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Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy, Genetic Homogeneity, and Mapping of the Locus within a 2-cM Interval

A. Ducros,[1] T. Nagy,[1] S. Alamowitch,[1] A. Nibbio,[1] A. Joutel,[1] K. Vahedi,[1,2] H. Chabriat,[2] M. T. Iba-Zizen,[3] J. Julien,[9] P. Davous,[4] J. Y. Goas,[10] O. Lyon-Caen,[5] B. Dubois,[5] X. Ducrocq,[11] F. Salsa,[12] M. Ragno,[13] P. Burkhard,[14] C. Bassetti,[15] M. Hutchinson,[16] M. Vérin,[17] F. Viader,[18] F. Chapon,[18] M. Levasseur,[6] J. L. Mas,[7] O. Delrieu,[1] J. Maciazek,[1] M. Prieur,[8] H. Mohrenweiser,[19] J. F. Bach,[1] M. G. Bousser,[2] and E. Tournier-Lasserve[1]

[1]INSERM U25, [2]Service de neurologie, CHU St. Antoine, [3]IRM Hôpital des Quinze-Vingt, [4]Hôpital d'Argenteuil, [5]CHU Pitié-Salpétrière, [6]Hôpital d'Orsay, [7]CH St. Anne, and [8]Laboratoire de cytogénétique, Paris; [9]Hôpital Haut L'Evêque, Pessac, France; [10]CHU de Brest, Brest; [11]CHU de Nancy, Nancy; [12]Ospedale Civile, Bassano del Grappa, Italy; [13]C&G Mazzoni Hospital, Ascoli Pisceno, Italy; [14]Hôpital cantonal de Genève, Geneva; [15]Hôpital de Bern, Bern; [16]St. Vincent's Hospital, Dublin; [17]CHU de Rennes, Rennes; [18]CHU de Caen, Caen; and [19]Lawrence Livermore, CA National Laboratories, Livermore, CA

Summary

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a recently identified autosomal dominant cerebral arteriopathy characterized by the recurrence of subcortical infarcts leading to dementia. A genetic linkage analysis conducted in two large families recently allowed us to map the affected gene on chromosome 19 in a 12-cM interval bracketed by D19S221 and D19S215. In the present study, these first 2 families and 13 additional ones, including a total of 199 potentially informative meiosis, have been genotyped with eight polymorphic markers located between D19S221 and D19S215. All families were linked to chromosome 19. The highest combined lod score (Z_{max} = 37.24 at θ = .01) was obtained with marker D19S841, a new CA_n microsatellite marker that we isolated from chromosome 19 cosmids. The recombinant events observed within these families were used to refine the genetic mapping of CADASIL within a 2-cM interval that is now bracketed by D19S226 and D19S199 on 19p13.1. These data strongly suggest the genetic homogeneity of this recently identified condition and establish the value of its clinical and neuroimaging diagnostic criteria. Besides their importance for the ongoing positional cloning of the CADASIL gene, these data help to refine the genetic mapping of CADASIL relative to familial hemiplegic migraine and hereditary paroxysmal cerebellar ataxia, conditions that we both mapped within the same chromosome 19 region.

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Address for correspondence and reprints: Dr. E. Tournier-Lasserve, INSERM U25, Faculté de Médecine Necker, 156 rue de Vaugirard, Paris 75015, France.

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Introduction

Since 1977, several families suffering from an autosomal dominant stroke condition of unknown etiology have been reported under various names such as hereditary multi-infarct dementia or familial sclerosing vasculopathy (Sourander and Walinder 1977; Stevens et al. 1977; Colmant 1980; Sonninen and Savontaus 1987). In 1991, the clinical, neuroimaging, and genetic parameters of this hereditary condition were precisely defined, on the basis of the analysis of a very large pedigree originating from France (Tournier-Lasserve et al. 1991). This autosomal dominant disorder, now designated under the acronym CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), is characterized, in the absence of any vascular risk factor, by the recurrence of subcortical infarcts starting in early/mid adulthood and leading to dementia. Cerebral magnetic resonance imaging (MRI) shows, in all clinically affected individuals, well delineated images of abnormal signal highly suggestive of small deep infarcts as well as a diffuse hypersignal on T2-weighted images of the cerebral white matter (Tournier-Lasserve et al. 1991). It is interesting that these white matter abnormalities (WMAs) are also observed in "at risk," but yet asymptomatic, individuals. It represents an early stage of the disease, and MRI scanning is an absolute requirement to establish accurately the status of an individual for linkage analysis (Tournier-Lasserve et al. 1993). This condition is underlaid by a nonatherosclerotic, nonamyloid angiopathy affecting mainly the small arteries of the white matter and the basal ganglia (Baudrimont et al. 1993). Linkage analysis of this first large French pedigree allowed us to map the affected gene on chromosome 19 in a 14-cM interval between D19S221 and D19S222. These results were confirmed in a second unrelated French family (Tournier-Lasserve et al. 1993).

