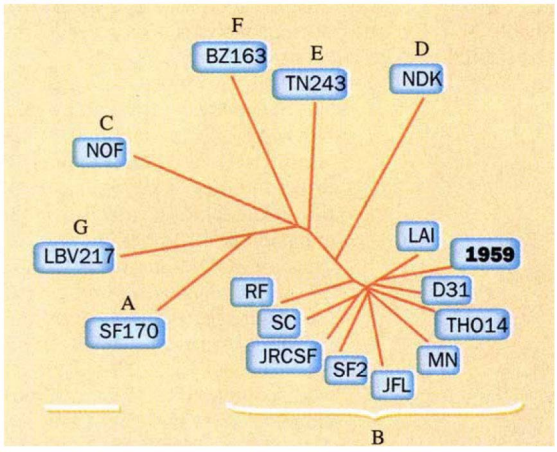
Was HIV present in 1959?

Sir — A previously healthy 25-year-old man died of a mysterious illness in September 1959 in Manchester, UK. An autopsy performed by Dr George Williams[1] revealed the presence of *Pneumocystis carinii* and cytomegalovirus pneumonia as well as perioral and perianal herpes infections, but an underlying cause was not found. Following the clinical description of AIDS, the possibility that this man had AIDS was raised [2]. With the advent of polymerase chain reaction (PCR) technology, the case was subsequently opened for re-examination.



Phylogenetic relationship between the virus amplified from the kidney DNA (1959) and other clade B and non-clade B strains of HIV-1. The phylogenetic tree was constructed using the neighbour-joining method. Scale bar, 5%.

Paraffin-embedded tissues from the case, as well as tissues from a matched case of accidental death in 1959, were coded by Williams and submitted to Dr Gerald Corbitt (The Virology Unit, University of Manchester), who reported the detection of *gag* sequences of human immunodeficiency virus type 1 (HIV-1) by PCR in kidney , bone marrow, spleen and pharyngeal mucosa tissues, but not in liver or brain or in any tissues from the control case[3].

SUMMARY OF PCR RESULTS

Samples	β-globin	HIV-1 gag	HIV-1 env	HIV-2 LTR	HIV-1, 2/SIV gag/pol	HLA DQ-α
DNA from Corbitt (Nov 1992)						
1959/2 (kidney)	+	+	+	ND	ND	1.2, 4 (2, 3)
1959/8 (bone marrow)	+	–	–	ND	ND	1.2, 3
Tissue sections from Williams (Feb 1994)						
C (thyroid)	+	–	–	–	–	3, 4
13 (liver)	+	–	–	–	–	3, 4
5 (kidney)	+	–	–	–	–	3, 4
17 (kidney)	+	–	–	–	–	ND
14 (myocardium)	ND	–	–	–	–	ND
12 (pancreas)	+	–	–	–	–	ND
16 (mucosa)	+	–	–	–	–	ND
A (brain)	ND	–	–	–	–	ND
B (brain)	+	–	–	–	–	ND
Tissue sections from Corbitt (Feb 1994)						
K1 (kidney)	ND	–	–	–	–	ND
K2 (kidney)	+	–	–	–	–	ND
P (pharynx)	ND	–	–	–	–	ND
L (larynx)	ND	–	–	–	–	ND
B (bone marrow)	ND	–	–	–	–	ND

Detection of human β-globin gene was performed using outer primers GH20 and GH21 plus inner primers KM29 and KM38 or PC03 and PC04 as described by Saiki *et al.*[4]. Detection of HIV-1 *gag* was performed using outer primers P59 (1,366–1,389 of pNL4–3; 5′ GGACATCAAGCAGCCATGCAAATG-3′) and P52′ (1,658–1,630; 5′-TTTGGTCCTTGTCTTATGTCCAGAATGCT-3′) plus inner primers P61 (1,395–1,428; 5′-AGAGACCATCAATGAGGAAGCTGC-3′) and P52′2 (1,652–1,630; 5′-CCTTGTCTTATGTCCAGAATGCT-3′). Detection of HIV-1 *env* was performed using outer primer P5 (ref. 5) and PV3 (7,368–7,341; 5′-CAGTAGAAAAATTCCCCTCCACAATTAA-3′) plus inner primers P7b (6,979–7,002; 5′-ATCAACTCAACTGCTGTTAAATGG-3′) and PV3. Detection of HIV-2 LTR was performed using outer primers LTR A and LTR B plus inner primers LTR C and LTR D as described by Gao *et al.*[6]. Detection of HIV-1 and HIV-2/SIV *gag–pol* was performed using outer primers LenG11 (1,929–1,957 of SIVMM251; 5′-TATGTAGACAGATTCTACAAAA-3′) and LenP2 (3,395–3,371; 5′-CTATTAAGATATCATCCATGTACTG-3′) plus inner primers LenG5 (2,016–2,035; 5′-AATGCTAACCCAGATTGCAA-3′) and LenP4 (3,298–3,275; 5′-TGGTGACCCCTTCCATCCCTGTGG-3′). With some exceptions, PCR conditions were similar to those described in Zhu *et al.*[5]. Rare positive results for HIV-1 were noted, but they were shown to be contaminants by seqencing of the PCR products. LTR, long terminal repeat; ND, not done.

