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Address for correspondence and reprints: Dr. Elaine A. Ostrander, Division of Clinical Research, Fred Hutchinson Cancer Research Center, M318, 1124 Columbia Street, Seattle, WA 98104

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The Gene for Nijmegen Breakage Syndrome (V2) Is Not Located on Chromosome 11

To the Editor:

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by oculocutaneous telangiectasia and cerebellar ataxia. Individuals with this disorder display immunological impairments, hypersensitivity to ionizing radiation, and a predisposition to cancer (for reviews, see Shiloh 1995). There has been reported genetic heterogeneity in AT, which appeared to include four genetic complementation groups in classical AT—i.e., A, B/C, D, E—and two variants, so-called Nijmegen breakage syndrome (NBS), V1 and V2 (Murnane and Painter 1982; Jaspers et al. 1988). Among the four groups of classical AT, no significant differences in clinical appearance have been seen. Familial linkage analyses have produced evidence that genes for all four complementation groups in classical AT reside in a narrow region on chromosome 11q22-23 (Gatti et al. 1988; McConville et al. 1994). On the other hand, NBS patients have neither cerebellar ataxia nor telangiectasia but do display microcephaly and a developmental delay (Weemaes et al. 1981; Wegner et al. 1988). However, patients share features with AT, such as high radiosensitivity, radioresistant DNA synthesis (RDS), and chromosome instability (Jaspers et al. 1988), suggesting that the same pathway (or part thereof) is impaired in both syndromes. The underlying gene for NBS has not yet been identified, and its location in the human genome is still unknown.

The genetic complementation groups were defined by somatic cell fusion between AT cells from different AT patients: if the fused cells retained RDS, then the two patients belonged to the same groups. Each genetic complementation group within classical AT as well as NBS were believed to arise from different mutated genes or at least from different mutations within one or several genes. However, the recently cloned ATM gene was found to be mutated in all complementation groups of AT, without any correlation to whatever the complementation group, indicating that, at least in classical AT, only one single gene is involved (Savitsky et al. 1995). Whether the same ATM gene is also involved in NBS remains, as yet, unclear, since the complementation studies based on the restoration of RDS obviously give conflicting results (Komatsu st al. 1989; Verhaegh et al. 1993, 1995; Zdzienicka et al. 1994; Savitsky et al. 1995).

To further study the genetic complementation of NBS and AT on the basis of high-radiation sensitivity of both diseases, we transformed several primary cultures of NBS skin fibroblasts with SV40 virus, and an immortal cell line was successfully established only from GM7166 cells belonging to AT-V2. The passage number of these GM7166VA7 cells is presently 80, and the life span was sufficiently long to allow them to be used for subcloning of somatic-cell and microcell hybrid. In addition, the radiation sensitivity of GM7166VA7 cells was nearly the same as that of the parental primary cultures.

Using the GM7166VA7 cell line, we examined the genetic heterogeneity of NBS V2 and classical AT by an introduction of chromosome via microcell-mediated chromosome transfer. A single copy of human chromosome 11 was introduced into GM7166VA7 cells, and the karyotypes of the resulting G418-resistant clones were analyzed. Figure 1 shows metaphase chromosomes of GM7166VA7 cells and the microcell hybrid clone 11/1. GM7166VA7 cells contained two chromosomes 11, whereas the microcell hybrid clone 11/1 revealed a trisomy of chromosome 11 in 80% of the cells and a disomy in the remaining 20%. This disomy probably was the result of loss of either of the original parental chromosomes 11 or of the introduced chromosome 11, as has been previously observed in chromosome transfers to AT5BIVA cells (Komatsu et al. 1990). In order to confirm the presence of chromosome 11 derived from A9(neo11)-1 in the microcell hybrid clone, CA-repeat polymorphisms at the D11S420 locus on human chromosome 11q23.3-24 were analyzed in both GM7166VA7 recipient cells and A9(neo11)-1 donor

