Diagnostic criteria of Creutzfeldt–Jakob disease (CJD), a rare and fatal transmissible nervous system disease with public health implications, are determined by clinical data, electroencephalogram (EEG), detection of 14-3-3 protein in cerebrospinal fluid (CSF), brain magnetic resonance imaging and prion protein gene examination. The specificity of protein 14-3-3 has been questioned. We reviewed data from 1,572 autopsied patients collected over an 18-year period (1992–2009) and assessed whether and how 14-3-3 detection impacted the diagnosis of sporadic CJD in France, and whether this led to the misdiagnosis of treatable disorders. 14-3-3 detection was introduced into diagnostic criteria for CJD in 1998. Diagnostic accuracy decreased from 92% for the 1992–1997 period to 85% for the 1998–2009 period. This was associated with positive detections of 14-3-3 in cases with negative EEG and alternative diagnosis at autopsy. Potentially treatable diseases were found in 163 patients (10.5%). This study confirms the usefulness of the recent modification of diagnosis criteria by the addition of the results of CSF real-time quaking-induced conversion, a method based on prion seed-induced misfolding and aggregation of recombinant prion protein substrate that has proven to be a highly specific test for diagnosis of sporadic CJD.

Background
Sporadic Creutzfeldt–Jakob disease (sCJD) is the most common form of human prion disease, a group of rapidly fatal untreatable and transmissible encephalopathies. Clinical diagnosis of sCJD has relied on criteria revised over time to incorporate the detection of 14-3-3 protein in the cerebro-spinal fluid (CSF) in 1998 [1] and the results of brain magnetic resonance imaging (MRI) in 2009 [2]. As a marker of acute neuronal injury that does not directly detect abnormal prion propagation, the specificity of 14-3-3 detection has been questioned [3]. This may lead to overdiagnosis of sCJD, and to misdiagnosis of potentially treatable forms of neurological disorders [4]. The introduction of this test into the diagnosis criteria for CJD has considerably impacted the organisation of CJD surveillance in France. Since 1998, patient referral for autopsy to the French National Network for CJD surveillance has relied mainly on the report of 14-3-3 detection performed in five affiliated laboratories.

In a series of 1,572 autopsied patients collected over an 18-year period, we assessed whether there was overdiagnosis of sCJD in France and how the 14-3-3 detection impacted on this, and could lead to the misdiagnosis of treatable disorders.

Methods
Cohort study
We reviewed clinical, laboratory, electroencephalographic (EEG) and pathological data for 1,572 patients who were referred to the French neuropathology CJD...
network for autopsy between 1992 and 2009, and were included in the CJD National Reference Centre register [5-11].

Diagnostic assessment
Data provided by physicians to the CJD National Reference Centre register and to the neuropathologists for autopsy request were analysed (ND-L, J-PB, J-JH), distinguishing between possible and probable cases according to the World Health Organization (WHO) criteria (Table 1). For EEG data (available for 1,266 cases: 888 sCJD and 378 non-CJD), periodic (or pseudo-periodic) sharp wave complexes considered to be typical of CJD by the referring physicians, were recorded (EEGs were not systematically reviewed). CSF 14-3-3 protein immunoreactivity was available in 1,123 cases (749 sCJD and 374 other diagnoses). Analysis for the prion protein gene (PRNP) was performed in 871 cases (705 sCJD and 166 non-CJD).

