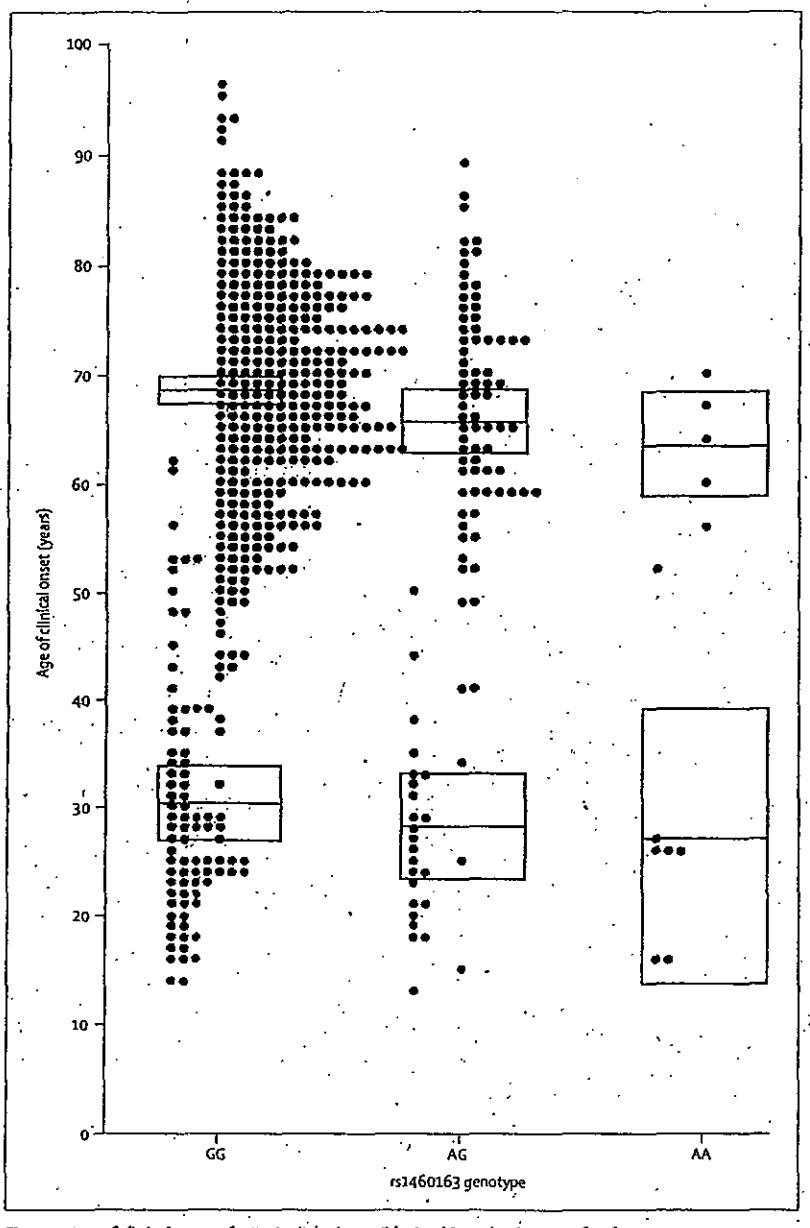

Figure 4: Age of clinical onset of vCJD (red) and sCJD (blue) patients against rs1460163 genotype
Clinical onset was defined as the age of the first symptom that progressed into a neurological or neuropsychiatric condition due to prion disease. The central bars indicate mean age of onset; boxes indicate 95% CI of the mean.