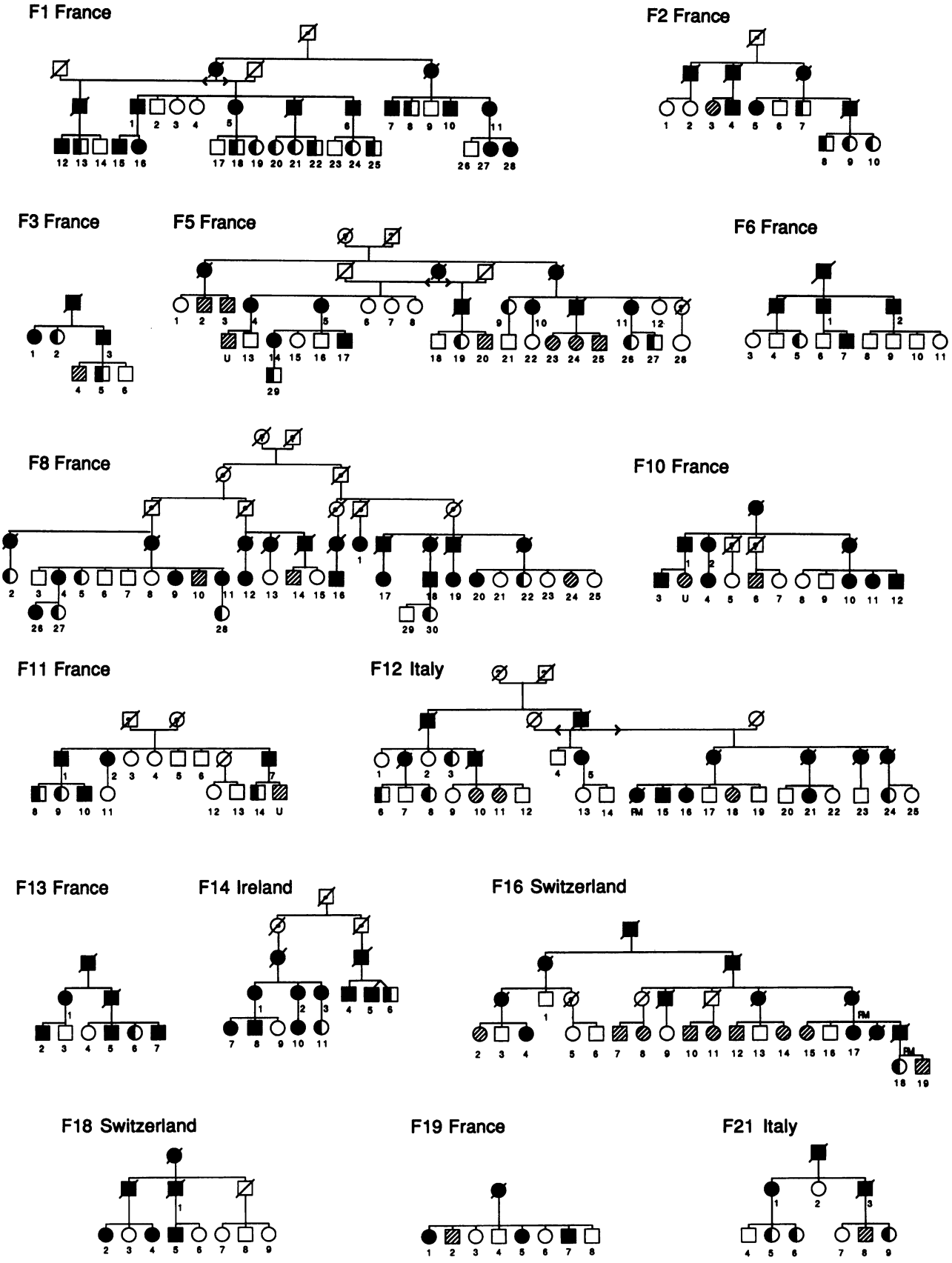

Figure 1 Pedigrees. Black-filled symbols represent clinically affected individuals; half-blackened symbols represent asymptomatic subjects with abnormal MRI; open symbols represent healthy subjects; cross-hatched symbols represent subjects of unknown status; symbols with a black central point represent ancestors who were most likely carriers of the affected gene; and question mark in the middle of an empty symbol represents individual for whom no history was available. The letter U represents individuals of unknown status that haplotype analysis contributed to the high-resolution genetic mapping of the D19S221-D19S215 interval. The origin of each pedigree is indicated.

