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New mutation in scrapie amyloid precursor gene (at codon 178) in Finnish Creutzfeldt-Jakob kindred

SIR,—We have reported an association between a mutation in codon 200 of the scrapie amyloid precursor gene (PRIP) and Creutzfeldt-Jakob disease (CJD) in patients of eastern European or Sephardic Jewish origin.[1,2] In this latest (but certainly not last) chapter in the story of genetic abnormalities in CJD, we report a mutation in codon 178 linked to CJD in a large Finnish kindred. The pedigree now includes 15 affected members in four generations. The disease shows a trend towards "anticipation"; successive generations have had a progressively earlier age at onset,[3] and the most recently affected member in the fourth generation is only 26 years old. Brain tissue from 1 patient transmitted disease to a capuchin monkey[4]; brain, spleen, and liver from another patient did not transmit disease to cats or other non-primates.

DNA was extracted from frozen brain specimens of 2 affected family members, and the PRIP coding region was amplified by polymerase chain reaction and inserted into plasmid pGEM5z, from which multiple subclones were sequenced in both directions.[5] A G-to-A mutation in codon 178 (resulting in a substitution of asparagine for aspartic acid) was present in two of four subclones in 1 patient and all of five subclones in the other. This mutation was then screened by restriction endonuclease cleavage patterns in DNA extracted from frozen brain, spleen, anticoagulated blood, or blood clots from other affected and unaffected family members, unrelated sporadic CJD patients, and non-CJD controls. We used the enzyme *Tth*111 I (New England Biolabs), visualising DNA fragment patterns by ethidium bromide after electrophoresis in 3% agarose gels.

Representative patterns are shown in the figure. A single mutation was found in all 8 affected members (including both the transmitting and untransmitting cases), but not in any of 8 currently healthy first-degree relatives of the patients, 5 unrelated Finnish sporadic CJD patients, 12 Finnish controls (6 with other neurological disease, 6 with non-neurological illness), or 69 healthy North American controls.

The clinical features of CJD in this family are in most respects typical of the familial disease described elsewhere. The mean age at onset is 50, and the illness evolves as a progressive dementia with associated cerebellar and upper motor neuron signs, muscular rigidity, and myoclonus. However, periodic EEG activity has not been observed, and the mean duration of illness of 21 months is longer than usual for either familial or sporadic CJD. Neuropathological examination has revealed spongiform change, neuronal loss, and astroglial proliferation, without amyloid plaques.

Thus, the codon 178 mutation in this family, like the codon 200 mutation, is clearly implicated in susceptibility to CJD, but does not seem to affect clinical course, pathological findings, or transmissibility. In contrast, the octapeptide repeat insertion found in two other CJD families is associated with unusual clinical heterogeneity,[6] and codon 102 and 117 mutations are associated with the pathologically distinctive Gerstmann-Sträussler-Scheinker syndrome.[7,8] As more patients with these mutations are studied, and additional mutations are found, a pattern may emerge that will permit a correlation between molecular genetics, pathogenesis, and susceptibility in transmissible amyloidotic spongiform encephalopathies as a whole.

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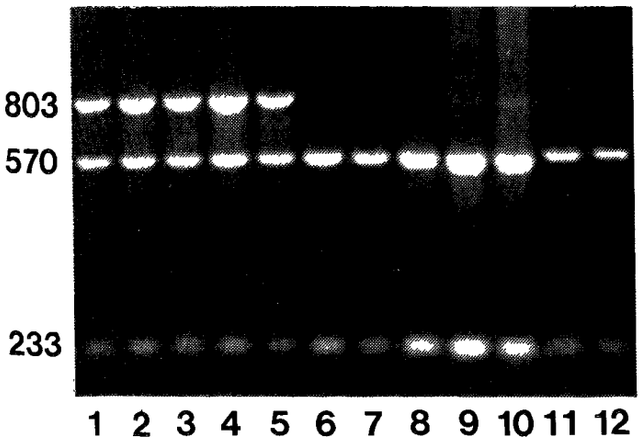

PRIP coding region fragments after digestion with *Tth*111 I.