Thus, it was concluded that this Manchester patient was the first documented case of AIDS in the medical literature. However, it remained unclear epidemiologically how he might have acquired HIV-1 infection. He was said to be a heterosexual who did not use intravenous drugs. He performed his National Service in the Royal Navy but was stationed exclusively in England, except for a brief voyage to Gibraltar in 1957.

Because the nucleotide sequence of the virus from a 1959 case of AIDS could shed light on the evolution of HIV-1 and related lentiviruses, we asked Corbitt for this information. His laboratory had determined the DNA sequence of two fragments of HIV-1 *env*, but was unsuccessful in obtaining more sequence data. We therefore offered to carry out a more extensive PCR and sequencing effort, as well as phylogenetic analysis. In November 1992, Corbitt sent us DNA extracted from kidney and bone marrow samples that had previously tested positive for HIV-1 (ref. 3).

In our hands, multiple attempts to amplify HIV-1 sequences by PCR from the bone marrow DNA were unsuccessful. However, we successfully amplified five fragments of HIV-1, covering the entire viral genome, from the kidney DNA. These PCR products were then cloned and sequenced. Sequence heterogeneity was observed among different clones, consistent with the presence of viral quasispecies typically seen in samples from infected people. Moreover, the short sequences of *env* obtained in Corbitt's laboratory closely matched our sequences for the corresponding region. All of our sequences from this case have been submitted to the HIV Database at Los Alamos National Laboratory and are available through Dr Gerald Myers (GenBank accession no. U23487).

The full-length nucleotide sequence was analysed independently by Myers of Los Alamos National Laboratory and Dr Eddie Holmes of Oxford University. Regardless of the region of genome examined, all phylogenetic analyses showed that the virus in the kidney sample was a member of HIV-1 clade B, the subtype currently prevalent in the United States and Europe (see figure). The branch length of the Manchester case was comparable to those found for recent HIV-1 clade B strains, suggesting that the 1959 sequence could not be distinguished from contemporary clade B sequences and thus raising the spectre of specimen contamination. Given the matched *env* sequences obtained in Manchester and New York plus the detection of quasispecies with full-length genomes, the evidence suggests that any contamination was more likely to be by another clinical specimen than by cloned HIV-1 sequences or amplicons. We therefore decided to repeat the analyses

using another set of tissues from the 1959 case.

We contacted Williams and obtained tissue sections of brain, thyroid, kidney, pancreas, liver, myocardium and mucosa (probably pharynx) in February 1994. Concurrently, sections of kidney, pharynx, larynx and bone marrow were provided by Corbitt. DNA extraction and nested PCR were performed to detect LTR, *gag*, *pol* and *env* sequences of HIV-1 and HIV-2/SIV (simian immunodeficiency virus). Appropriate negative controls were included in each experiment, and positive controls were used to show that the PCR assays were sensitive (detection limit of about 2 copies) for all subtypes of HIV-1 (including group O) and HIV-2/SIV. As summarized in the table, PCR results were uniformly positive for β-globin in samples tested but negative for HIV-1 and HIV-2/SIV in all samples.

To investigate the apparent discrepancy, we performed tissue typing using the Amplitype HLA DQ-alpha PCR Amplification and Typing Kit (Perkin Elmer Cetus) to define the HLA DQ-α alleles in each DNA sample. As shown in the table, the first kidney DNA contained DQ-α-1.2 and -4 sequences, with traces of DQ-α-2 and -3, whereas the accompanying bone marrow sample contained DQ-α-1.2 and -3 sequences. In contrast, thyroid, liver and kidney samples provided directly by Williams contained sequences of DQ-α-3 and -4. These findings suggest that the collection of tissues examined was derived from at least two individuals. Although we cannot state with certainty which set of tissues is from the Manchester patient, the first set does appear to be more problematic in that a mixture of HLA DQ-α alleles was present. In our opinion, this finding invalidates the conclusion reached in the 1990 *Lancet* report[3].