Table 1

CADASIL Families

	No. of Sampled Individuals					
	Affected					
Family	Clinical	MRI Only	Healthy	Unknown Status	Spouses	Total
F1	11	9	8	0	10	38
F2	2	4	3	1	2	12
F3	2	2	0	2	2	8
F5	6	5	12	6	4	33
F6	3	1	7	0	3	14
F8	9	8	10	3	2	32
F10	7	0	4	1	1	13
F11	4	3	7	0	3	17
F12	4	4	14	3	4	29
F13	4	1	2	0	2	9
F14	8	2	1	0	2	13
F16	2	1	7	9	7	26
F18	4	0	3	2	2	11
F19	3	0	4	1	0	8
F21	2	3	3	1	1	10
Total	71	43	85	29	45	273

NOTE.—Detailed clinical descriptions are available for most of these 15 families: F1 (Tournier-Lasserve et al. 1991); F2 (Davous et al. 1991); F6, F8, F10, F11, and F13 (Chabriat et al. 1995); F12 (Ragno et al. 1995); F18 (Jung et al. 1995); and F21 (Gray et al. 1994).

Subsequently, the size of the mapping interval was reduced to 12 cM on 19p between D19S221 and D19S215 (Joutel et al. 1993).

Since 1991, an increasing number of families originating from different countries and sharing clinical and neuroimaging features strikingly similar to those previously described have been reported (Davous et al. 1991; Mas et al. 1992; Salvi et al. 1992; Gray et al. 1994; Gutiérrez-Molina et al. 1994; Jung et al. 1995; Ragno et al. 1995; Sabbadini et al. 1995; Chabriat et al. 1995). Fifty such families have been referred to our lab since 1993, which suggests that the prevalence of this disorder is much higher than anticipated. Detailed clinical analysis of these families showed that the "stroke phenotype" present in >80% of the patients was not the only clinical phenotype of CADASIL patients, since >30% of them also suffer from migraine with aura (as compared to 1%–6% in control populations), and 15% have mood disorders (Chabriat et al. 1995). Incidentally, this observation led us to suspect the implication of the CADASIL gene in the pathophysiology of migraine and to map a gene responsible for familial hemiplegic migraine (FHM) within the same region on chromosome 19 (Joutel et al. 1993). The presence of a cerebellar ataxia in ~20% of FHM families led us to suspect and establish the genetic mapping of another neurological autosomal dominant disease, hereditary paroxysmal cerebellar ataxia (HPCA), on chromosome 19 in close vicinity to the FHM and CADASIL loci (Vahedi et al. 1995).

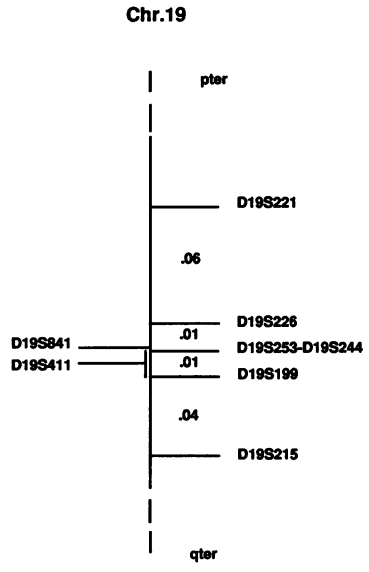

Figure 2 Schematic regional map of chromosome 19p. Published markers used for linkage analysis are indicated with their respective distances on the right (θ). Markers positioned by genetic analysis of the CADASIL pedigrees are on the left. According to our haplotyping results, D19S411, which was previously mapped at θ = 0 from D19S226 (Gyapay et al. 1994), is now positioned between D19S841 and D19S199. The position of D19S841, a new marker that we isolated from a chromosome 19 cosmid, is indicated according to multipoint and haplotyping results.