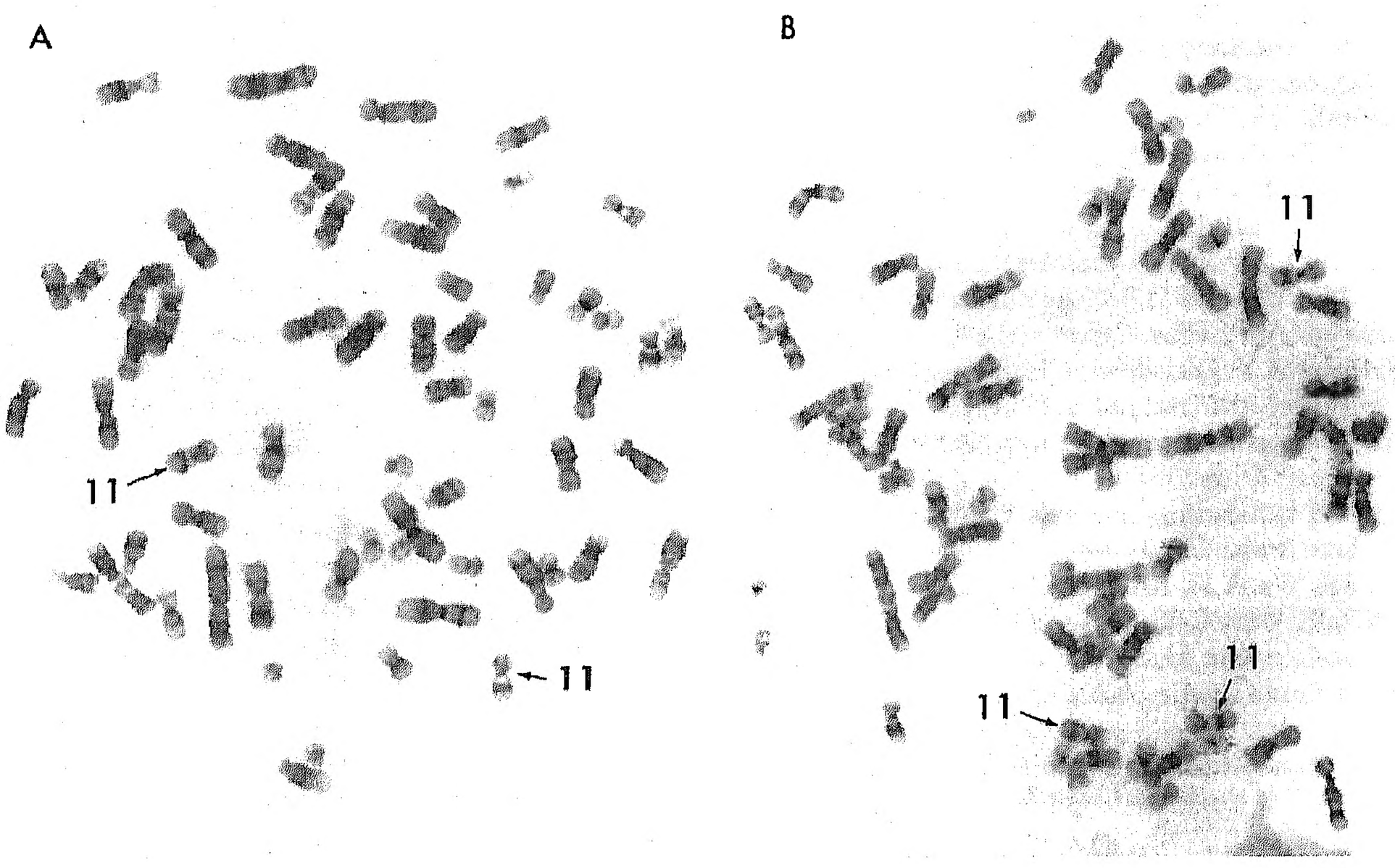

Figure 1 Representative GTG-stained metaphase spreads of a parental GM7166VA7 cell (*A*) and a microcell hybrid from clone 11/1, which contains an additional copy of a normal human chromosome 11 (*B*).

cells. Since CA-repeat lengths in these cell lines were different by two bases, the origin of the chromosome 11 copies in the hybrid clones could be identified as coming from either the recipient cells or the donor cells. Both hybrid clones, 11/1 and 11/41, contained two main bands in this assay, which indicated that at least one single chromosome 11 from the A9(neo11)-1 donor cells was successfully transferred.

Survival curves of normal control cells MRC5 and GM7166VA7 and of the microcell hybrid clones of 11/1, 11/21, and 11/41 are presented in figure 2. GM7166VA7 cells were highly sensitive to radiation, compared with normal cells, and this sensitivity was unchanged even after the transfer of an extra copy of normal chromosome 11. Although only three microcell hybrid clones are shown in figure 2, all of the seven clones that we generated lacked any restoration of radiation resistance. This is in contrast to the previous observation with AT cells, where AT5BIVA and AT4BINE1.3 cells containing an additional chromosome 11 from A9(neo11)-1 cells had normal levels of radiation sensitivity (Komatsu et al. 1990; Jongmans et al. 1995). Since analysis of microsatellite DNA and karyotyping proved successful chromosome transfer of chromosome 11, and since A9(neo11)-1 cells were adequate donors for functional complementation studies with AT, our results indicate that the gene responsible for NBS V2 is not located on chromosome 11 (it is a single human

