A 90 kb DNA deletion associated with neurofibromatosis type 1

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Abstract
A deletion of 90 kb of DNA has been identified in a patient with neurofibromatosis type 1, using pulsed field gel electrophoresis. The deletion lies between probes 17L1A and AC5 in the critical region of chromosome 17 and represents the only molecular alteration found by PFGE in a series of 90 unrelated patients. The subject showing the deletion is an isolated case, shows typical clinical features, and represents one of the first examples of a molecular deletion to be found in this disorder.

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with a prevalence of 1 in 3000 to 4000 of the population. The major clinical features include café au lait spots, multiple neurofibromas, and Lisch nodules of the iris, and diagnostic criteria have been established.[1] Virtually all gene carriers show signs of the disorder by 5 years of age.[2] The gene for NF1 has been localised on chromosome 17 by linkage studies[3][4] and close flanking DNA markers have been defined.[5] Two patients with translocations

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involving the region 17q11.2 have been reported, and it has been suggested that the gene has been disrupted, leading to loss of normal function.[6][7] A physical map of 2·3 Mb of the NF1 region has been constructed, the translocation breakpoints have been identified, and two HTF islands have been defined.[8][9] These translocation breakpoints are 60 kb apart.[10] Several potential coding sequences have been identified in the region flanked by the two HTF islands and the translocation breakpoints (Cawthon *et al*, in preparation).[10–12] Very recently, two groups have independently reported the isolation of cDNA clones within what is likely to be the NF1 gene itself.[13][14]

The mutation rate for NF1 is very high (10^{-4}/allele/generation) and around 50% of cases represent new mutations. One possible explanation for this observation is that the NF1 locus may contain highly mutable sequences which may become duplicated or deleted, as is the case in Duchenne muscular dystrophy. Because most new cases of DMD are the result of DNA deletions, we decided to investigate the possibility that deletions may be associated with new mutations in NF1. We have screened a large panel of clinically well characterised, independent NF1 mutations using the DNA markers which map between the two HTF islands, using both conventional and pulsed field gel electrophoresis, and have discovered one case in which mutation to the NF1 phenotype is accompanied by deletion of DNA from the critical region.

Patients and methods
The panel consists of 125 unrelated NF1 patients, of which 45 were new mutations. All the patients were analysed by conventional electrophoresis. A sub-set of 90 was studied by pulsed field gel electrophoresis (PFGE). All the patients satisfy the NIH diagnostic criteria for NF1.[1] Clinical details on each patient are well documented so that variation in the severity or the nature of clinical features, in particular mental retardation, can be correlated with specific NF1 mutations at the molecular level, once these are defined.

DNA was extracted from peripheral blood,[15] and high molecular weight DNA was prepared in agarose

blocks. Each block contained 5 to 7 µg DNA which was digested to completion with 20 units of the rare cutter restriction enzymes, NotI, SacII, or BssHII, and fractionated on a 1% agarose gel using a 'CHEF' apparatus (Contour Clamped Homogeneous Electric Field, Biorad) in 0·5×TBE (0·045 mol/l Tris, 0·045 mol/l boric acid, 0·001 mol/l EDTA). The pulse time was maintained at 60 seconds for 14 hours and 90 seconds for 10 hours at 200 volts. Gels were stained in ethidium bromide, ultraviolet nicked for one minute by medium wave length UV light, and transferred to Hybond N membranes (Amersham). DNA probes were labelled by the random primer method.[16] Initially the linking clone 17L1A[8] and cDNA NF1–c2[12] (Cawthon et al, in preparation) were used to screen the panel. Membranes were prehybridised in 6×SSC (1×SSC=0·15 mol/l NaCl, 0·015 mol/l Tris sodium citrate), 5×Denhardt's, 0·5% SDS (sodium dodecyl sulphate), 7% dextran sulphate for two hours. The labelled probe was added to the prehybridised membranes and hybridisation continued in the same solution for 18 hours at 65°C. Post hybridisation washes were carried out at 65°C in 2×SSC, 0·1% SDS, increasing the stringency as required. Membranes were then exposed to Fuji x ray film at −70°C with intensifying screens for several days. To screen for smaller scale deletions or rearrangements, DNA was digested with the restriction enzymes EcoRI, HindIII, PvuII, PstI, BglII and TaqI, subjected to standard agarose gel electrophoresis, and hybridised with DNA probe 17L1A, a linking clone[8]; NF1c2

cDNA (Cawthon et al, in preparation); pEH1, a jump clone from cosmid EVI20[11]; pDV1.9, a genomic subclone near the t(17;22) translocation breakpoint[12]; PRC 9·4, a cDNA clone of EVI2[12]; 2B/B35I, a subclone of 2B/B35[17] and 1F10.[9] The localisation of these probes is depicted in fig 1.

