The improved thermal stability and increased catalytic rate of MC3 have clearly contributed to its ability to inactivate prions. The ability of proteases to degrade prions appears to be dependent on the use of reaction conditions or additives that open up the structure of the infectious molecule to allow access to the peptide bonds. Based on previous results we continued to use alkaline conditions as this appeared to be generally more efficient at allowing protease digestion. This was supported by bioassay results showing a reduced log inactivation when experimentally the pH dropped below pH 12 (results not shown). Other groups have used detergent, principally SDS, usually in the presence of heat to effect similar conformational changes to promote protease digestion.[27,28,39]

There are many examples of the use of genetic engineering to enhance the properties of naturally occurring enzymes, and subtilisin-type proteases have been among the foremost of those modified.[22] Properase and MC3 are all engineered versions of the *B. lentus* subtilisin backbone and were selected for these studies on the basis of their stability and activity at alkaline pH. Clearly such an approach has applications in healthcare management with the methods being simple and safe to use and non-destructive to medical instruments.

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Conflict of interest statement

The views expressed in the publication are those of the authors and not necessarily those of the Health Protection Agency or any other funding body.

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研究報告の概要

〔変異型クロイツフェルト・ヤコブ病の遺伝的危険因子：ゲノムワイド（全ゲノム）でのアソシエーション（関連性）研究〕

＜背景＞

ヒトおよび動物のプリオン病は遺伝子の管理下にあるが、PRNP（プリオンたん白をコード化する遺伝子）以外については変異型クロイツフェルト・ヤコブ病（vCJD）の原因物質である牛海綿状脳症（BSE）プリオンに対するヒト罹患しやすさについてはほとんどわかっていない。

＜方法＞

我々はvCJDのリスクのゲノムワイドアソシエーション研究（GWAS:genome-wide association study ゲノム全体を対象とした疾病との関連性の研究）を行い、また、我々の知見の再現性確認のためにヒトプリオン病の多くのカテゴリーからのサンプル（929サンプル）および英国（UK）とパプアニューギニアから得られたコントロールサンプル（4254サンプル）で調べた。そのUKコントロールにはWellcome Trust Case Control Consortium（WTCCC）によってジェノタイピングされたものが含まれている。我々はまた、プリオン病の臨床的表現型の遺伝的調節に関してフォローアップ分析も行い、プリオン感染マウス細胞モデル中での候補遺伝子発現を分析した。

＜調査結果＞

PRNP遺伝子座はプリオン病のいくつかのマーカーと全てのカテゴリーを通じてリスクに強く関連していた（最も関連性が強いとされた単一のSNP〔一塩基多型〕のvCJDにおけるアソシエーションは$p=2.5\times10^{-17}$;vCJDにおいてハプロタイプアソシエーションは$p=1\times10^{-24}$）。疾病リスクへの主な寄与はPRNP多型コドン129によって付与されるものではあるが、別の近傍のSNPによってvCJDのリスクの増大がもたらされた。PRNPに加えて、RARB（レチノイン酸受容体βをコードする遺伝子）の上流に技術的にバリデートされたSNPアソシエーションが1つあり、それはゲノムワイドな有意性を示した（$p=1.9\times10^{-7}$）。類似のアソシエーションが医原性CJD（iCJD）患者の小規模なサンプルで見出されたが（p=0.030）、孤発性CJD（sCJD）やクールーでは認められなかった。培養細胞では、レチノイン酸はプリオンタンパク質の発現を調節している。我々はSTMN2（SCG10をコードする遺伝子）の上流の領域中に獲得性プリオン病とのアソシエーションを1つ見出し、そのアソシエーションは、vCJD（$p=5.6\times10^{-5}$）、クールー潜伏期間（p=0.017），およびクールーに対する抵抗性（$p=2.5\times10^{-4}$）であった。そのリスクジェノタイプはsCJDとは関連していなかったが発症年齢の早期化をもたらしていた。さらに、Stmn2の発現はプリオン病のマウス細胞モデルにおいては感染後30分の1に低減していた。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリンーIHの記載を示す。

2. 重要な基本的注意

(1) 略

1) 略

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

報告企業の意見	今後の対応
vCJDの原因病原体であるBSEプリオンに対するヒトの感受性について遺伝子レベルでの解析を行ったことについての報告である。 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、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、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。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Genetic risk factors for variant Creutzfeldt–Jakob disease: a genome-wide association study

Simon Mead, Mark Poulter, James Uphill, John Beck, Jerome Whitfield, Thomas E F Webb, Tracy Campbell, Gary Adamson, Pelagia Deriziotis, Sarah J Tabrizi, Holger Hummerich, Claudio Verzilli, Michael P Alpers, John C Whittaker, John Collinge

Summary

Background Human and animal prion diseases are under genetic control, but apart from *PRNP* (the gene that encodes the prion protein), we understand little about human susceptibility to bovine spongiform encephalopathy (BSE) prions, the causal agent of variant Creutzfeldt–Jakob disease (vCJD).