CJD family S affected members (lanes 1–5);[3] unaffected members (lanes 6–8), unrelated sporadic CJD cases (lanes 9, 10), non-CJD controls (lanes 11, 12)

*Tth*111 I cleaves the normal 803 bp PCR product into two fragments of 570 and 233 bp, the GAC-to-AAC mutation in codon 178 abolishes this cleavage site Individuals heterozygous for the mutation show these two fragments plus 803 bp (from uncleaved mutated allele)

Seroepidemiological study of filovirus related to Ebola in the Philippines

SIR,—Since November, 1989, several isolates of a filovirus closely related to Ebola virus have been recovered from cynomolgus monkeys *(Macaca fascicularis)* imported into the USA from the Philippines.[1,2] The resemblance of the virus, tentatively named Reston strain, to Ebola virus is a concern because more than 600 registered cases with 71% case fatality ratio caused two outbreaks in Sudan and Zaire in 1976.[3,4] This has prompted the Philippine Department of Health to investigate the risk of transmission from animals to man and to assess the public health implications of this virus. We investigated all the export facilities and some wildlife trapping areas from December, 1989, to May, 1990. Most filovirus epizootics in a US quarantine facility were associated with monkeys from facility A in the Philippines. We therefore paid special attention to workers in the animal hospital at this facility, where the risk of exposure appeared highest.

We did a serological survey of people exposed to cynomolgus monkeys in the Philippines. By interview we gathered information about possible risk factors for infection. Any history of recent illness, such as fever of sudden onset, diarrhoea, vomiting, and haemorrhagic manifestations, was also obtained. Sera were tested against Reston strain antigen in three laboratories (Research

Institute for Tropical Medicine, Metro Manila, US Army Medical Research Institute for Infectious Disease, and US Centers for Disease Control) by the indirect fluorescent antibody test (IFAT), with a titre cut-off of 16 or more, and by western blot.

Of the 186 people studied, 48 were from wildlife collection areas and 138 were from the four primate export facilities (A 13%, B 14%, C 6%, D 67%). 12 (6%) had serological evidence of infection. There was no illness or death attributable to filovirus infection. 22% of workers in facility A had positive IFAT titres, significantly more than the 4% at other facilities (risk ratio 5·6, 95% CI 1·09–24·14). Of the 5 workers in the animal hospital at facility A, 4 had positive IFATs, and for 3 of these titres were above 256. Workers in the hospital had more positive titres than the rest of facility A (RR undefined, 95% CI 2·98 to very large). There was no association between seropositivity and other risk factors such as exposure to primates before employment, type of work, bites, scratches, and eating monkey meat.

Serological and epidemiological evidence suggests that simian filovirus can be transmitted to heavily exposed individuals but does not cause illness. Lack of illness after exposure to this filovirus was also reported from the USA, where a worker seroconverted after being cut with a scalpel contaminated with virus. He did not become ill.[5-7] There has been concern over the specificity of the IFAT. However, we think that the high titres found for the 3 hospital workers reflect true positivity because these people were clustered around the known area of active transmission in the hospital at facility A and because their titres were similar to those seen in primates with confirmed Reston virus infections. This study and similar reports from the USA indicate that this filovirus does not represent an immediate public health menace on the scale of the African Ebola virus.