Results
No anomalous fragments were detected in 125 NF1 patients using conventional gel electrophoresis. However, in one of the 90 patients screened using PFGE, an altered fragment was observed with the probe NF1-c2 using NotI, SacII, NarI, EaqI, BssHII, SalI, and NruI (fig 2). The normal fragment length with the enzymes NotI, SacII, NarI, EaqI, or BssHII is 290 kb, but in the patient a new fragment of 200 kb was observed in addition to the normal one (fig 2). The normal fragment length with enzymes SalI and NruI is 500 to 600 kb and an altered fragment was also seen with these enzymes, which was around 90 kb smaller than the normal one. The new fragment was not present in either of the patient's unaffected offspring (fig 2) nor has it been observed in 89 other NF1 patients or 20 normal controls.

The patient probably represents a new mutation. Her mother has been clinically examined and found to be normal. Her father, now dead, did not have any skin abnormalities or other symptoms of NF1. Her clinical features include six café au lait spots, multiple neurofibromas, 10 Lisch nodules, scoliosis, and
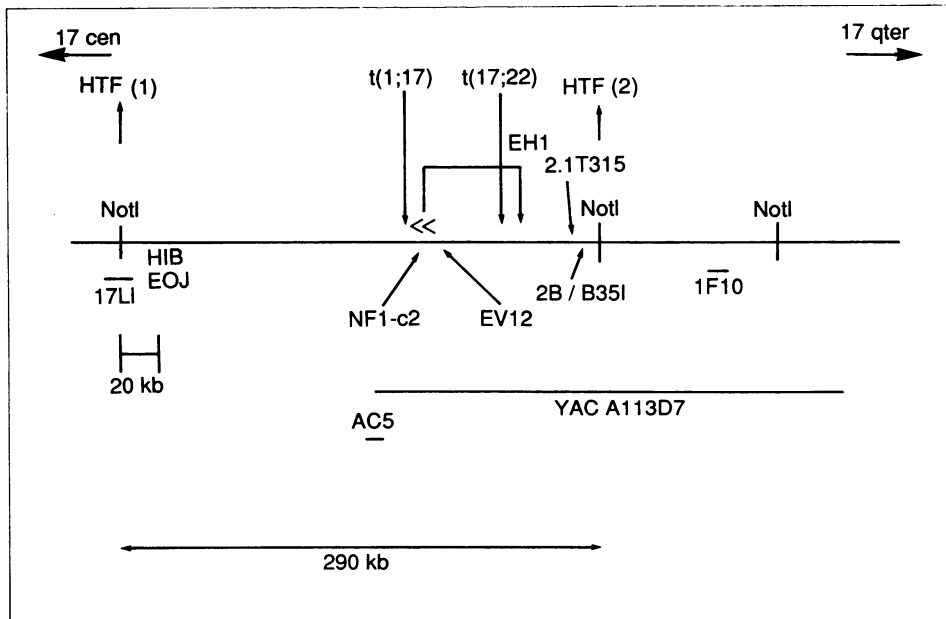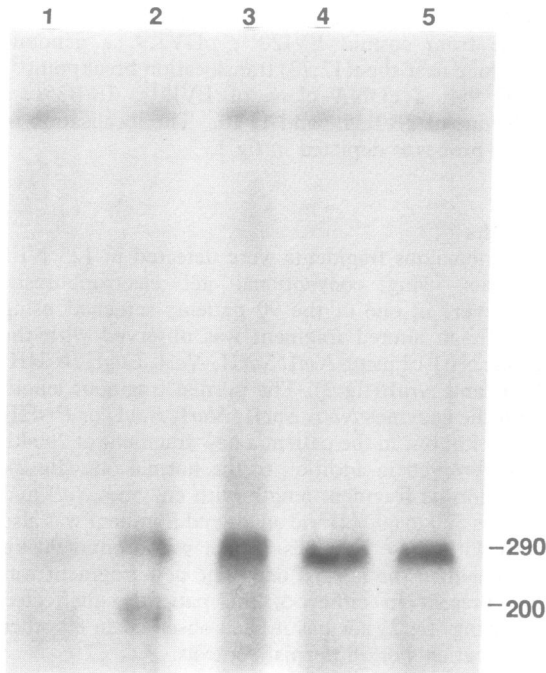

Figure 1   Physical map of NF1 region.