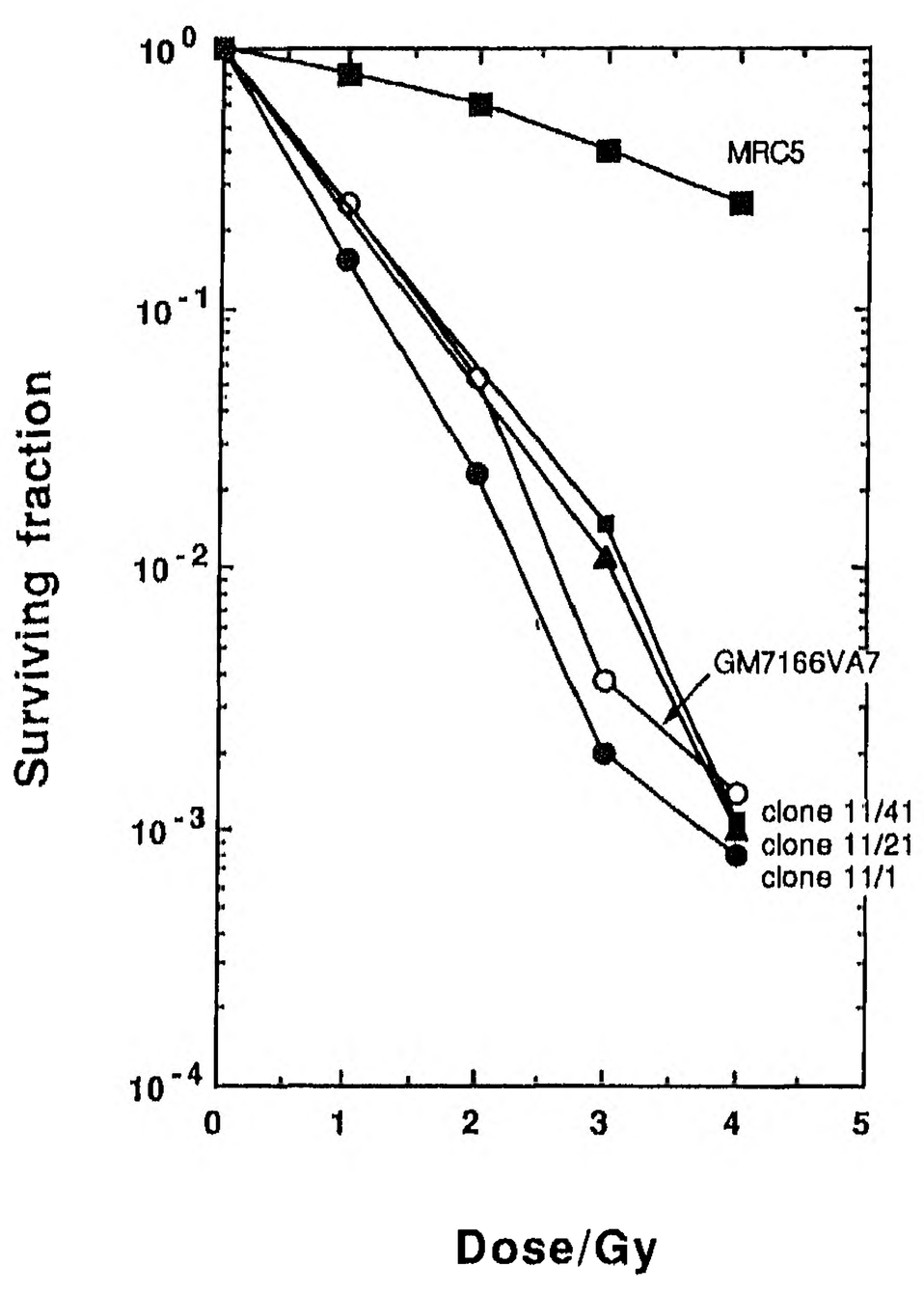

Figure 2 Survival curves for X-irradiated GM7166VA7 cells, control MRC5 cells, and microcell hybrid clones containing an additional human chromosome 11: 11/1, 11/21, and 11/41.