Table 2

Pairwise Linkage Data for CADASIL and Chromosome 19 Markers

Locus and Family	Z at θ = .00	.01	.05	.10	.20	.30	.40	Z_{max} Total	θ
D19S221:									
F1	−99.00	1.12	2.80	3.16	2.90	2.14	1.08		
F2	2.64	2.59	2.40	2.14	1.61	1.03	.44		
F3	−3.09	−1.10	−.44	−.19	.01	.07	.06		
F5	−99.00	1.99	3.00	3.09	2.61	1.75	.70		
F6	2.90	2.85	2.64	2.37	1.78	1.14	.45		
F8	−99.00	6.08	6.16	5.68	4.36	2.86	1.30		
F11	−99.00	.07	.63	.74	.64	.37	.10		
F12	3.14	3.15	3.11	2.93	2.33	1.53	.62		
F13	1.77	1.73	1.59	1.41	1.04	.63	.22		
F14	1.08	1.05	.90	.73	.44	.21	.06		
F16	3.18	3.12	2.88	2.56	1.91	1.24	.58		
F18	1.72	1.68	1.54	1.36	.98	.56	.18		
F19	...	...	...	...	...	...	...		
F21	1.80	1.77	1.69	1.44	1.04	.60	.19		
Total	−99.00	26.10	28.90	27.42	21.65	14.13	6.00	28.90	.05
D19S226:									
F1	−99.00	6.42	6.58	6.19	5.00	3.50	1.74		
F2	1.71	1.68	1.52	1.32	.92	.52	.16		
F3	−3.40	−1.40	−.72	−.44	−.19	−.08	−.02		
F5	−99.00	3.11	3.43	3.25	2.51	1.54	.51		
F6	2.77	2.72	2.52	2.26	1.70	1.09	.44		
F8	−99.00	4.25	4.46	4.14	3.18	2.07	.93		
F10	2.33	2.28	2.09	1.85	1.33	.78	.25		
F11	−99.00	−.19	.40	.55	.50	.29	.08		
F12	3.88	4.42	4.63	4.38	3.55	2.48	1.22		
F13	...	...	...	...	...	...	...		
F14	3.93	3.86	3.56	3.17	2.36	1.50	.64		
F16	2.05	2.01	1.85	1.65	1.21	.74	.28		
F18	−.07	−.06	−.56	−.04	−.02	−.01	−.00		
F19	1.51	1.48	1.35	1.18	.83	.47	.14		
F21	.55	.54	.50	.43	.30	.16	.04		
Total	−99.00	31.12	32.11	29.89	23.18	15.05	6.41	32.24	.03
D19S841:									
F1	7.32	7.12	6.71	6.07	4.71	3.20	1.53		
F2	2.75	2.70	2.50	2.24	1.67	1.07	.43		
F3	.66	.65	.59	.52	.37	.23	.10		
F5	−99.00	2.27	2.71	2.65	2.14	1.39	.52		
F6	2.73	2.69	2.49	2.23	1.68	1.07	.43		
F8	3.72	3.64	3.39	3.10	2.38	1.54	.67		
F10	2.66	2.61	2.41	2.15	1.58	.97	.35		
F11	2.91	2.86	2.65	2.37	1.76	1.08	.36		
F12	2.96	2.95	2.82	2.59	1.97	1.22	.42		
F13	1.39	1.36	1.24	1.10	0.79	.46	.15		
F14	2.39	2.33	2.09	1.79	1.19	.64	.20		
F16	2.54	2.49	2.27	1.98	1.40	.85	.33		
F18	1.78	1.74	1.60	1.42	1.01	.58	.18		
F19	−.01	−.01	−.01	−.01	−.01	.00	.00		
F21	1.81	1.77	1.63	1.44	1.04	0.60	0.19		
Total	−99.00	37.24	35.08	31.61	23.69	14.89	5.88	37.24	.01
D19S253:									
F1	5.37	5.82	4.91	4.34	3.41	2.29	1.06		
F2	.56	.55	.52	.49	.26	.10	.01		
F3	.70	.68	.62	.53	.39	.24	.10		
F5	2.36	2.33	2.18	1.94	1.55	1.07	.56		
F6	.35	.34	.29	.24	.15	.07	.02		

(*continued*)

Table 2 (continued)