Prion protein gene, Western blotting and neuropathological studies
PRNP analysis was performed as described [5,6] when informed consent was obtained from either the patient or the next of kin. Western blotting and neuropathology were performed following established methods [8-10]: after gross examination, one hemi-brain (left or right at random) was frozen at autopsy for PrPsc displaying (Western blot), the other fixed in formalin for 1 month. Hemi-brains were kept in the brain bank. At least eight formalin-fixed samples included: (i) transverse sections of cerebral cortex (middle frontal and superior temporal gyri, medial temporal gyrus including hippocampal formation and parahippocampal region, and cuneus); (ii) transverse sections of basal ganglia (head of caudate nucleus and anterior putamen, thalamic centromedian nucleus and mammillary bodies); (iii) sections perpendicular to the axis of the brains stem (cerebellar vermis including the nucleus dentatus; brain stem including the substantia nigra). After partial inactivation by 96% formic acid for 1 hour and paraffin embedding, 4 μm-thick sections were stained with haematein-eosin and periodic acid-Schiff...
techniques. Since 1998, a minimum of two sections (cerebral and cerebellar cortices) have been immuno-
labelled with PrP antibodies, using 12F10 monoclonal
antibody (Bertin, Montigny le Bretonneux, France) [10].
Each specimen was examined by at least two neuro-
pathologists (VS, DS, CD, J-JH), and, in most cases,
neuropathologists from other centres of the French
neuropathology network of Creutzfeldt-Jakob disease).
Combined disorders were classified considering both
therapeutic possibilities and prion diseases unique
infection control problem. For example, a case with
lesions of Wernicke’s encephalopathy associated with
a few Alzheimer lesions was classified as encephalop-
athy. However, when lesions of Wernicke’s encephalop-
athy were associated with CJD, the case was classified
as CJD.

Ethics
No autopsy can be performed in France unless the
patient’s next of kin can testify that the deceased did
not object to autopsy. The national computerised regis-
try of objection to autopsy is systematically consulted.
Another specific consent is needed for genetic stud-
ies. The Commission Nationale de l’Informatique et
des Libertés allows the surveillance register to publish
anonymised data [11].

Statistics
Statistical analysis was performed using STATA 12.1
(StataCorp. 2011. Stata Statistical Software: Release
12. College Station, Texas: StataCorp LP). Data are
described as mean (standard deviation) and percent-
age, and compared with means comparison test and
Fisher’s exact test. A p value ≤ 0.05 was considered to
be statistically significant.

Results
Data from 1,572 autopsies, performed between January
1992 and December 2009, were studied. This corre-
sponds to 11.8% of the 13,384 patients referred to the
CJD surveillance network. Excluding genetic (n=96),
iatrogenic (n=55), and variant CJD (vCJD) (n=18), there
were 920 sCJD cases and 483 cases of non-prion dis-
eseases (Figure 1).

Among these cases there were 230 patients with neu-
rodegenerative diseases (47.6%). As detailed in Table
2, these were mainly Alzheimer’s disease (74.8%),
Lewy body dementia and Parkinson’s disease (14.8%),
and fronto-temporal lobar degeneration (5.7%). There
were 47 cases of non-prion anoxic/metabolic/toxic
encephalopathy (9.7%), 45 cases of vascular demen-
tia (9.3%), 39 cases of cancer-related disorder (8.1%),
30 cases of encephalitis (6.2%) and 19 cases of non-
convulsive status epilepticus with no cause found at
autopsy (3.9%). In 39 patients, no history of dementia
and no histopathological lesions were found and
we categorised them as ‘normal brain’. In 28 patients,
there was a history of dementia and no typical histo-
pathological lesions were found. We categorised them

Figure 2
Evolution of the number of suspected cases of Creutzfeldt-Jakob disease and of the percentage of cases with 14-3-3 detection,
France,1992–2009
as ‘unspecified dementia after clinicopathological correlation’ (Table 2). A total of 163 patients with potentially treatable diseases had been suspected of having sCJD. Of these, 36 patients fulfilled criteria for probable sCJD (Table 3).

**Impact of 14-3-3 detection on the activity of surveillance network**

In 1992 14-3-3 detection was performed in under 20% of patients referred to the French neuropathology CJD network for autopsy, but by 1999 it was performed for 85% of such patients, and for over 90% after 2000 (Figure 2). Simultaneously, surveillance was reorganised to integrate this test: all patients in whom 14-3-3 was detected were referred to the French network and their diagnostic status was systematically analysed according to the WHO criteria (Table 1). This was associated with an important increase of the number of suspected cases between 1997 and 2001 (Figure 2).

**Accuracy of diagnosis criteria in suspected Creutzfeldt-Jakob disease cases and detection of 14-3-3 protein**

From January 1992 to December 1997, the proportion of definite CJD among patients classified as probable CJD (diagnosis accuracy) was 92%. This proportion significantly decreased to 85% from January 1998 to December 2009 (p = 0.014) (Table 3). This was due to the detection of 14-3-3 protein in cases with negative EEG and alternative diagnosis at autopsy. Among 686 autopsied patients classified as probable CJD, 36 were false probable CJD cases due to CSF 14-3-3 detection only (Table 4).

