Research letters

ACDP/SEAC/WG/TSE/P52
ANNEX B

Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease

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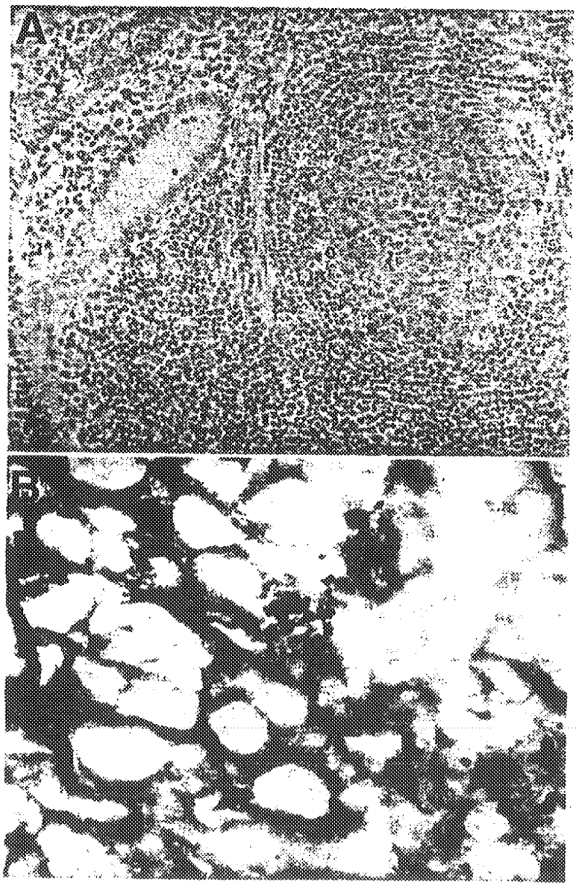
A variant of Creutzfeldt-Jakob disease (CJD) was identified in 1996;[1] there is evidence for a link between variant CJD and bovine spongiform encephalopathy (BSE).[2,3] The proportion of the population exposed to infectious amounts of the BSE agent through consumption of infected meat is unknown, therefore it is difficult to predict the number of future cases of variant CJD. Prion protein (PrP) has been found in the tonsillar tissues from sheep infected with scrapie,[4] and at necropsy from a patient with variant CJD.[5] We describe our findings in an appendix, removed 8 months before the onset of disease, from a patient with variant CJD.



Low-power view of appendix after immunocytochemistry for PrP (A) and high-power view of germinal centre after double labelling for PrP and CD21 (B)

Scattered cytoplasmic PrP immunoreactivity (brown reaction product) is seen within germinal centre cells which have morphology of follicular dendritic cells. Coarse deposits of PrP (black reaction product) are seen within the cytoplasm of CD21 immunoreactive cells (brown reaction product).

A 45-year-old man developed numbness of his face and right hand in May, 1996. Investigations included T2-weighted cranial magnetic-resonance imaging (MRI) which showed three small high-signal white-matter lesions. Multiple sclerosis was suspected. His sensory disturbances spread to his trunk and legs. He was treated for depression in April, 1997. Later that year he became hyperactive, disinhibited, and had aggressive outbursts. He also had intermittent deafness. In November, 1997, he had difficulty writing, slurred speech, and ataxia. A repeat MRI was unchanged. Visual evoked responses and examination of cerebrospinal fluid were normal. By early in 1998, his ataxia had deteriorated and his symptoms led to assessment in a psychiatric unit. Although systemic markers for vasculitis were negative, a brain biopsy was done in April, 1998, to exclude this treatable condition. Brain biopsy showed changes of variant CJD with scattered small cortical plaques surrounded by vacuoles and immunocytochemistry (with monoclonal anti-PrP antibodies 3F4 and KG9) showed extensive PrP deposition within plaques, and around neurons and blood vessels.