Locus and Family	Z at θ = .00	.01	.05	.10	.20	.30	.40	Z_{max} Total	θ
F8	5.35	5.24	4.80	4.24	3.07	1.85	.66		
F10	.69	.67	.60	.48	.32	.16	.04		
F11	1.81	1.77	1.63	1.40	1.03	.58	.16		
F12	−99.00	.34	1.50	1.80	1.66	1.22	.68		
F13	...	...	...	...	...	...	...		
F14	1.22	1.19	1.08	0.91	.64	.35	.10		
F16	...	...	...	...	...	...	...		
F18	...	...	...	...	...	...	...		
F19	1.20	1.18	1.09	.95	.72	.44	.15		
F21	1.20	1.19	1.07	.90	.63	.33	.08		
Total	−99.00	19.37	19.58	18.07	13.66	8.63	3.20	19.79	.03
D19S244:									
F1	−99.00	1.10	1.62	1.68	1.47	1.09	.58		
F2	1.34	1.35	1.34	1.27	1.00	.65	.29		
F3	.70	.68	.62	.55	.39	.24	.10		
F5	−99.00	.97	1.42	1.42	1.14	.78	.37		
F6	.35	.34	.29	.25	.15	.08	.02		
F8	6.16	6.05	5.59	5.00	3.75	2.46	1.16		
F10	.51	.50	.45	.39	.26	.13	.04		
F11	.36	.35	.28	.23	.16	.11	.03		
F12	−99.00	.56	1.71	1.97	1.83	1.35	.66		
F13	1.80	1.76	1.62	1.44	1.05	.64	.23		
F14	3.07	3.01	2.75	2.41	1.72	1.02	.38		
F16	1.51	1.47	1.34	1.18	.90	.62	.31		
F18	.85	.84	0.80	.74	.56	.33	.11		
F19	−.01	−.01	−.01	−.01	−.01	−.00	−.00		
F21	1.51	1.47	1.35	1.18	.83	.46	.13		
Total	−99.00	20.46	21.18	19.69	15.22	9.95	4.66	21.43	.04
D19S411:									
F1	3.44	3.37	3.10	2.75	2.00	1.22	.48		
F2	.38	.37	.33	.29	.19	.10	.03		
F3	.70	.68	.62	.55	.39	.24	.10		
F5	−99.00	.06	.65	.79	.76	.57	.29		
F6	2.25	2.20	2.02	1.79	1.30	.80	.30		
F8	2.46	2.43	2.26	2.00	1.39	.78	.56		
F10	.81	.79	.71	.61	.41	.21	.06		
F11	1.72	1.69	1.57	1.42	1.05	.61	.18		
F12	3.17	3.15	3.03	2.81	2.24	1.50	.68		
F13	...	...	...	...	...	...	...		
F14	1.35	1.32	1.20	1.05	.73	.39	.11		
F16	...	...	...	...	...	...	...		
F18	...	...	...	...	...	...	...		
F19	1.20	1.18	1.07	.94	.65	.36	.11		
F21	.90	.88	.62	.55	.39	.24	.10		
Total	−99.00	18.13	17.18	15.56	11.50	7.02	2.67	18.13	.01
D19S199:									
F1	−99.00	4.80	5.04	4.75	3.78	2.55	1.14		
F2	2.62	2.57	2.38	2.13	1.59	1.02	.44		
F3	.39	.38	.34	.29	.18	.09	.02		
F5	−99.00	1.83	2.28	2.24	1.81	1.16	.40		
F6	.47	.46	.41	.35	.23	.12	.03		
F8	7.58	7.43	6.84	6.08	4.51	2.89	1.30		
F10	2.10	2.05	1.85	1.59	1.04	.49	.12		
F11	−99.00	1.16	1.64	1.66	1.36	.86	.29		
F12	−99.00	−2.24	−.85	−.33	.03	.10	.07		
F13	1.79	1.76	1.62	1.43	1.05	.64	.23		
F14	2.54	2.48	2.24	1.93	1.33	.75	.25		

(*continued*)

Table 2 (continued)

Locus and Family	Z at θ = .00	.01	.05	.10	.20	.30	.40	Z_{max} Total	θ
F16	2.52	2.45	2.20	1.87	1.19	.49	−.03		
F18	1.13	1.10	1.01	.89	.62	.33	.09		
F19	−.33	−.11	.22	.34	.33	.21	.07		
F21	1.51	1.47	1.35	1.18	.19	.09	.02		
Total	−99.00	27.59	28.57	26.40	19.24	11.79	4.44	28.57	.05
D19S215:									
F1	−99.00	.24	2.57	3.17	3.07	2.32	1.20		
F2	−99.00	−1.44	−.20	.19	.36	.24	.07		
F3	...	...	...	...	...	...	...		
F5	−99.00	−2.97	−.47	.47	.97	.80	.35		
F6	2.36	2.31	2.14	1.90	1.41	.87	.32		
F8	1.25	1.55	1.81	1.74	1.33	.81	.27		
F11	−99.00	−1.63	−.37	.04	.25	.19	.06		
F12	−99.00	−.56	1.81	2.46	2.48	1.88	.96		
F13	−99.00	−.53	.07	.23	.25	.16	.05		
F14	−99.00	−1.66	−.45	−.08	.09	.07	.02		
F16	−4.64	−2.28	−1.26	−.69	−.18	.02	.05		
F18	.47	.45	.40	.33	.19	.08	.02		
F19	...	...	...	...	...	...	...		
F21	...	...	...	...	...	...	...		
Total	−99.00	−6.51	6.02	9.76	10.22	7.44	3.38	10.45	.16

Herein, the analysis of 13 additional CADASIL families allowed us to demonstrate the genetic homogeneity of this condition. The responsible gene is now mapped within a 2-cM interval bracketed by D19S226 and D19S199.