**Alternative diagnoses found in patients with probable sCJD and detection of 14-3-3 protein**

Since the introduction of 14-3-3 detection in 1998, the present study reveals a decrease in the proportion of neurodegenerative diseases and an increase of the proportion of cancers, vascular diseases, encephalitides and epilepsy among patients with an alternative diagnosis (Table 5). In those patients with non-neurodegenerative diseases, 14-3-3 was detected in 50 cases (44%) of the total of 114 cases. In neurodegenerative diseases, 49 (31%) of the total of 160 patients had positive 14-3-3 detection.

**Potentially treatable diseases with positive detection of 14-3-3 protein**

Possibly treatable cases (163, 33.7% of non-CJD cases) included 39 cancer-related disorders, 27 encephalopathies (Wernicke’s encephalopathy or pellagra), 30 encephalitides (some viral diseases, such as subacute sclerosing panencephalitis, have poor outcomes, but may benefit from therapeutic trials), 39 normal brains (e.g. unrecognised delirium, drug abuse, withdrawal syndrome, psychiatric disease), 19 non-convulsive status epilepticus with no cause found at autopsy and a number of other disorders (Figure 1). Interestingly, with the introduction of 14-3-3 detection, the number of patients classified as probable sCJD with a final diagnosis of potentially treatable diseases significantly increased from 1 to 35 (p = 0.01) (Table 3). Among these 35 patients, 26 showed positive detection of 14-3-3 and 17 were classified as probable sCJD according to positive 14-3-3 only without periodic sharp wave complexes on EEG.

The mean age at death in sCJD and non-CJD cases were similar: (age +/− standard deviation (SD) = 68.7 years+/−9.5, vs 68.9 years+/−14.7). However, before the age of 50 and after the age of 80 years, misdiagnoses were more numerous than between 50 and 80 years (p<0.0001). Regarding specifically treatable diseases, 68% (111/163) of the cases were aged 60 years and older.

The distribution of the PRNP genotypes at codon 129 in 705 sCJD and 166 non-CJD cases revealed significant differences. Among sCJD patients, the genotype was: Met-Met (421/705) (59.7%), Met-Val (139/705) (19.7%), Val-Val (145/705) (20.6%). Among the other cases,

### Table 1

| 1992–1998 | 1999–2009 |
|---|---|
| **Definite CJD**: neuropathological diagnosis | **Definite CJD**: neuropathological diagnosis |
| **Probable CJD**: I + 2 from column II + III | **Probable CJD**: I + 2 from column II + III, or possible CJD + CSF 14-3-3 detection |
| **Possible CJD**: I + 2 from column II + disease duration ≤ 2 years | **Possible CJD**: I + 2 from column II + disease duration ≤ 2 years |

| I | Rapidly progressive dementia | I | Rapidly progressive dementia |
|---|---|---|---|
| A | Myoclonus | A | Myoclonus |
| B | Cerebellar or visual signs | B | Cerebellar or visual signs |
| C | Pyramidal or extrapyramidal signs | C | Pyramidal or extrapyramidal signs |
| D | Akinetic mutism | D | Akinetic mutism |

| III | Typical EEG | III | Typical EEG |

CJD: Creutzfeldt-Jakob disease; CSF: cerebrospinal fluid; EEG: electroencephalogram.
it was Met-Met (75/166) (45.2%), Met-Val (73/166) (44.0%), Val-Val (18/166) (10.8%); (p < 0.002).

**Discussion**

As compared with the other European countries belonging to the EuroCJD network, France shows one of the highest number of 14-3-3 protein referrals. This largely contributed to the high incidence of sCJD in France and is regarded as a marker of high surveillance intensity [12]. An intensive surveillance fits well with the primary aim of the EuroCJD network which is to identify vCJD cases among the CJD population. However, while the highest sensitivity of diagnosis criteria is commendable, the present study shows that the introduction of 14-3-3 detection in the definition of probable cases coincided with a loss of specificity leading to the misdiagnosis of potentially treatable diseases. Without 14-3-3, the diagnostic sensitivity in probable cases was 56.1% and the specificity 95.6%. With 14-3-3 detection, the sensitivity was 82.4%, and the specificity 75.6%.