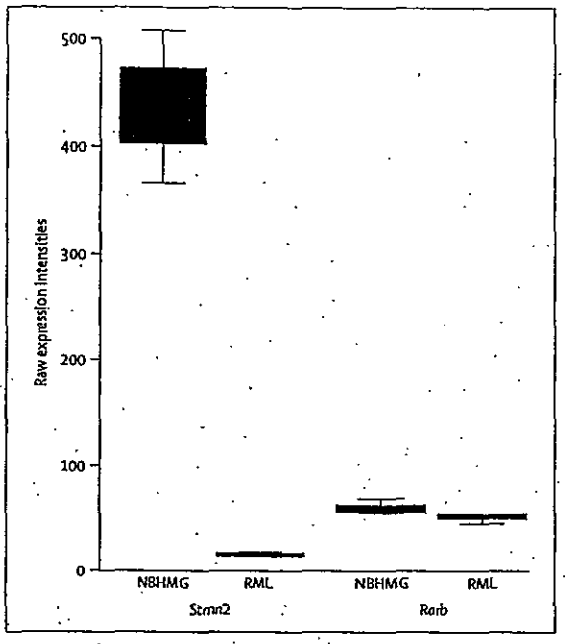

Figure 5: Boxplot of *Stmn2* and *Rarb* expression
Expression of *Stmn2* and *Rarb* in mouse neuronal cells (GT-1) treated with homogenate of healthy brain (NBHMG) or Rocky Mountain Laboratory scrapie brain homogenate (RML). Median is shown as a thick red horizontal line, IQR by boxes, and largest and smallest observations by whiskers.

tests]). rs1460163 was associated with age of kuru onset ($p=0\cdot017$) and resistance to kuru ($p=2\cdot5\times10^{-4}$), with the same highest-ranking risk allele for vCJD and kuru. rs1460163 is located in a large block of linkage disequilibrium that extends just 5′ to *STMN2* (figure 3). Other SNPs tested in the replication phase were either poorly genotyped in the discovery phase (concordance <99%), or showed no evidence of association in any prion disease category additional to vCJD ($p>0\cdot001$; best from four risk models).

We then analysed the clinical and molecular phenotype of UK prion disease for rs1460163, rs6116492, and rs6794719. In patients with sCJD, there was a significant modifying effect of the risk allele, with clinical onset 5 years earlier for those with risk genotype rs1460163AA compared with those with GG (linear regression of log-transformed age of onset against genotype $p=0\cdot02$; figure 4). In patients with vCJD, the mean age of onset for genotype AA was 3 years earlier than for those with GG, but this was not statistically significant ($p=0\cdot26$). By use of a linear regression model with disease type as a factor, the rs1460163AA allele was associated with age of onset in sCJD and vCJD (log-transformed $p=0\cdot01$) and was also independent of rs1799990. No effect was seen on year of presentation, which in part will determine incubation time in vCJD, but this analysis is confounded by uncertainty in the time of exposure. No effect was seen on sCJD PrP^{Sc} strain type as defined by partial protease K digestion and western blot. rs6116492 and rs6794719 had no effect on prion disease phenotype.

In a cellular model of mouse prion disease, the expression of *Stmn2* was profoundly altered by infection with prions. This difference was shown by comparison of the transcriptome of prion-infected and prion-uninfected cells in culture. Mouse hypothalamic neuronal (GT-1) cells that were infected with mouse brain homogenate (NBHMG) or RML-infected brain homogenate were analysed with the Affymetrix Mouse Expression Array 430_2.0. Comparison of the expression between NBHMG and RML showed that

Stmn2 is significantly ($p=3\cdot6\times10^{-18}$) downregulated by a factor of about 30 and ranked tenth out of more than 21537 genes that were represented by one or more transcripts on the array (figure 5). In this study, the expression of 543 of 21537 (2·5%) genes was altered, with a fold change of more than 2·83 (corrected Benjamini–Hochberg method). Neither *RARB* nor *STMN2* is significantly expressed in human blood cells, which obviates the analysis of the correlation of gene expression with genotypic risk in a large collection of samples.

Discussion

We describe the first genome-wide study of genetic risk in a human prion disease and replication of a small number of top-ranking candidate SNPs. Further genetic studies of human prion disease, including more extensive replication studies, are warranted because our power was limited by the small size of the vCJD sample and an early generation platform was used. Owing to the rarity of the disease, all available samples were used; the use of amplified DNA in a proportion of cases might have also affected the quality of genotyping. For these reasons, we used highly stringently filtered data and verified genotypes from candidate SNPs with an in-house assay. The potential exists for a larger scale study in sCJD that capitalises on decades of surveillance for human prion diseases across Europe and the rest of the world; however, this disease is undoubtedly more heterogeneous than vCJD.

