Hereditary multi-infarct dementia unlinked to chromosome 19q12 in a large Scottish pedigree: evidence of probable locus heterogeneity

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Abstract
Hereditary multi-infarct dementia is a rare autosomal dominant disorder that predominantly affects the cerebral white matter. A locus was recently mapped in French pedigrees to chromosome 19q12. We have examined a large Scottish pedigree with neuropathologically confirmed hereditary multi-infarct dementia using polymorphic DNA markers spanning the 19q12 region and found no evidence of linkage. This suggests that, as in familial Alzheimer's disease, there is more than one locus.

Multi-infarct dementia is the second commonest neurodegenerative condition in the Western world, next only to Alzheimer’s disease. In the Orient it may be the most common. Unfortunately almost nothing is known about the genetics or molecular pathology of the disorder. Nevertheless just as the study of families with rare autosomal dominant forms of Alzheimer’s disease have contributed enormously to unravelling its molecular genetics, so it is hoped a similar approach can be applied to multi-infarct dementia with equally fruitful results.

Hereditary multi-infarct dementia is a rare autosomal dominant disorder that leads to recurrent small predominantly subcortical ischaemic strokes. Various known as chronic familial vascular encephalopathy, hereditary multi-infarct dementia, and cerebral autosomal dominant arteriopathy with subcortical infarcts and leuкоencephalopathy, reports of seven families have been published to date. Clinically the disorder resembles Binswanger’s encephalopathy but is distinguished by its mendelian inheritance pattern and normotension. Males and females are equally affected with onset usually in the fifth and sixth decade (range 20 to 70 years). There is slow progressive mental deterioration (3 to 20 years), associated with focal deficits, often transient, including parapareses, paraesthesia, headaches, and visual disturbance. Psychiatric symptoms are common and may be the sole manifestation. However, many cases eventually progress to a terminal picture of dementia, pseudo bulbar palsy, incontinence, and ataxia. Magnetic resonance imaging (MRI) of the brains of affected persons show, on T2 weighting, multiple high intensity signal lesions of the subcortical white matter. As the disease progresses these coalesce to produce patchy areas of confluence. Necropsy shows multiple small subcortical infarcts, diffuse myelin loss, and pallor of the hemisphere white matter. This is associated with vascular changes affecting the deep perforating vessels to the white matter and basal ganglia; the defining features are thickening of the arterial wall, widening of the Virchow Robin spaces, and a non-congophilic hyalinisation of the media. It is uncertain whether these are primary or secondary aspects of the disease.

A locus for the disorder was recently mapped to chromosome 19q12 in two unrelated French families. Clinical evaluation included MRI of the brains of both clinically symptomatic and apparently normal members of the pedigrees. Disease status was assigned to a number of asymptomatic pedigree members on the basis of the demonstration on T2 weighting of multiple high intensity signal lesions of the subcortical white matter. Since this report, linkage to chromosome 19q12 has been confirmed in further French families (E Tournier-Lasserve, personal communication). Multipoint analysis locates the affected gene within a 14 CM interval bracketed by markers D19S221 and D19S222. The highest lod score is obtained using the probe D19S226. The following study attempts to determine whether a large Scottish family with neuropathologically confirmed hereditary multi-infarct dementia resembles the French families and is also linked to chromosome 19q12 or whether, as in familial Alzheimer’s disease, there is more than one locus.

Materials and methods

Subjects
Fig 1 displays 87 members of the pedigree, which was initially identified in 1986 when several members were found to be dementing. Subsequent neuropathological examination of two affected family members showed gross white matter disease and infarction plus non-congophilic hyalinisation of the media of the deep perforating arteries (fig 2). On this evidence a diagnosis was made of hereditary multi-infarct dementia. The family was recently re-approved and reassessed for further evidence of illness. Several previously uninvestigated branches were examined, and at the same time venesection was performed. MRI scanning of symptomatic members of the pedigree is continuing. Of the 87 members of the pedigree displayed in fig 1, DNA is at present available for 43 persons including three who have now
Figure 1  Scottish pedigree with neuropathologically confirmed hereditary multi-infarct dementia (CADASIL"). All symptomatic members of the pedigree, regardless of liability class (see text), are fully shaded for purposes of confidentiality.

died. Symptomatic persons were divided into three classes: (1) definite or highly probable, (2) probable, and (3) possible, corresponding to the likelihood of having a diagnosis of hereditary multi-infarct dementia. Criteria for definite or highly probable (n=22) included neuropathological or radiological evidence of white matter disease and/or recurrent early onset neurological symptoms in at risk persons consistent with the diagnosis including early onset strokes or dementia; probable (n=8) included neurological symptoms suggestive of demyelination or combined neurological and psychiatric symptoms of unknown origin in an affected first degree relative; possible (n=16) included unexplained psychiatric or neurological symptoms in an at risk person. These included recurrent severe headaches, urinary incontinence, transient focal deficits, psychosis, and epilepsy. Asymptomatic persons were not included in the analysis.

