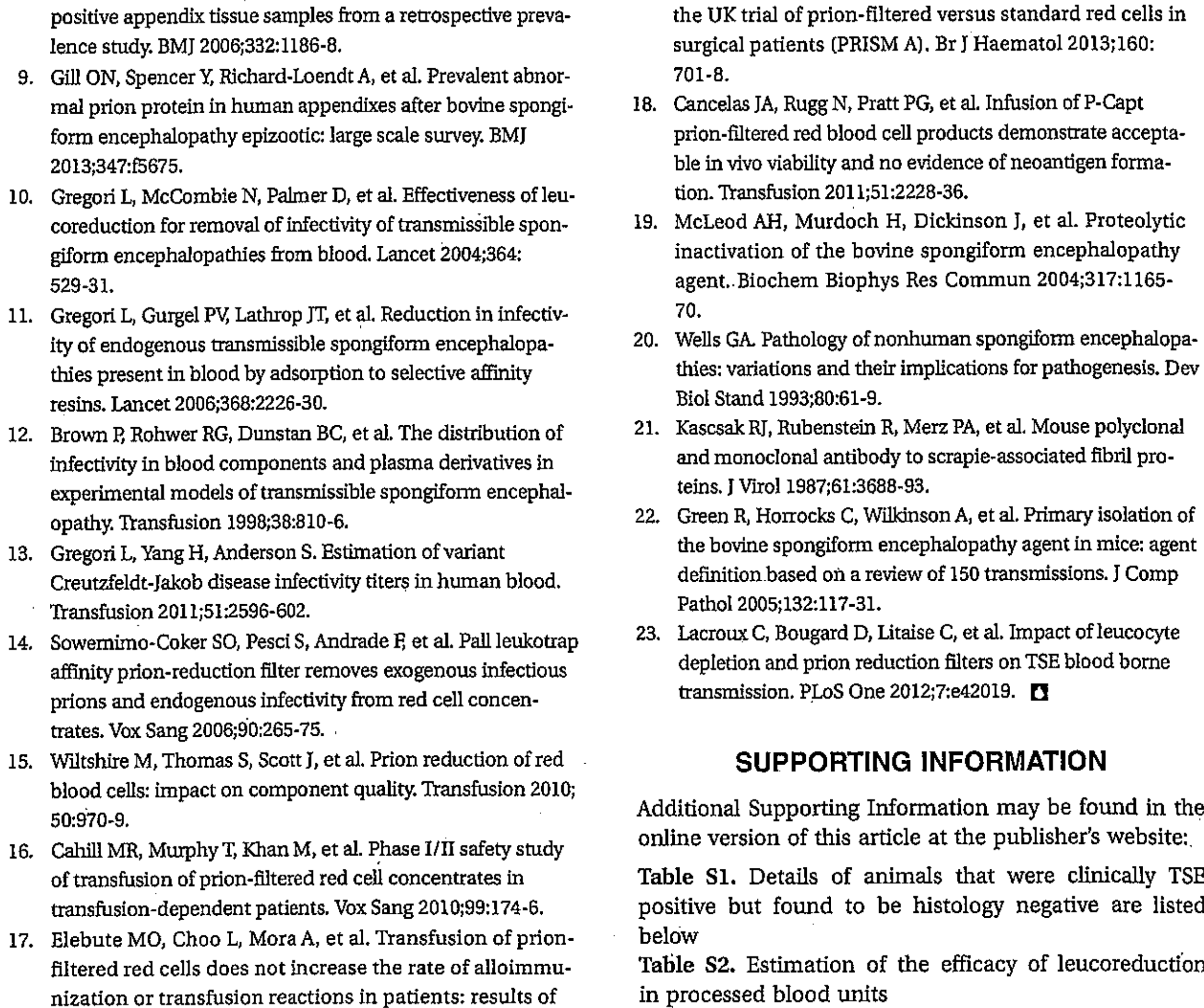

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Details of animals that were clinically TSE positive but found to be histology negative are listed below