In September, 1995, he had had an appendectomy after 2 days of right iliac-fossa pain and fever. Histology of the appendix did not show acute appendicitis. Immunocytochemistry in May, 1998, with monoclonal antibodies 3F4 and KG9, showed immunoreactivity for PrP in the cytoplasm of scattered cells, predominantly in germinal centres (figure A). No staining was seen after omission of antibodies. The morphology of these immunoreactive cells suggested that they were follicular dendritic cells, which was confirmed by double labelling with antibodies to CD21, which co-localised to PrP immunoreactive cells (figure B). Immunoreactivity for PrP was not seen in any of 10 control appendices investigated.

Demonstration of PrP within the cytoplasm of follicular dendritic cells of the appendix mirrors the findings in tonsillar lymphoid tissue.[4,5] Involvement of the tonsillar tissue before onset of disease has been shown from the age of 10 months in sheep infected with scrapie; however, our findings are the first demonstration of PrP in tissue in human beings during the incubation period of CJD. Involvement of gut-associated lymphoid tissue before the clinical onset of disease is in keeping with an enteric route of entry for the variant CJD agent.

An implication of the presence of PrP in the appendix during the incubation period of variant CJD is that it offers the opportunity for large scale screening of appendectomy and, presumably, tonsillectomy, specimens removed since the onset of the BSE epidemic. Appendectomy specimens are routinely sent for histological examination and are usually available for further study. Although the incidence of human exposure to the BSE agent may be small, approximately 44 000 appendectomies are done in the UK each year (data from Royal College of Surgeons). Such a study would provide new data on the proportion of the

population at risk of developing variant CJD, although it is not known at what stage during the incubation period of variant CJD that lymphoid tissue becomes involved or whether this involvement will inevitably lead to the development of neurological disease.

1 Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996; **347**: 921–25.
2 Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 1997; **389**: 498–501.
3 Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. Molecular analysis of prion stain variation and the aetiology of 'new variant' CJD. *Nature* 1996; **383**: 685–90.
4 Schreuder BEC, van Keulen LJM, Vromans MEW, Langeveld JPM, Smits MA. Preclinical test for prion diseases. *Nature* 1996; **381**: 563.
5 Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* 1997; **349**: 99–100.

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Epidermal mosaicism producing localised acne: somatic mutation in *FGFR2*

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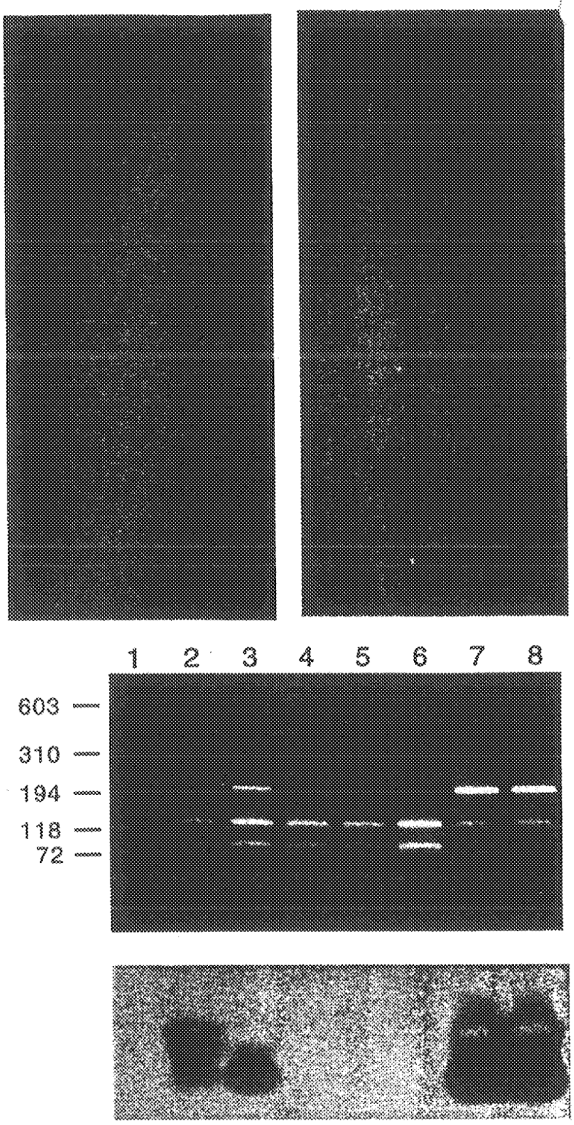
A 14-year-old boy with otherwise unremarkable acne for 2 years presented because the acne was much more severe in a lesion extending down his left arm (figure). This linear and atypically distal distribution, with a comedone in virtually every follicle of the affected area, suggests an unusual pathological basis. Disorders following this kind of linear or whorled pattern (lines of Blaschko) are thought to reflect the epidermal distribution of somatic mosaicism, whether due to X-inactivation in females carrying X-linked dominant mutations, or to mutation in embryonic precursors of the keratinocyte lineage.[1] Supporting this theory, somatic mutations of the keratin gene (*K10*) have been detected in naevoid epidermolytic hyperkeratosis.[2] In considering candidate genes for somatic mutation in our patient, we recalled the atypical generalised acne seen in Apert syndrome.[3] This complex congenital malformation, characterised by craniosynostosis and syndactyly, is due to specific germline mutations in the gene for fibroblast growth factor receptor 2 (*FGFR2*).[4] We examined this patient's epidermal naevus for comparable somatic mutations in *FGFR2*.