Subjects and Methods

Families and Status Definition

A total of 15 unrelated families originating from five different European countries were genotyped (fig. 1). Two of them, F1 and F2, have already been genotyped with several chromosome 19 markers and were further investigated with new markers (Tournier-Lasserve et al. 1993). Thirteen additional families were analyzed. They have all been selected on the basis of the following criteria: (1) a history of recurrent strokes without any vascular risk factors for the propositus and at least one relative, (2) a leukoaraiosis with or without small, deep infarcts on brain MRI in all clinically affected patients, and (3) a pattern of inheritance consistent with an autosomal dominant mode. Fifty such families were referred; 13 included at least six potentially informative meiosis; they were therefore considered as potentially informative and genotyped. Two hundred twenty-eight subjects (>18 years of age) from these 15 families gave their informed consent for this study. They were examined by a board-certified neurologist, underwent cerebral MRI, and were blood drawn. As described elsewhere, MRI was used to establish the status for genetic linkage analysis (Tournier-Lasserve et al. 1993). In brief, individuals with an abnormal MRI were considered as affected, whether or not clinically symptomatic; asymptomatic individuals with a normal MRI were considered as healthy when ≥35 years of age and as having an unknown status when <35 years of age (table 1). One hundred fourteen individuals had an abnormal MRI; among them, 71 members experienced neurological symptoms, and 43 were totally asymptomatic. Neurological symptoms included transient ischemic attacks or completed strokes (51 patients), migraine with aura (26 patients, including 15 patients suffering from both strokes and migraine with aura and 11 patients suffering only from migraine with aura), dementia (22 patients with preceding strokes and 6 patients with an isolated progressive dementia), and mood disorders (8 patients with associated strokes and 3 patients with isolated mood disorders). One hundred fourteen individuals had an MRI showing neither WMA nor subcortical infarcts: 85 of them ≥35 years of age were classified as healthy, 29 of them were <35 years of age and classified as having an unknown status. Among the 85 patients classified as healthy, 2 individuals had an MRI showing sequelae from large vessel cerebral infarcts due to atherosclerosis, and 2 patients suffered from migraine with aura.

Pathological data were available in six of these families (F1-11, F10-PM, F12-PM, F16-PM, F18-1, and F21-3) and showed in all cases a nonatherosclerotic, nonamyloid angiopathy affecting the small arteries of the cerebral white matter and the basal ganglia strikingly similar to previously reported lesions (Sourander and Walinder 1977; Baudrimont et al. 1993; Gray et al. 1994; Jung et al. 1995). Karyotype analysis performed in one index case from eight families (F1, F2, F5, F8, F10, F11, F13, and F14) was unremarkable.

Markers

All 15 families were analyzed with eight polymorphic microsatellite markers chosen from the Généthon linkage map (D19S221, D19S226, D19S411, and D19S215) (Gyapay et al. 1994) and other published maps (D19S253, D19S244, and D19S199) (Hudson et al. 1992; Weber and May 1989). The last one, D19S841, was recently isolated in our group from a cosmid previously mapped by FISH to 19p13.1 (De Jong et al. 1989; Human Genome Center, Lawrence Livermore National Laboratory, URL http://www.bio.llnl.gov/bbrp/genome/chrom_map.html). D19S841 is a $(CA)_{24}$ repeat contained within cosmid 12909 (Ducros 1995; Genome Data Base [GDB] identification G00-593-305). All oligonucleotide sequences are avaible through the GDB (John Hopkins University, Baltimore). D19S253 and D19S244 are $(GATA)_n$ repeats, and the others are $(CA)_n$ repeats. A genetic partial map of chromosome 19 is presented in figure 2.

Genotyping and Linkage Analysis

We extracted DNA from peripheral blood from all consenting members including 199 potentially informative meiosis, 45 spouses, and 29 subjects of unknown status (Miller et al. 1988). For subjects F18-1 and F21-3, DNA was extracted from autopsy material. Polymorphic genomic sequences were amplified by PCR as described elsewhere (Tournier-Lasserve et al. 1993). Linkage analysis was performed using version 5.1 of the LINKAGE program package (Lathrop et al. 1984) using published alleles frequencies from CEPH pedigrees. The frequency of the D19S841 alleles was determined by genotyping 70 unrelated subjects, including 28 CEPH families founders (Ducros 1995; GDB polymorphism identification G00-593-357). CADASIL gene frequency was set at .0001. Lod scores obtained with markers D19S221 and D19S226 for families F1 and F2 have been reported elsewhere (Tournier-Lasserve et al. 1993) and were incorporated here in the combined lod score calculation. Haplotype studies were performed on all families, and the most likely haplotypes were inferred by minimizing the number of crossover events in each sibship.

To facilitate the multilocus linkage analysis, genotypes were processed to produce a maximum of five alleles at each marker locus while preserving linkage information. The most likely position of D19S841 with respect to the others markers was established within a subset of informative CADASIL families by using the LINKMAP program. Then, the same program was run with all pedigrees to establish the best estimate of the CADASIL gene location. Because of computational limitations, two subintervals were analyzed successively. Homogeneity was examined using the admixture test from the HOMOG program package (Ott 1991).

Results

Genetic Homogeneity

Two-point linkage analysis data are shown in table 2. Significant lod scores (>3) were obtained in families F1, F5, F8, F12, F14, and F16 with several markers, and positive lod scores were obtained in all other families. A maximum combined lod score of 37.24 was reached with marker D19S841 at $\theta = .01$. Admixture analysis of the two-point data with the HOMOG package showed evidence for linkage with homogeneity for all eight markers, with a significance level (P) of the χ^2 test $<.0001$ for all of them. Admixture analysis of the multipoint data also supported a strong evidence for genetic homogeneity ($P < .0001$) and estimated the proportion of linked families to be 1.0 (95% confidence interval .81–1.00).