Methods We did a genome-wide association study of the risk of vCJD and tested for replication of our findings in samples from many categories of human prion disease (929 samples) and control samples from the UK and Papua New Guinea (4254 samples), including controls in the UK who were genotyped by the Wellcome Trust Case Control Consortium. We also did follow-up analyses of the genetic control of the clinical phenotype of prion disease and analysed candidate gene expression in a mouse cellular model of prion infection.

Findings The *PRNP* locus was strongly associated with risk across several markers and all categories of prion disease (best single SNP [single nucleotide polymorphism] association in vCJD $p=2\cdot5\times10^{-17}$; best haplotypic association in vCJD $p=1\times10^{-24}$). Although the main contribution to disease risk was conferred by *PRNP* polymorphic codon 129, another nearby SNP conferred increased risk of vCJD. In addition to *PRNP*, one technically validated SNP association upstream of *RARB* (the gene that encodes retinoic acid receptor beta) had nominal genome-wide significance ($p=1\cdot9\times10^{-7}$). A similar association was found in a small sample of patients with iatrogenic CJD ($p=0\cdot030$) but not in patients with sporadic CJD (sCJD) or kuru. In cultured cells, retinoic acid regulates the expression of the prion protein. We found an association with acquired prion disease, including vCJD ($p=5\cdot6\times10^{-5}$), kuru incubation time ($p=0\cdot017$), and resistance to kuru ($p=2\cdot5\times10^{-4}$), in a region upstream of *STMN2* (the gene that encodes SCG10). The risk genotype was not associated with sCJD but conferred an earlier age of onset. Furthermore, expression of *Stmn2* was reduced 30-fold post-infection in a mouse cellular model of prion disease.

Interpretation The polymorphic codon 129 of *PRNP* was the main genetic risk factor for vCJD; however, additional candidate loci have been identified, which justifies functional analyses of these biological pathways in prion disease.

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See Reflection and Reaction page 25

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Introduction

Prion diseases are transmissible, fatal, neurodegenerative conditions of human beings and animals that are caused by the autocatalytic misfolding of host-encoded prion protein (PrP).[1] An epizootic prion disease, bovine spongiform encephalopathy (BSE), widely exposed the population of the UK (and, to a lesser extent, many other populations) to prion infection. The subsequent diagnosis of variant Creutzfeldt–Jakob disease (vCJD) in young British adults, and the experimental finding that this was caused by BSE-like prions,[2–4] resulted in a major public and animal health crisis.

Although the number of recorded clinical cases of vCJD to date has been small (~200) in relation to the millions of people who were potentially exposed, how many individuals were infected is unclear. The clinically silent incubation period in human beings can exceed 50 years,[5] and estimates of the prevalence of subclinical infection made on the basis of screening archived surgical specimens predicts that thousands of individuals in the UK are infected.[6] Blood transfusion seems to be an efficient route of secondary transmission[7] but no screening test to ensure the safety of blood products is yet available. Case control studies have identified no unusual occupational, dietary, or other exposure to BSE prions among patients with vCJD,[8] which suggests that genetic factors might be crucial.

A known genetic factor for susceptibility to prion disease is the common single nucleotide polymorphism (SNP) at codon 129 in *PRNP*, the gene that encodes PrP in human beings. Here, either methionine (~60% allele frequency in Europeans) or valine is encoded.[9] All patients with vCJD who have been genotyped are homozygous for methionine,[10] which represents the strongest association to date of a common genotype with any disease. Although this is a powerful effect, about a third of the exposed UK population have this genotype. An important role for other genetic loci is supported by the results of mouse quantitative trait locus studies, which have identified many regions that are not linked to *Prnp* but control the highly variable prion disease incubation periods,[11,12] including that of BSE prions.[13] The importance to public health of understanding susceptibility to BSE prion infection in human beings is therefore clear.

We undertook a genome-wide association study with 100K and 500K Affymetrix arrays with all available samples

from white British patients with vCJD (n=119) compared with our own and publicly available UK control data, which was genotyped by the Wellcome Trust Case-Control Consortium (WTCCC). Because all available vCJD samples from the UK were included in the discovery phase, we went on to compare the top-ranked SNP associations and additional SNPs at the *PRNP* locus with a large and diverse collection of patients with prion disease, including those with iatrogenic CJD (iCJD), sporadic CJD (sCJD), and kuru.