The potential overlap in pathogenesis between vCJD and the other prion disease categories used in the replication phases of the study must also be considered. The pathogenesis of vCJD contrasts with the replication cohorts in terms of prion strain (all groups), tissue distribution, and route of infection (for iCJD and sCJD). Furthermore, in the case of our large collection from Papua New Guinea, the linkage-disequilibrium relationship between candidate SNPs and a putative functional SNP is not known and can therefore differ from that in the UK. For these reasons, an absence of association in one or more replication categories does not preclude a genuine association in vCJD.

The precedent of codon 129 was important to inform the comparisons in the replication phase. All UK prion diseases have strong associations with homozygous genotypes; for vCJD, only the methionine homozygous genotype. However, the groups from Papua New Guinea are the most relevant in the replication phase because our only precedent of a major acquired human prion disease epidemic is kuru, which was historically transmitted by cannibalism and had a devastating effect on the Fore and neighbouring linguistic groups of the Eastern Highland region of Papua New Guinea.[5] Kuru was extensively documented at its peak in the mid-20th century.[22] We amplified DNA from this archive and continued surveillance of kuru in the Fore in the late 20th century to identify recent cases of kuru with long incubation times and elderly Fore women with long-term survival after exposure to high doses of prions. At *PRNP* codon 129, elderly Fore women survivors of the kuru epidemic showed a profound Hardy–Weinberg disequilibrium, with an excess of the prion disease-resistance genotype 129MV relative to both homozygous genotypes 129MM and 129VV. The patients with kuru show an age stratification of codon 129, with young patients being mostly genotype MM or VV and adult or elderly patients being mostly MV, consistent with a powerful effect of codon 129 MV in extending kuru incubation time.[5,23,24] Our study thus confirms the strong association of *PRNP* codon 129 (rs1799990) across acquired and sporadic prion diseases as the outstanding genetic risk factor in human prion disease. Notably, the effect was detectable in a small sample, which should be encouraging for those contemplating studies of rare diseases with well characterised patients and a distinct pathogenesis.

The additional associations we report are not as strong or robust as those we confirm for *PRNP* codon 129 but each of these are beyond what would have been expected by chance when taking into account the problem with multiple testing. Although we cannot be certain that any of the three candidate SNPs we describe altered the expression of their nearest gene (*PRNP*, *STMN2*, or *RARB*), in each case these are excellent candidates for involvement in prion pathobiology. The risk conferred by rs6116492T could act through altered expression of *PRNP* owing to the crucial role for PrP in prion disease pathobiology; however, we have no direct evidence that a putative genetic risk conferred by rs1460163 or rs6794719 is manifest through their nearest genes (*STMN2* or *RARB*) because these SNPs have no linkage disequilibrium with coding regions. Regulatory regions often act on nearby genes but can also act over great distances or even on different chromosomes, implicating other genes.[25]

In the absence of further cohorts of orally acquired prion disease and taking into account the aforementioned caveats, we turn to functional evidence of a role for these candidate genes in prion disease. The expression of PrP in cultured neuronal and lymphoid cells is regulated by retinoic acid.[26–28] Furthermore, the production of the disease-associated isoform of PrP (designated PrP^{Sc}) in cultured mouse neuronal cells infected with mouse prions is increased by treatment with retinoic acid.[26] Whether retinoic acid acts through the receptor encoded by *RARB* or another retinoic acid receptor for these biological activities is not known at present. In addition to *PRNP*, the strongest overall genetic evidence we found is for a SNP association upstream of *STMN2*. SCG10, the protein product of this gene, is a regulator of microtubule stability in neuronal cells, with potential implications for aggresome formation and modulation of prion neurotoxicity.[29] We found that *Stmn2* is turned off by prion infection in mouse neuronal cells, in keeping with an early study,[30] but different from a recent and rigorously conducted study.[31] Whether prion infection or unknown experimental factors are responsible for this large effect is unclear; a role for SCG10 in prion infection has not

been established and speculation about a mechanism in prion disease would be premature.