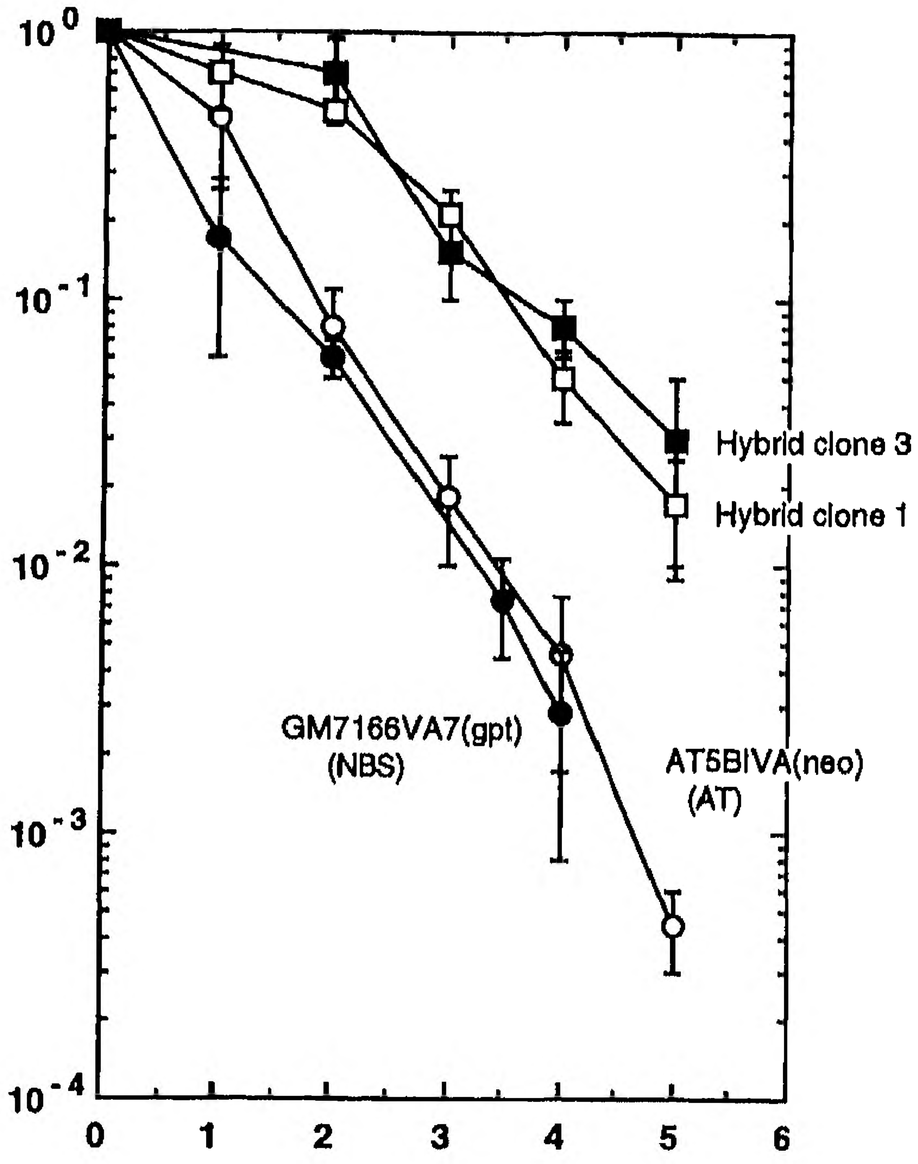

Figure 3 Survival curves of X-irradiated hybrid clones 1 and 3, produced by somatic cell fusion between GM7166VA7(gpt) and AT5BIVA(neo), and of both the parental cells.

chromosome that complements radiation sensitivity of AT in all complementation groups; Jongmans et al. 1995).

The genetic heterogeneity of both diseases is supported by functional complementation of hybrid cells generated by fusing NBS cells and AT-D cells. All hybrid clones independently obtained by somatic cell fusion of GM7166VA7(gpt) and AT5BIVA(neo) exhibited a significant restoration of radiation resistance, as shown in figure 3. This result is in agreement with that of haplotype studies of sib pairs with NBS that use polymorphic markers of chromosome 11q22-23 from the AT region. The alleles of affected siblings did not match in any of the six families with NBS (whether V1 and V2) (Stumm et al. 1995). Taken together, these results indicate that the locus of the NBS V2 gene is genetically distinct from the AT locus on chromosome 11 and, therefore, that the ATM-gene is not implicated in NBS.