DNA was extracted from peripheral blood lymphocytes, from scrapings and keratinous plugs of lesional epidermis, and from banal follicular keratoses on the other arm. Scrapings routinely yielded 200–500 ng high-quality DNA. The region of *FGFR2* containing the two major Apert syndrome mutations was amplified by PCR and digested with *Mbo*I and *Bgl*I, each of which has a single restriction site in the normal sequence abolished by the major Apert syndrome mutations 934C→G and 937→G, respectively. All samples showed normal digestion with *Bgl*I, but samples from lesional skin were partially resistant to *Mbo*I digestion whereas samples from the opposite arm and blood digested normally with *Mbo*I (figure). We cloned the PCR product and sequenced eight independent *Mbo*I-resistant clones. All contained the 934C→G mutation (predicting a Ser252Trp substitution) identical to that in Apert syndrome. This was confirmed by blot hybridisation of the PCR product with a mutant oligonucleotide (figure). From two independent samples of the lesion, 56% and 34%, respectively, of cells were mutated (not shown).



Clinical features of acneiform naevus and analysis of *FGFR2*
(Top left) Sharply demarcated linear lesion extending from left shoulder to antecubital fossa. Close-up (top right) shows confluent comedones; scrapings taken from this region.
(Middle) Digestion of *FGFR2* PCR product with *Mbo*I. Lane 1, water control; 2 and 3, independent scrapings from lesional skin (left arm), taken 1 year apart; 4, skin scraping from right arm; 5, skin scraping from normal individual; 6, peripheral blood DNA from patient; 7, 8 unrelated Apert patients with 934C→G mutation in *FGFR2*. Primers used were 5'-GGAATTCCTTGCAGACAAACTCTACGTCTC-3' and 5'-GGAATTCAAAGGTGTCAGCCAGCAG-3'.
(Bottom) Blot hybridisation of undigested PCR product with radiolabelled 934C→G mutant oligonucleotide 5'-AGAGCGATGGCCTCACCG-3'. Lanes as above. Procedures pre and post PCR were conducted in different laboratories using separate reagents and equipment. Other methodological details available from the authors.

We conclude that the acneiform naevus is due to a somatic mutation of *FGFR2* identical to one which, if present in the germline, causes Apert syndrome. The germline mutation exhibits several unusual properties, including a very high mutation rate, exclusive paternal origin, and association with advanced paternal age.[5] The hypothesis that the mutation confers a selective advantage to male germ cells[5] might also apply to mutant epidermal cells. Other germline mutations of *FGFR2* and *FGFR3* are associated with acanthosis nigricans and cutis gyrata,[3,4] and