Figure 2  (Top) Brain slices from pedigree member 47 showing infarct holes in basal ganglia in several sites plus several white matter cavities and diffuse white matter atrophy with sparing of the subcortical arcuate fibres. (Bottom) Microscopy on pedigree member 49. Haematoxylin and eosin preparation showing thick layers of homogeneous fibrilair, non-consophilic material, leading to occlusion, in wall of deep perforating blood vessel of brain.

Methods
DNA was extracted from frozen blood samples. Oligonucleotide primer pairs were synthesised on an ABI PCR Mate with a 5’ FITC label on one. After deprotection, the primers in ammonium hydroxide solution were passed through a NAP-10 column (Pharmacia LKB) and eluted in 10 mmol/l sodium phosphate pH 6-8. Polymerase chain reaction (PCR) was performed in 200 μmol/l dNTPs, 10 mmol/l Tris HCl, pH 9, 1-0 mmol/l MgCl2, 50 mmol/l KCl, 0-1% Triton X-100, 1-0 μmol/l primers, 1 unit Taq polymerase (Promega), and 100 ng DNA in 25 μl. PCR was carried out using a Cetus thermal cycler as follows: cycle 1 four minutes, 94°C; cycles 2-27 one minute, 94°C, two minutes, 55°C, 2-5 minutes, 72°C; cycle 28 one minute, 94°C, 30 seconds, 55°C, 7-5 minutes, 72°C.

After the PCR, stop mix (95% formamide, dextran blue, and 25 mmol/l EDTA pH 9) was added before heat denaturation at 94°C for two minutes, followed by rapid cooling on ice before loading onto the gels.
Electrophoresis was carried out using an automated laser fluorescence (ALF) sequencer (Pharmacia LKB). Gels were made up with 6% Hydrolink (Long Ranger™) in 1 x TBE (0-1 mol/l Tris, 0-083 mol/l borate, 1 mmol/l EDTA) containing 7 mol/l urea and run at 55 W constant power, ca 1200 V at 50°C. The alleles were read and sized automatically with our in house computer programs, then checked manually.

**Linkage Analysis**

Two point linkage was performed using the LINKAGE package,^12^ Lod scores were separately calculated using affected members only with both broad and narrow definitions of the phenotype. Model I included definite and highly probable cases only. Model II was as model I plus probable cases. Model III was as model II plus possible cases. The seven markers chosen for genotyping spanned the region reported as showing linkage to 19q12^20^ and included the three probes included in the original study. All were taken from the updated Genethon linkage map.^13^ Oligonucleotide sequences were available through the Genethon Data Bank.

**Results and Discussion**

The table provides pairwise linkage data for the seven probes. Each is analysed using the three separate models of diagnostic certainty as indicated.

Fig 3 is a multipoint map of the region using the seven markers with analysis also restricted to symptomatic family members. We found no evidence of linkage on any of the models and indeed on all models the region of reported linkage can be excluded with a lod score of less than -2.

There are several explanations for our findings. Misclassification of family members or errors of genotyping or both may have produced a false negative result. While the former is possible on account of incomplete neuroimaging or pathology on much of the family, we have tried to minimise error by assigning members to classes of diagnostic certainty and by analysing our data using conservative affected only models. None of the analyses shows linkage to the region of interest. Similarly, genotyping errors are possible but cannot account for the negativity. Genotyping was performed at least twice for each marker on all family members, and read independently by two raters blind to disease status. Concerning the original diagnosis of the family, the neuropathology is identical to that previously published for inherited multi-infarct dementia (fig 3). Features included white matter infarction, demyelination, and non-congophilic hyalinisation of the deep perforating vessel walls. We therefore conclude that locus heterogeneity is present in hereditary multi-infarct dementia. However, until a second locus is mapped this will remain a hypothesis. Fortunately, the above family is large enough to generate a significant lod score in its own right, and mapping this new locus is now our priority.
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