KENSHI KOMATSU,[1] SHINYA MATSUURA,[1] HIROSHI TAUCHI,[1] SATORU ENDO,[1] SEIJI KODAMA,[2] DOMINIQUE SMEETS,[3] CORRY WEEMAES,[4] AND MITSUO OSHIMURA[5]

[1]*Department of Radiation Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima;* [2]*Department of Radiation Biology, Faculty of Pharmaceutical Science, Nagasaki University, Nagasaki; Departments of* [3]*Human Genetics and* [4]*Paediatrics, University Hospital of Nijmegen, Nijmegen; and* [5]*Department of Molecular and Cell Genetics, School of Life Science, Tottori University, Tottori, Japan*

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Address for correspondence and reprints: Dr. Kenshi Komatsu, Department of Radiation Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan.

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Codon 219 Polymorphism of PRNP in Healthy Caucasians and Creutzfeldt-Jakob Disease Patients

To the Editor:

A number of point and insert mutations of the PrP gene (PRNP) have been linked to familial Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker disease (GSS) (for a review, see Pocchiari 1994; Goldfarb and Brown 1995). Moreover, the methionine/valine homozygosity at the polymorphic codon 129 of PRNP may cause a predisposition to sporadic (Palmer et al. 1991; Salvatore et al. 1994) and iatrogenic (Collinge et al. 1991; Brown et al. 1994) CJD or may control the age at onset of familial cases carrying either the 144-bp insertion (Poulter et al. 1992) or codon 178 (Goldfarb et al. 1992), codon 198 (Hsiao et al. 1989), and codon 210 (Pocchiari et al. 1993) pathogenic mutations in PRNP. In addition, the association of methionine or valine at codon 129 and the point mutation at codon 178 on the same allele seem to play an important role in determining either fatal familial insomnia or CJD (Goldfarb et al. 1992). However, it is noteworthy that a relationship between codon 129 polymorphism and accelerated pathogenesis (early age at onset or shorter duration of the disease) has not been seen in familial CJD patients with codon 200 mutation (Gabizon et al. 1993) or in GSS patients with codon 102 mutation (Barbanti et al. 1994; Hainfellner et al. 1995), arguing that other, as yet unidentified, gene products or environmental factors, or both, may influence the clinical expression of these diseases.

Recently, Furukawa et al. (1995) found a new G→A polymorphism in the first position of codon 219 of PRNP in 12 of 100 healthy Japanese people, with an allele frequency of 6%. This polymorphism results in the substitution of the most common negatively charged glutamic acid by the positively charged lysine. They also reported one GSS family whose affected members carried the GSS-related codon 102 mutation and the polymorphic lysine at codon 219 on the same allele. The authors stated that the clinicopathological features of these patients clearly differ from those of previously reported GSS patients with codon 102 mutation (Furukawa et al. 1995). These findings prompted us to analyze this polymorphism in our control and CJD Caucasian populations.

To determine the incidence of codon 219 polymorphism, we screened DNA samples of the following: 100 randomly selected unrelated healthy adult individuals of both sexes, collected from all over Italy; 59 sporadic CJD patients with no known PRNP mutation (in 36 patients the clinical diagnosis was confirmed by neuropathological examination and/or western blot detection of the disease-specific, partially protease-resistant, prion protein); 8 familial CJD patients with codon 210 mutation; 2 familial CJD patients with codon 200 mutation; and 1 GSS patient with codon 102 mutation. We also screened 34 healthy members (mutated and nonmutated individuals) of these families.

DNA was extracted from blood, according to standard procedures. Since the substitution of G by A in the first position of codon 219 of PRNP (Glu→Lys) does not create or abolish any restriction site, the restriction site–generated PCR was used to screen for the presence of this polymorphism. A mismatched sense primer (Sc-7) containing a C→A change at nucleotide 650 and a matched antisense primer (Sc-4) were prepared as described elsewhere (Furukawa et al. 1995). This substitution creates a restriction site for BsiW I (98 and 20 bp, respectively) in the PCR product (118 bp) only when the G nucleotide is present in the first position of codon 219. The accuracy of the test was assessed by using DNA samples whose entire open reading frames of PRNP were fully sequenced (Pocchiari et al. 1993; Barbanti et al. 1994).

All 100 Italian controls had Glu/Glu at codon 219, with a Glu:Lys allele frequency of 1:0, significantly different from that in the Japanese population (.94:.06; $P = .004$, Fisher exact test; Furukawa et al. 1995). Moreover, none of the 59 sporadic CJD patients, 11 familial CJD/GSS patients, or 34 healthy members of these families showed the polymorphic lysine at codon 219.

This discrepancy may be related to ethnic background. However, the finding that the Japanese and Caucasian populations have different gene frequencies at the polymorphic codons 219 and 129 (Owen et al. 1990; Doh-ura et al. 1991) may be highly relevant in the clinicopathological phenotype of CJD and related disorders. This is supported by the different influence