Refining of the CADASIL Interval

High-resolution genetic map of the D19S221-D19S215 interval.—The previous order of the polymorphic markers within the D19S221-D19S215 interval was the following: tel–D19S221–(D19S226/D19S411)–(D19S253/D19S244)–D19S199–D19S215–cen. The analysis of inherited haplotypes, both high-and low-risk haplotypes inherited from the affected parent and haplotypes inherited from the unaffected parent, revealed six crossover events, which led us to refine the position of marker D19S411 as well as to map marker D19S841 precisely (fig. 2). These six crossover events, shown in figure 3, strongly suggest that the most likely order of the markers is the following: tel–D19S221–D19S226 – D19S841 – (D19S253/D19S244/D19S411)–D19S199–cen. The multipoint linkage analysis conducted in order to estimate the most likely position of the new marker D19S841 throughout the fixed map tel–D19S226–(.01)–D19S253–(.01)–D19S199–cen showed the following maximum-likelihood order: tel–D19S226–(.009)–D19S841–(.001)–D19S253–(.01)–D19S199–cen, odds against all alternatives being at least 10,000:1.

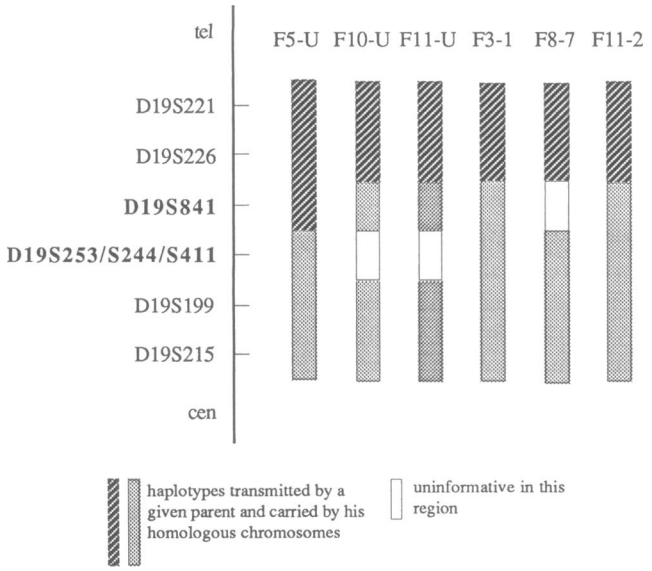

Figure 3 Crossover events observed within the D19S221-D19S215 interval. Haplotype analysis of the 15 families revealed six crossover events that led to refine the position of marker D19S411, previously mapped at $\theta = 0$ from D19S226, and to precisely locate the new marker D19S841. A schematic diagram of the relevant region on 19p is shown on the left (not to scale). Because no recombination event was observed between markers D19S253, D19S244, and D19S411, they are represented as a single locus. For each individual in whom haplotype analysis revealed a crossover event, the involved chromosome is represented by a bar. Above the bar, the family and the position in the pedigree of this individual are indicated. Three crossover events were observed in individuals of unknown status (F5-U, F10-U, and F11-U) on the chromosome inherited from their unaffected parent. These three subjects are indicated in fig. 1 by a letter U.

Genetic mapping of the CADASIL locus between D19S226 and D19S199.—Haplotype analysis in recombinant individuals established that the CADASIL locus is flanked proximally by marker D19S199 (three recombination events: F1-24, F11-5, and F12-17) and distally by marker D19S226 (three recombination events: F3-1, F8-7, and F11-2). These recombination events were observed in three affected individuals (two clinically affected individuals having an abnormal MRI [F3-1 and F11-2] and one asymptomatic individual with an obviously abnormal MRI [F1-24]) and three healthy subjects with a normal MRI, aged >40 years (F8-7, F11-5, and F12-17) (fig. 4).

Two clinically asymptomatic subjects, F1-28 (27 years old) and F5-19 (40 years old), diagnosed as affected on the basis of the MRI, have inherited the low-risk haplotype from their affected parent. F1-28 is uninformative at three markers from the interval (D19S841, D19S253, and D19S411), and F5-19 is uninformative for D19S253. These two individuals could be double recombinants but most likely are in fact misclassified, since their MRI showed only one small hypersignal, which may be due to another cause. Another asymptomatic subject, F12-25, classified as unaffected, had inherited the high-risk haplotype from her affected mother; she is uninformative at D19S841 and D19S411. This individual, age 42 years, may either be a double recombinant or represent a rare case of late onset of this disease. Except for these three individuals, no recombinant event was observed between D19S841, D19S253, D19S244, D19S411, and the CADASIL locus.

Multipoint analysis was used to best estimate the position of the CADASIL gene within two intervals: tel–D19S221–(.06)–D19S226–(.02)–D19S199–cen and tel–D19S226–(.009)–D19S841–(.011)–D19S199–cen. As shown in figure 5, the best estimate of the CADASIL gene location is between D19S226 and D19S199. The odds against the placement beyond D19S226 and D19S199 are, respectively, 209:1 and 3,715:1.