Methods

Samples

Figure 1 shows the four tiers of genotyping in the study. Samples were obtained from 119 patients with vCJD (ten patients with probable vCJD and 109 patients with definite vCJD) who were diagnosed at the National Prion Clinic (NPC), London, or the National CJD Surveillance Unit (NCJDSU), Edinburgh, between 1995 and 2005 according to established criteria. Patients who acquired iatrogenic vCJD through blood transfusion were not included in this series. All patients with vCJD were thought to have acquired the disease in the UK and were of white British ethnic origin (60% were men; mean age of disease onset was 29·8 [SD 10·9] years).

For more on the criteria see http://www.advisorybodies.doh.gov.uk/acdp/tseguidance/tseguidance_annexb.pdf

Samples were obtained from 506 patients with probable or definite sCJD diagnosed according to established criteria and from 28 patients with iCJD related to exposure to cadaver-derived growth hormone in the 1980s or earlier; these samples were obtained from the NPC or the NCJDSU or from other clinical colleagues in the UK. All patients were from the UK or elsewhere in northern Europe. Although most patients were of white British ethnic origin, and all patients of known non-white ethnic origin were excluded, this information was based on names and geographical location for some samples. 325 patients had pathologically confirmed sCJD and 181 patients had a diagnosis of probable sCJD with a high specificity according to published WHO criteria, although some of these patients might have had a neuropathological diagnosis made elsewhere.[14] Mean age of disease onset was 68·2 (SD 12·0) years for the patients with sCJD and 31·1 (6·3) years for the patients with iCJD. 50% of the samples from patients with sCJD were from men.

For WTCCC genotype data see http://www.wtccc.org.uk/info/access_to_data_samples.shtml

Before 1987, kuru surveillance was done by many different investigators; however, from 1987 to 1995 surveillance was done solely by the Kuru Surveillance Team of the Papua New Guinea Institute of Medical Research. From 1996, kuru surveillance was strengthened: a field base and basic laboratory for sample processing and storage were established in the village of Waisa in the South Fore, and a wide collection of population control samples were taken.[5] The samples from patients with kuru (n=151) were taken from young children, adolescents, and adults during the peak of the epidemic and from recent cases of kuru with long incubation times in elderly patients. The patients lived in the South Fore (n=53), North Fore (n=40), Gimi (n=3), and Keiagana (n=10) regions; linguistic group was not known in 45 patients.

Elderly women who had been exposed to kuru were defined as aged older than 50 years in 2000 and from a region that had been exposed to kuru: South Fore (n=74), North Fore (n=36), Gimi (n=13), and Keiagana (n=2). The modern-day healthy population from the exposed region was obtained by matching each elderly woman to at least two current residents of the same village who were aged less than 50 years in 2000. These mostly came from the South Fore, with some from the North Fore, and a small number of individuals from Gimi, Keiagana, and Yagaria linguistic groups, as indicated. First-degree relatives of the elderly women, identified by either genealogical data or microsatellite analysis, were excluded from these groups.

155 samples were from volunteers recruited by the Medical Research Council Prion Unit from the National Blood Service (NBS). Information was collected about their sex, age, ethnic origin, and birthplace divided into 12 regions. 90 samples genotyped with Affymetrix arrays were selected to match the vCJD collection for white British ethnic origin, birthplace (by 12 regions in UK, each region was represented in patients and controls with the same ranking), and sex (proportion of men with vCJD was 60%, and the proportion of men in the NBS controls was 57%).

A further 575 UK control samples were obtained for the replication phases of the study (730 healthy controls in total) from the NBS (95 white, random, healthy young blood donors) and from the European Collection of Cell Cultures (ECACC) human random control DNA collection (480 blood donors of known age and sex). No selection was done in the replication phase of the study. Not all control samples were genotyped for all replication studies; however, there is no reason to expect significant genetic heterogeneity in our collections of UK blood donors based on analyses of the UK population done by the WTCCC and others.[15] All UK control samples contained good quality unamplified DNA. The mean age at sampling was 38·7 (SD 10·8) years, and 51% were men. In addition, we used publicly available UK control data generated by the WTCCC. In brief, 1500 samples from the 1958 British Birth Cohort and 1500 samples from the UK Blood Service Control Group were genotyped with commercial Affymetrix 500K arrays with a Bayesian robust linear model with Mahalanobis distance (BRLMM) algorithm. We did not detect any duplicate individuals between the UK control collections nor any significant differences in allele frequency between our in-house UK control collections or those genotyped by the WTCCC.

The clinical and laboratory studies were approved by the local research ethics committee of University College London Institute of Neurology and National Hospital for Neurology and Neurosurgery and by the Medical Research Advisory Committee of the Government of Papua New Guinea. The full participation of the Papua New Guinea communities was established and maintained through discussions with village leaders, communities, families,