Figure 2   Pulsed field gel analysis with probe NF1–c2. Lanes 1 and 2 contain DNA from the NF1 patient digested with the enzyme NarI or NotI. Lane 3 represents DNA from her spouse digested with NotI. Lanes 4 and 5 contain DNA from the unaffected children digested with NotI. The normal fragment length with the enzymes NarI or NotI is 290 kb, but in the NF1 patient a new fragment of 200 kb is observed in addition to the normal one.

neurofibromas of the gum. She is of above average intelligence and no cytogenetic abnormalities have been detected on GTG and RBG banding.[18] The patient has two clinically normal children both over 20 years of age.

In order to define the ends of the deletion, we have analysed the family using PFGE with further DNA markers mapping to the region of the previously identified translocation breakpoints (fig 1). The markers used were H1BEOJ (end of jump clone towards the translocation from 17L1A) (Wallace *et al*, unpublished data), AC5 (end of YAC clone A113D7) (Marchuk *et al*, unpublished data), EH1 (end of jump clone from cosmid EVI 20),[11] 2B/B35I,[17] 2.IT315 (subclone of cosmid T315) (Viskochil *et al*, unpublished data), and IF10,[9] and their presumptive localisations are shown in fig 1. All these markers, except 1F10, map between the two HTF islands on the physical map of the NF1 region reported previously.[10] [19] The same 200 kb variant band was detected after digestion with *NotI* or *SacII* using all of the above markers except 1F10. A 90 kb *NotI* fragment was seen in the proband and her children with probe 1F10.

Discussion

The high mutation rate in NF1 has led to expectations that, as with Duchenne muscular dystrophy, a significant number of cases would prove to result from gene deletion, but the patient reported here represents the first instance of a purely molecular deletion to be found in this disorder before isolation of the gene. One case of a ring chromosome 17 with NF1 showing loss of chromosomal material in the relevant region has been described.[20] Two NF1 patients with translocations in 17q11.2 have also been reported.[8] [9] In the present study, we have used PFGE to study a large panel of NF1 patients and, using probes derived from the region of the translocations, we have discovered this single case in a total of 90 unrelated patients in which there has apparently been a deletion of DNA. An altered fragment, approximately 90 kb smaller than the normal one, was detected using various restriction enzymes and all of the DNA probes whose positions are shown in fig 1, with the exception of 1F10. The new band is unlikely to be the result of sequence polymorphism or methylation differences, as it is detected with different restriction enzymes and has never been observed in a sample of 200 other chromosomes.

The distance between the two translocation breakpoints is about 60 kb[10] and since none of the probes that map between the breakpoints (NF1–c2 and EV12) is itself deleted, the deletion cannot encompass both translocation breakpoints. The probes 17L1A, which represents the proximal HTF island (fig 1) and H1BEOJ, which is a jump from 17L1A, are not deleted and hence the deletion is excluded from the region between these two markers. The probe 1F10 maps distal to the second HTF island (fig 1) but does not detect the altered fragment; therefore, the deletion must be completely within the interval bounded by the two HTF islands. None of the remaining probes that detect the altered fragment (AC5, NF1–c2, EV12, EH1, 2B/B35I, 2·1 T315) is itself deleted. Thus, there must be a region of at least 90 kb in which none of the probes used in this study is located, but which contains the deletion. The precise definition of this region depends on the exact location of the probe loci, but apparently lies between H1BEOJ and AC5. Since it is likely that the deletion is the cause of NF1 in the patient, it must disrupt the NF1 gene, either directly by causing an alteration in the expression of the gene or indirectly as a result of the loss of regulatory sequences or a disruption in the higher order structure of DNA. We are investigating the patient to define the two ends of the deletion further. Since the completion and preparation for publication of the work described here, two groups[13] [14] have independently reported the isolation of cDNA clones that are likely to represent part of the NF1 gene itself. The gene shows a large (13 kb) transcript and appears to have other genes embedded within its introns;

deletions have been identified in three patients and an insertion of DNA in a further one. The patient described here will provide a further advance in our understanding of the range of molecular pathology found in neurofibromatosis.

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