Our data lend considerable support to the hypothesis that genetic susceptibility in addition to *PRNP* codon 129 genotype has contributed significantly to the outbreak of vCJD to date. Whether these effects are on the incubation period rather than susceptibility, such that further waves of BSE-associated prion disease with longer incubation periods might occur in the years ahead and be associated with different genotypes at many risk loci, is unknown.[32]

Contributors

SM assessed patients, conceived and designed the study, managed the data acquisition, undertook the quality control and some statistical analyses, and drafted the manuscript. MP, JU, and JB were involved in the design and conduct of the array and replication studies. GA and TW were involved in design and conduct of the MGB probe replication study. PD and SJT were involved in the design and conduct of the mouse cell expression work. JW and MPA did the Papua New Guinea field work and commented on the manuscript. CV and JCW advised on and conducted statistical analyses and commented on the manuscript. JC assessed patients, established the study and sample collections, provided overall direction, and finalised the manuscript. HH provided bioinformatics and database support.

Conflicts of interest

J Collinge is a director and shareholder for D-Gen, a company in the field of prion diagnosis, therapeutics, and decontamination. The other authors have no conflicts of interest.

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 12 月 24 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			研究報告の公表状況	A vCJD blood test achieves 100% accuracy www.News-Medical.Net, 22 Dec 2008	公表国 オーストラリア	
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
昨年 12 月，Amorfix Life Sciences 社（カナダ）は異型クロイツフェルト・ヤコブ病(vCJD)のプリオンについて検討したヒト血液検体に関する第 2 回目の盲検試験の結果を公表した。当該試験は英国で実施された。同社は，多量の正常タンパク質がある検体中において非常に低レベルの凝集した異常折りたたみタンパク質を選択的に検出することができる，独自の特許権をもつ Epitope Protection™技術を開発した（詳細については，www.amorfix.com を参照のこと）。本試験の結果から，当該検査は 100%の感度および 100%の特異性を有すると主張している。試験では新鮮血漿および凍結血漿検体を用い，その一部に脳由来の vCJD プリオンを添加した。実に，脳ホモジネートを $1/10^6$ まで希釈したものを添加した検体を検出することに成功した。この技術は，vCJD 感染した集団を対象とした血漿を見極めるための大規模な検査にも適用できる。これらの結果は，以前は疾患に対し抵抗性を示すと考えられていた MV 遺伝子型をもつ人における vCJD の発症が，最近，初めて報告されたことを考慮すると，特に重要である。また，この検査法は明らかに献血および血液製剤の安全性を高めるのに役立つと考えられる。	BYL-2009-0366

報告企業の意見	今後の対応
この検査法はプリオン検出の向上をもたらす可能性がある。輸血用血液の安全性を高めるために有用であることが証明された場合，血漿分画製剤に用いる血漿プールにも使用される可能性がある。 弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されており，弊社製剤による vCJD 感染リスクは極めて低いと考えられる。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も Amorfix の輸血および血漿分画製剤のスクリーニングに関する情報収集に努める。

vCJD blood test achieves 100% accuracy

Published: Monday, 22-Dec-2008

Disease/Infection News

Amorfix Life Sciences has announced it achieved 100% sensitivity and 100% specificity in a second blinded trial of human blood samples using its EP-vCJD blood test in collaboration with the National Institute for Biological Standards and Control (NIBSC) in the United Kingdom.

"We have now successfully completed both fresh and frozen human plasma testing, as part of a test validation process facilitated by NIBSC," said Dr. George Adams, Chief Executive Officer of Amorfix. "The company has 50,000 test kits available to begin large-scale testing to determine the fraction of the population infected with vCJD. This information is vital for determining the need for routine testing of blood donations."

The UK Spongiform Encephalopathy Advisory Committee (SEAC) yesterday announced the first clinical case of vCJD in a patient with an MV genotype (all previous vCJD clinical cases were MM genotype) and suggested that 50 to 250 further cases might arise in the UK. This is consistent with a recent editorial in a leading medical journal, Lancet Neurology, published last week suggesting "waves" of vCJD cases could be expected.

"This first MV case of vCJD now shows people with MV genotypes are not resistant to vCJD, but may incubate the disease for a longer time before developing neurological symptoms. Yesterday's report of vCJD with MV genetics shows we are not out of the woods with this tragic epidemic, and also raises the possibility of ongoing blood-borne transmission of vCJD from silent carriers of the infection," said