Although the condition we now call sporadic Creutzfeldt-Jakob disease (sCJD) was the first human prion disease to be described, it remains in many important respects the least well understood. In the familial forms of CJD (fCJD) the close association with mutations in the prion protein gene (PRNP) limits speculation concerning their aetiology. For the acquired forms of human prion disease (kuru, iatrogenic CJD and variant CJD), we have a good understanding of aetiology in terms of the source of infection and the routes of exposure, and we can model these diseases in experimental animals with a degree of confidence. However, exactly upon what aetiological factors the apparently ‘sporadic’ nature of sCJD depends is open to a number of possible interpretations; and these points of view tend to reflect deep-seated assumptions in the minds of those working on prion diseases or, as they have also been known, the transmissible spongiform encephalopathies.

A traditional view of sCJD (often informed by analogies with animal transmissible spongiform encephalopathies and their modelling in mice) tends towards viewing all of these diseases as acquired. In this scenario, sCJD represents cases of human transmissible spongiform encephalopathy for which the human (or animal) source of infection is yet to be determined. By extension, the PRNP mutations associated with familial forms of CJD are proposed to represent susceptibility factors that make infection (presumably by an ubiquitous agent in the environment) and consequent disease almost certain within an individual’s lifetime.

An alternative view, often favoured by those who subscribe to the prion hypothesis, regards prion protein metabolism as an error prone process. Rarely (and if the incidence of sCJD is a reliable indication, it is a very rare event indeed), the normally benign or positively beneficial cellular prion protein acquires a self-perpetuating abnormal folding state, which ultimately leads to neuronal dysfunction and neurodegeneration. Under this scenario the presence of mutations in PRNP make this change far from unlikely, in fact close to certainty within the lifetime of the mutation carrier.

It is against this background that the debate about sCJD sub-classification occurs. It is an important debate, partly in order to provide a more precise diagnosis but, more fundamentally, because sub-classification might identify particular risk factors (genetic or environmental) for certain forms of sCJD and the identification of such risk factors may help to prevent future cases.

sCJD has long been known to be heterogeneous in its clinicopathological phenotype, but a rational molecular classification system for the disease was not proposed until the mid-1990s. The fact that two competing systems were proposed and developed in parallel should not obscure the fact that these differed in detail, not in substance. Both classification systems were based on the proposition that the clinicopathological heterogeneity in sCJD (and other human prion diseases besides) depends upon a combination of the sequence of the patient’s PRNP gene and on the physico-chemical properties of the abnormal prion protein (PrPSc) that accumulates in the patient’s brain (Parchi et al., 1999; Hill et al., 2003).

Genotyping is relatively unambiguous and there is a clear consensus that (both in the presence and the absence of pathogenic mutations) the methionine (M)/valine (V) polymorphism at codon 129 of PRNP exerts a powerful effect on disease susceptibility and phenotype. The relevant physico-chemical properties of PrPSc are much less clear cut, but for most practical purposes these have, thus far, been taken to mean the size and glycoform ratio of the fraction of prion protein from sCJD brain that survives proteolytic degradation under defined condition (termed PrPSc), as determined by western blotting. The PrPSc glycoform ratio does not vary dramatically between different patients with sCJD, but the extent of N-terminal truncation does, resulting in at least two major size classes—one predominately truncated at glycine 82, resulting in a ~21 kDa fragment, termed type 1, and the other further truncated to serine 97, resulting in a ~19 kDa fragment, and termed type 2. The sCJD disease classification proposed by Parchi and Gambetti (Parchi et al., 1999) recognizes six phenotypic variants (MM1/MV1, VV1, MM2 cortical, MM2 thalamic, MV2 and VV2), each with characteristic incidence, clinical features and pathological phenotypes. There is a moderately good correspondence between this classification system and that proposed by Collinge and colleagues (Hill et al., 2003), although the nomenclature differs.