The results of our analyses reported here raise serious doubts about the authenticity of the 1959 Manchester man as the first documented case of AIDS due to HIV-1 infection. We suggest a further independent study of the remaining tissue samples to help resolve the matter, as this case is potentially important to our understanding of the evolution of primate immunodeficiency viruses.

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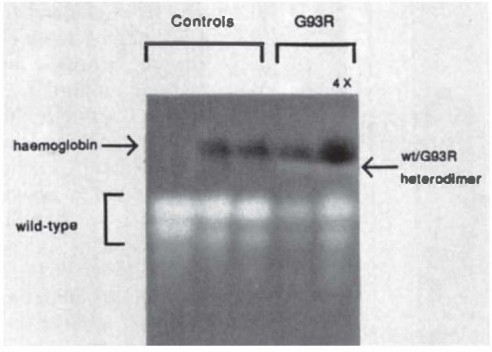
A novel SOD mutant and ALS

SIR — No effective treatment exists for amyotrophic lateral sclerosis (ALS), a fatal degenerative disease characterized by the death of large motor neurons. About 5% of cases are familial, with the vast majority apparently occurring sporadically. The two forms are clinically indistinguishable and the underlying mechanisms may be related. The discovery of mutations in the SOD-1 gene, encoding CuZn superoxide dismutase (SOD), in about 20% of familial ALS cases[1,2] has thus stimulated efforts to understand the disease mechanism in these cases[3,4].



Equal amounts (except 4× and standard) of erythrocyte proteins separated on a native gel and stained for superoxide dismutase activity. Controls, left to right: CuZn SOD standard, normal human, spouse of proband. The assignment of wt/G 93R is based on comparison with other mutants we have made that have gained one positive charge[7]. Disease onset in the proband occurred at age 29 years. Family members had onsets at 36, 37 and 41 years leading to death within 12, 3 and 2 years, respectively. Solution assays for SOD-1 enzyme activity were determined directly on erythrocyte lysates by measuring loss of superoxide spectrophotometrically[8]. Many other assays are inhibited by haemoglobin which must be removed by precipitation with organic solvents. These solvents cause some precipitation of the less stable SOD-1 familial ALS mutants resulting in an underestimate of total activity (our unpublished results). Amounts of erythrocyte lysate for enzyme assay and for activity gels[9] were standardized by assaying haemoglobin.

Two general mechanisms by which SOD-1 mutants cause disease have been proposed: loss of enzyme function, or the gain of an adverse function. Support for the former comes from the finding that SOD-1 familial ALS patients, who are heterozygotes, have total enzyme levels reduced to 50–80 % of normal[4]. However, loss of enzyme activity could be incidental to the gain of a new adverse function. Strong evidence for such an adverse function comes from transgenic mice overexpressing SOD-1 familial ALS enzymes. These animals develop an ALS-like disease, yet the SOD enzyme activity in their central nervous systems is increased compared to controls[3,4].

Dominant negative mutations cause the loss of activity contributed by wild-type subunits in multimeric proteins (SOD is a homodimer) by direct interaction or by increasing the proteolytic turnover of the complex. After screening erythrocytes for SOD-1 enzyme activity in 27 UK families with familial ALS (see figure legend), we found one individual with only 30% of wild type levels (17.3 ± 0.93 U mg^{-1}), when compared to 71 control individuals, showing that we had detected a dominant negative effect. DNA sequencing[1] revealed that the SOD-1 familial ALS mutation responsible encodes a Gly 93 to Arg change (G93R)[5].

To confirm the dominant negative nature of G93R we separated homodimers from heterodimer on a native polyacrylamide gel and stained for SOD activity (see figure). An equal interaction of native and mutant dimer interfaces would produce a 1:2:1 ratio of native homodimer to heterodimer to mutant homodimer. Comparison of the 4× G93R lane with the 1× controls indicates that an equal interaction is occuring and that approximately 25% of SOD molecules in the G93R erythrocytes are present as wild-type homodimers. If G93R were completely dominant negative, only native homodimers should be active and enzyme levels would be 25% of control, rather than the 30% observed. Separation of the more positively charged wild-type/G93R heterodimer on the activity gel indicates that it has some residual activity which probably accounts for the additional 5% observed in the accurate solution assay. No activity corresponding to the G93R homodimer was observed. Thus, the activity gel confirms that G93R is a dominant negative mutation, although that it is not completely dominant negative in erythrocytes.

If the disease mechanism in SOD-1 familial ALS involves superoxide radicals, then increased severity of disease would be predicted for individuals with the G93R mutation, which is only 30% active, compared with the 50–80% activity of most mutants. In the family reported here

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