Table S2. Estimation of the efficacy of leucoreduction in processed blood units

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

1　基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

2　具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

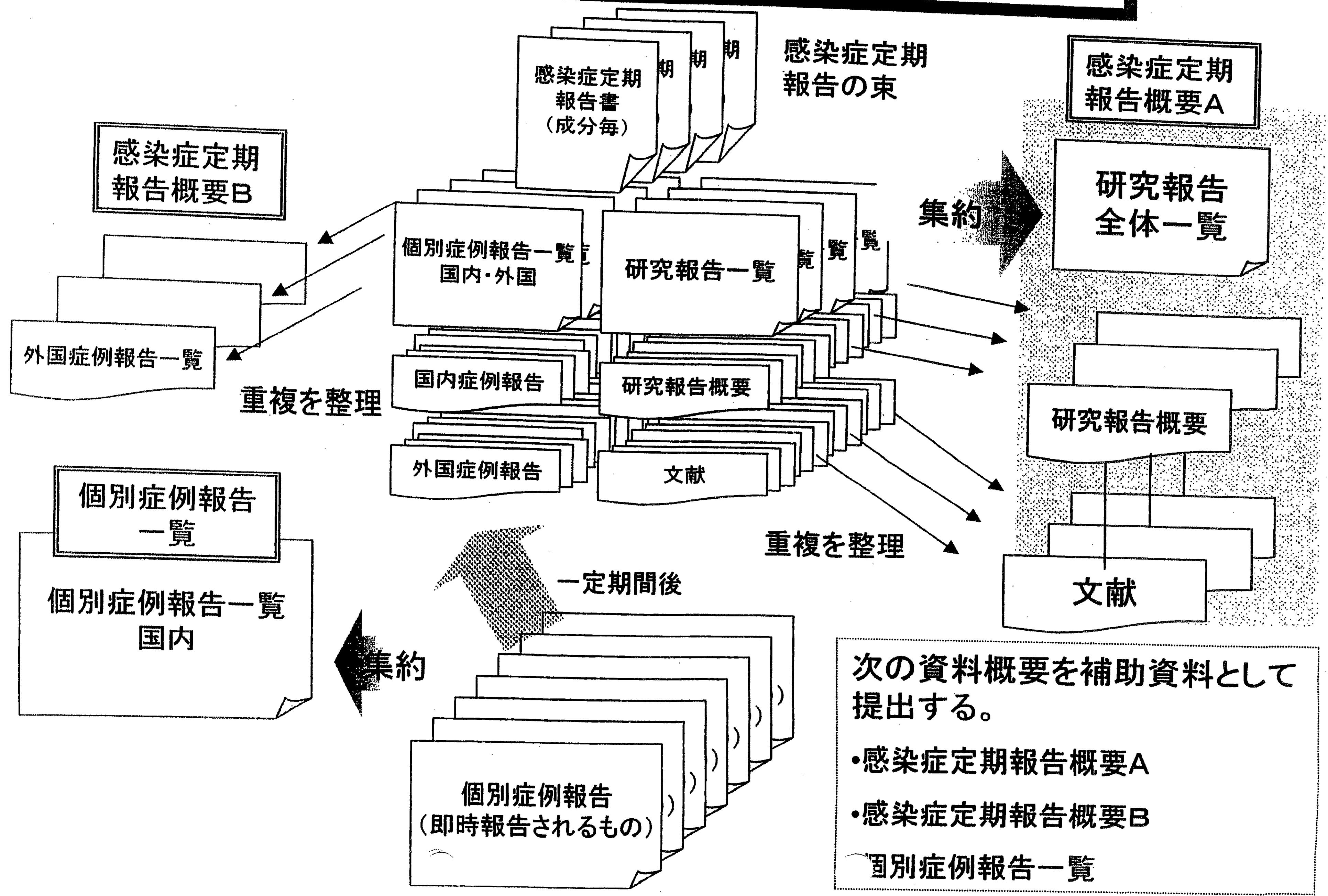
① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式(別紙様式第2)及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧(別紙様式第4)をまとめた**「資料概要B」**を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症的報告について(目次)」資料は廃止することとする。



感染症定期報告・感染症個別症例報告の取り扱い
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