Other factors may be responsible for the high number of misdiagnoses. In all European countries, special attention was paid to CJD from March 1996 onwards, when vCJD was first reported in the United Kingdom [13], introducing a public health motivation for diagnosis.
coinciding with the introduction of CSF 14-3-3 in the diagnostic criteria. Specific to our research, another possible bias may be the study of autopsy cases only. Medical staff may ask for an autopsy to be performed because there is doubt about the CJD diagnosis. As a consequence, other diagnostic alternatives may have been discussed before the patient's death without formal report to the neuropathologist. Also, it may be pointed out that in France, in case of suspicion of CJD, any medical device that has come into contact with the patient should be destroyed unless autopsy dismisses the diagnosis. An increase in the use of CSF 14-3-3 and in the percentage of probable sCJD solely with positive CSF 14-3-3 is not specific to France, however, as shown by the study of national databases of 11 members of EUROCJD-Consortium, including nine European countries, Australia and Canada during the period 1993–2002 [14].

The 14-3-3 proteins are a group of 30-kDa proteins involved in signal transduction and apoptosis and are normally expressed by brain neurons. Their detection in CSF is regarded as a marker of subacute neuronal suffering. It thus can be observed in various neurological diseases including epilepsy, cancer, and paraneoplastic encephalitis. In our series, during the 1998–2009 period, when performed, 14-3-3 protein was detected in 15/18 cases of epilepsy, 15/37 cases of cancer and 7/28 cases with encephalitis, all potentially treatable diseases. Because of its lack of specificity, interpretation of 14-3-3 detection results must be performed within the context of the patient's clinical history and must not result in potentially useful therapies being dismissed. Importantly, a positive 14-3-3 may be drug-induced [15], which could explain some of the ‘normal brains’ found at autopsy.

Table 3: Ante-mortem classification of patients with potentially treatable diseases and definite diagnosis in patients with a diagnosis of probable sporadic Creutzfeldt-Jakob disease before and after introduction of 14-3-3 detection, France, 1992–2009

| Period of time | Ante-mortem classification of patients with potentially treatable diseases | Definite diagnosis among patients with a probable diagnosis of sCJD |
|---------------|-------------------------------------------------|---------------------------------------------------|
|               | Probable sCJD | Possible sCJD | Total | sCJD | Other diagnosis | Total |
| 1992–1997     | 1 | 4% | 21 | 96% | 22 | 151 | 92% | 13 | 8% | 164 |
| 1998–2009     | 35 | 26% | 102 | 74% | 137 | 586 | 85% | 100 | 15% | 686 |
| Total         | 36 | 23% | 123 | 77% | 159 | 737 | 87% | 113 | 13% | 850 |

sCJD: sporadic Creutzfeldt-Jakob disease.

In 2009, the presence of high signals of the striatum on T2-weighted sequences and diffusion-weighted imaging in brain MRI was introduced among the major criteria of probable sCJD [2]. It is too early to assess the impact of this improvement on the accuracy of these criteria. However, since these MRI alterations are rarely observed in most of the treatable alternative diagnoses, an increase in specificity should be expected.

Among the various researches for newer, more predictive, and informative biomarkers [25], a direct method for the diagnostic of sCJD that detects the seeding activity of abnormal PrP assemblies in the CSF has been proposed [26]. Real-time quaking-induced conversion (RT-QuIC) showed a specificity of 99 to 100% and a sensitivity similar to that of 14-3-3 detection [27,28]. Its validation using large series in European countries is pending. According to the results and to the reliability and the robustness of the method [27], RT-QuIC results in CSF or other tissues were recently added to the diagnosis criteria for sCJD by the EuroCJD consortium [28,29]. In the event that a specificity of 100% should be confirmed, especially in series including treatable alternative diagnoses, this method should contribute to considerably improve the diagnosis of CJD and other disorders leading to rapidly progressive dementia, including treatable diseases.
TABLE 4
Alternative diagnoses of treatable diseases in autopsied patients classified as probable sporadic CJD while alive on account of 14-3-3 detection only, France, 1992–2009 (n=36)