What has threatened to undermine confidence in this form of sub-classification is a phenomenon first reported by Parchi et al. (1999) when the system was given its first full description in a
large cohort of cases. Around 5% of these were mentioned to contain both types 1 and 2 PrPres, although the potential effects of this phenomenon on classification were not thoroughly explored. Further, direct examination of the phenomenon of mixed PrPres isotypes or ‘co-occurrence’ as it has come to be known has confirmed its existence and produced consistently higher estimates of the prevalence, typically ~30%, among different cohorts of sCJD cases (Puoti et al., 1999; Schoch et al., 2006; Uro-Coste et al., 2008). Such findings have led to the proposition that the incidence of sCJD cases with both types 1 and 2 PrPres is a function of the extent of brain sampling and the sensitivity with which a minority type may be detected in the presence of larger amounts of the other protein (Head et al., 2004; Yull et al., 2006).

Indeed, the use of antibodies that are selective for type 1 over type 2 have led to the proposition that PrP typing is a quantitative, not a qualitative, phenomenon (Polymeridou et al., 2005; Yull et al., 2009), although the validity of this approach is contested on technical grounds (Notari et al., 2008).

The significance of the manuscript by Cali et al., published in the current issue of *Brain* is, therefore, clear: it places firm restrictions on the prevalence of cases with mixed PrPres isotypes and proposes that they constitute a defined subtype of sCJD with a distinct clinico-pathological phenotype. In their report, the authors provide an in depth study of a cohort of sCJD cases of the (most frequently occurring) PRNP codon 129 MM genotype. Extensive sampling of different brain regions and the use of antibodies that have differential affinities for types 1 and 2 PrPres in western blots were used to show that the actual incidence of cases with both types 1 and 2 is ~40% within this genetic group, but that the remaining cases can safely be considered to be ‘pure type 1’ or ‘pure type 2’. Moreover, evidence is presented that these three groups (MM1, MM2 and MM1+2) are distinct in terms of neuropathology.

The study methodology employed histopathological examination of fixed tissue from one cerebral hemisphere, and biochemical analysis of frozen tissue from the other. The correlation of these data assumes a highly symmetrical pattern of pathology in the hemispheres in sCJD, for which supporting evidence is not presented. Earlier studies by Tagliavini and co-workers (Puoti et al., 1999, 2005) in which adjacent sCJD brain tissue samples were analysed by histopathology and biochemistry (in order to obtain as close a correlation between these variables as possible) found that the distribution of mixed PrPres isoforms in the brain is variable, accompanied by the corresponding and often focal changes in patterns of PrP accumulation in the brain.

An intriguing component of this study is the application of a method termed conformational stability immunoassay, in which chaotropic salts are used to probe the PrPSc structure. The results of this part of the study seem to suggest that the behaviour of pure and mixed prion protein types is not explicable simply in terms of their conventional types as determined by proteinase K digestion and western blotting. This appears to suggest that there may be other physico-chemical properties of PrPSc relevant to disease phenotype, a point of view shared by Uro-Coste et al. (2008) who have used a related enzyme-linked immunosorbent assay (ELISA)-based technique to investigate mixed types in CJD.

If we speculate that Gambetti and co-workers will next turn their attention to sCJD in *PRNP* codon 129 heterozygous and valine homozygous patients, and that they will identify the same phenomenon in these groups, the net result will be a corresponding increase in the subtypes of sCJD. In terms of routine diagnostic neuropathology, it is doubtful whether every individual case of suspected sCJD could be analysed in sufficient detail to say with certainty how it conforms to such a system. Nevertheless, the underlying conceptual approach is of some certain significance. Either sCJD can be sub-classified by prion protein genotype and isotype into discrete clinico-pathological entities or it cannot, and consequently the brain PrPres type would simply become another aspect of the regional pathological phenotype, contributing to the overall phenotypic spectrum of disease. The former proposition is not yet certain, whereas the latter view appears regressive. Irrespective of the eventual consensus on the role of molecular typing in sCJD classification, the report by Cali and colleagues makes a significant contribution to this fascinating and ongoing debate.

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