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β_2-agonists

SIR,—One issue raised in correspondence (Jan 5, p 43) about our report that regular use of an inhaled β-agonist was deleterious in bronchial asthma (Dec 8, p 1391) is whether or not our findings with fenoterol apply to other β-agonist drugs. Dr Palmer and Dr Jenkins and Professor Clark cite evidence suggesting that regular salbutamol and salmeterol reduce symptoms and improve lung function. In our study daytime measurements of pulmonary function were also improved in most patients by regular β-agonist therapy but at the expense of deterioration in other markers of asthma control. However, parallel group studies are inappropriate to the detection of adverse effects of β-agonists. Our findings would be statistically non-significant when analysed as if parallel groups had been entered, given that the inter-individual differences (and hence SD of means) considerably exceed intra-individual differences seen when each patient acts as his or her own control. Although comparisons of regular salmeterol with regular salbutamol are reassuring in respect of long-term adverse effects, both could be marginally deleterious. Further studies using a crossover design versus placebo (ie, salbutamol as needed) are required before we can be sure that the adverse effects of regular fenoterol are not shared, perhaps to a lesser degree, by salbutamol, terbutaline, or the new long-acting agents salmeterol and formoterol.

The anti-inflammatory effects of salmeterol remain to be proven, but even if confirmed as clinically useful they may not counter the possible adverse effects of regular β-agonist. We found that even high doses of inhaled corticosteroid, for which anti-inflammatory activity is well established, did not reduce the adverse effect of regular β-agonist. Of 8 patients using more than 1500 μg inhaled corticosteroid daily, 7 were better controlled on an intermittent rather than a regular β-agonist. Hence it seems unlikely that the anti-inflammatory effect of salmeterol would counter any adverse effect of the β-agonist.

We share the concern of Dr Crompton that the use of long-acting β-agonists as sole therapy may not be appropriate. Likewise claims that they may facilitate a reduction in inhaled or oral corticosteroid therapy, because of alleged anti-inflammatory activity cannot be justified and may be dangerous. Further studies are urgently needed. Meanwhile, caution in changing drug therapy, and careful documentation of all therapy used by patients with asthma, is to be commended.

Among the second batch of correspondence (Feb 2, p 300) Dr Ayres and his colleagues question whether our finding that regular inhalation of a β-agonists is deleterious in asthma relates to tachyphylaxis to β-agonists. We think this unlikely. If the explanation were tachyphylaxis one would expect control of asthma to be maintained provided additional β-agonist was used. However, even though considerably more β-agonist was used during the period of regular fenoterol treatment, control of asthma was significantly worse in most patients. Furthermore, most patients showed higher evening peak flow rates during regular fenoterol treatment, suggesting that the drug continued to be effective during regular treatment. If tachyphylaxis explained the overall worse control during regular fenoterol, we would have expected evening peak flow to have fallen below that found during intermittent treatment. In some patients there was reversibility of airflow obstruction when they were tested periodically throughout the period of regular fenoterol treatment, again arguing against tachyphylaxis.

Dr Niggemann and Dr Wahn (Feb 2) misinterpret our study design. 89 patients entered a crossover study in which for 6 months they received regular dry powder fenoterol with additional aerosol bronchodilator if needed, and for the other 6 months they used regular dry powder placebo with aerosol bronchodilator if needed. Hence the questions about the comparability of the "two groups" in respect of severity, intrinsic versus allergic asthma, and percentage on inhaled steroids—questions appropriate to a parallel group design—are not relevant here.

The high mortality rate from asthma in New Zealand may be directly related to the findings from our study, in that regular moderately high dose inhaled β-agonist treatment has been used in New Zealand over the past decade or more. Other countries may have had lesser problems with morbidity and mortality from asthma because of lesser usage of β-agonist. Our study would suggest that reducing the usage of β-agonist should improve morbidity, and possibly mortality, from asthma, and we believe these recommendations are applicable to other countries.

We agree that similar studies are required with other β-agonists, especially salbutamol, and the new long-acting β-agents. In the meantime, however, we believe that we have sufficient evidence from our study, and from the incomplete evidence available on other β-agonists cited in our paper, to recommend that fenoterol, salbutamol, or terbutaline should not be used for regular bronchodilator treatment and that the new long-acting β-agonist drugs should not be used until studies indicate that they do not share these adverse effects. We cannot concur with the view of Niggemann and Wahn that regular β-agonist treatment is safe if used in combination with anti-inflammatory agents; in our study even high-dose inhaled corticosteroid did not prevent the adverse