| Alternative diagnosis                  | n (with Metabolic encephalopathy) |
|----------------------------------------|----------------------------------|
| Encephalopathies                      | Metabolic encephalopathy*        |
| (n=6)                                  | 3                                |
| Vascular dementia                     | Unspecified vasculitis           |
| (n=1)                                  | 1                                |
| Cancer                                 | Primary Intracranial tumour      |
| (n=12)                                 | 9                                |
| Encephalitides                         | Meningoencephalitis              |
| Status epileptic                       | Rasmussen's encephalitis         |
| Others                                 | Malignant neuroleptic syndrome   |
| n=1                                    | 1                                |
| Normal brain                           | NA                               |
| (n=6)                                  | 6                                |

* One patient with a metabolic encephalopathy was the only probable CJD case in the period 1992–1997; CJD: Creutzfeldt-Jakob disease; NA: not applicable.

TABLE 5
Evolution of alternative diagnoses for sporadic Creutzfeldt-Jakob disease, France, 1992–2009

| Non-CJD cases                        | 1992–1997 | 1998–2009 | Total |
|--------------------------------------|-----------|-----------|-------|
| n                                    | %         | n         | %     |
| Degenerative diseases                | 39        | 53.4      | 191   | 46.6 |
| Encephalopathies                     | 7         | 9.6       | 40    | 9.8  |
| Cancer                               | 2         | 2.7       | 37    | 9.0  |
| Vascular diseases                    | 4         | 5.5       | 41    | 10.0 |
| Normal brain                         | 11        | 15.1      | 28    | 6.8  |
| Encephalitides                       | 2         | 2.7       | 28    | 6.8  |
| Epilepsy                             | 1         | 1.4       | 18    | 4.4  |
| Others                               | 7         | 9.6       | 27    | 6.6  |
| Total                                | 73        | 100       | 410   | 100  |

CJD: Creutzfeldt-Jakob disease.

Authors’ contributions
Conceived and designed the study: ND-L, J-PB, SH, J-JH. Collected and analysed data provided by the physicians: LP, ND-L, J-PB, DS, SH. Performed 14-3-3 detection: J-LL. Examined neuropathological specimen: VS, CD, DS, J-JH. Wrote the paper: LP, J-PB, SH, J-JH.

Acknowledgements
We thank all members of the Cellule nationale de référence de la maladie de Creutzfeldt-Jakob, and the French neuropathology network of Creutzfeldt-Jakob disease: H Sevestre (Amiens), F Dubas, F Letournel (Angers), G Viennot (Besançon), A Vital, C Vital (Bordeaux), F Chapon (Caen), JL Kémény (Clermont-Ferrand), F Labrousse, JM Vallat (Limoges), CA Maurage, MM Ruchoux (Lille), A Jouvet, N Kopp, D Meyeronnent, N Streichenberger, F Thivolet-Béjui (Lyon), D Figarella-Branger, A Maues de Paula, JF Pellissier (Marseille), J Floquet, JM Vignaud (Nancy), JF Michiels, F Vandebost (Nice), A Heitzmann (Orléans), F Gray, D Hénin, C Lacroix-Jousselin, J Mikol (Paris), P Baldet, V Rigau (Montpellier), P Levillain (Poitiers), MD Diébold, P Fornes, M Pluot (Reims), D Chiforeanu, N Rioux-Leclerc, S Saikaili (Montpellier), P Levillain (Poitiers), MD Diébold, P Fornes, C Lacroix-Jousselin, J Mikol (Paris), P Baldet, V Rigau (Montpellier), P Levillain (Poitiers), MD Diébold, P Fornes, M Pluot (Reims), D Chiforeanu, N Rioux-Leclerc, S Saikaili. We also thank the patients who gave their consent for autopsy and genetic studies.

Funding: This study was supported by Santé Publique France.

Conflict of interest
None declared.
10. Privat N, Laffont-Proust I, Faucheux BA, Saizdovitch V, Frobert Y, Laplanche JL, et al. Human prion diseases: from antibody screening to a standardized fast immunodiagnosis using automation. Mod Pathol. 2008;21(2):140-9. PMID: 18084251

11. Brandel JP, Peckeu L, Hall S. The French surveillance network of Creutzfeldt-Jakob disease. Epidemiological data in France and worldwide. Transfus Clin Biol. 2013;20(4):395-7. https://doi.org/10.1016/j.traci.2013.02.029 PMID: 23787616

12. Klug GM, Wand H, Simpson M, Boyd A, Law M, Masters CL, et al. Intensity of human prion disease surveillance predicts observed disease incidence. J Neurol Neurosurg Psychiatry. 2013;84(12):1737-7. https://doi.org/10.1136/jnnp-2012-304820 PMID: 23906290

13. Court L, Hauw JJ. Le docteur Françoise Cathala Pagesy et l'histoire des maladies à prions. Rev Neurol (Paris). 2015;171(6):805-11. https://doi.org/10.1016/j.neurol.2016.02.003 PMID: 24703683

14. de Pedro-Cuesta J, Glatzel M, Almazán J, Stoeck K, Mellina K, Shi S, et al. Cerebrospinal fluid real-time quaking-induced conversion is a robust and reliable test for sporadic Creutzfeldt-Jakob disease. Ann Neurol. 2012;72(2):278-85. https://doi.org/10.1002/ana.23589 PMID: 22926858

15. Jansen C, Parchi P, Capellari S, Ibrahim-Verbaas CA, Schuur M, Heinzl H, Hoftberger R, Unterberger U, Strobel T, Heinemann U, Krasnianski A, Meissner B, Varges D, Kallenberg C, Van Everbroeck B, Dobbeleir I, De Waele M, De Deyn P, Martin W, Zanusso G, Fiorini M, Farinazzo A, Gelati M, Benedetti MD, de Pedro-Cuesta J, Glatzel M, Almazán J, Stoeck K, Mellina K, Shi S, et al. Cerebrospinal fluid real-time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol. 2011;69(12):1578-82. https://doi.org/10.1001/2013.jamaneurol.79 PMID: 23229042

16. Gao C, Shi Q, Tian C, Chen C, Han J, Zhou W, et al. The epidemiological, clinical, and laboratory features of sporadic Creutzfeldt-Jakob disease patients in China: surveillance data from 2006 to 2010. PLoS One. 2011;6(6):e21278. https://doi.org/10.1371/journal.pone.0021278 PMID: 21619267

17. Paterson RW, Torres-Chae CC, Kuo AL, Ando T, Nguyen EA, Wong K, et al. Differential diagnosis of Jakob-Creutzfeldt disease. Arch Neurol. 2012;69(12):1350-9. https://doi.org/10.1001/archneurol.2012.21278748

18. Ferrazzi A, Ferraboschi P, Ferrari S, et al. Phosphorylated 14-3-3eta protein in the CSF of neuroleptic-treated patients. Neurology. 2005;64(9):1618-20. https://doi.org/10.1212/01.WNL.0000160397.81314.84 PMID: 15883127

19. Van Everbroeck B, Bobbelleir I, De Waele M, De Deyn P, Martin JJ, Cras P. Differential diagnosis of 201 possible Creutzfeldt-Jakob disease patients. J Neurol. 2004;251(3):298-304. https://doi.org/10.1007/s00415-004-0311-9 PMID: 15015009

20. Jansen C, Parchi P, Capellari S, Ibrahim-Verbaas CA, Schuur M, Strammelli R, et al. Human prion diseases in the Netherlands (1998-2009): clinical, genetic and molecular aspects. PLoS One. 2012;7(4):e36333. https://doi.org/10.1371/journal.pone.0036333 PMID: 22558438

21. Zanusso G, Monaco S, Pocchiari M, Caughey B. Advanced tests for early and accurate diagnosis of Creutzfeldt-Jakob disease. Nat Rev Neurol. 2016;12(6):325-33. https://doi.org/10.1038/nnreuro.2016.65 PMID: 27174240

License and copyright

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0) Licence. You may share and adapt the material, but must give appropriate credit to the source, provide a link to the licence, and indicate if changes were made.

This article is copyright of the authors, 2017.