Discussion

We previously mapped the CADASIL locus in a 12-cM interval on chromosome 19 by a linkage analysis conducted on two pedigrees. Herein, we report genetic linkage data obtained in 13 additional families. These data strongly suggest the genetic homogeneity of this condition and allow us to refine the mapping of the affected locus to a 2-cM interval bracketed by D19S226 and D19S199.

Recently, strong linkage to 19p was also reported in an Italian CADASIL pedigree (Sabbadini et al. 1995). By contrast, negative linkage results were reported on a Scottish pedigree affected by hereditary multi-infarct dementia, and the authors suggested that hereditary multi-infarct dementia, another name used to designate CADASIL, may be genetically heterogeneous (St. Clair

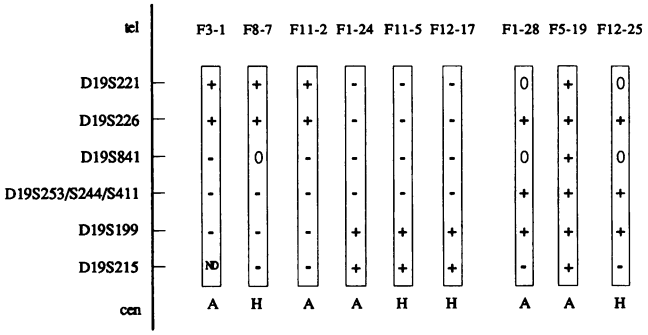

Figure 4 Recombination events leading to the precise mapping of the CADASIL locus. For each individual in whom haplotype analysis revealed a recombination event, the chromosome inherited from the affected parent is represented by a bar. The code above each bar indicates the family and the position in the pedigree (F3-1: individual 1 from family 3). A schematic map of 19p (not to scale) is shown on the left. Because no crossover event occurred between markers D19S253, D19S244, and D19S411, the latter are represented as a single locus. A = affected; H = healthy; plus sign (+) = recombinant; minus sign (−) = nonrecombinant; 0 = uninformative; and ND = not done.

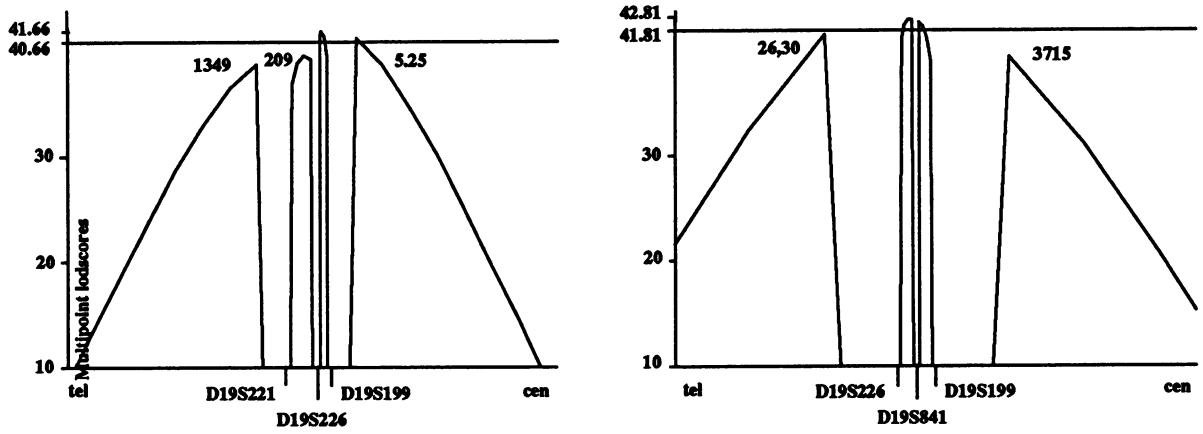

Figure 5 Multipoint analysis. Multipoint lod scores for different positions of the CADASIL locus are shown with respect to two subsets of three markers, on the left panel: D19221–(.06)–D19S226–(.02)–D19S199; on the right panel: D19S226–(.009)–D19S841–(.011)–D19S199. The solid lines indicate the 10:1 odds (1 – lod unit) interval for the placement of the gene. Odds against alternatives are shown for the most likely placement of the CADASIL locus in each interval.

et al. 1995). Unfortunately, in the latter study, the status data used for genetic linkage analysis have not been established on the basis of MRI for many family members, leading to a high risk of misclassification and therefore false recombinants. Until this major requirement is fulfilled, it will be difficult to draw any firm conclusion in favor or against linkage to the CADASIL locus in this family.

These data establish the value of combined clinical and neuroimaging diagnosis criteria for CADASIL. The follow-up of subjects carrying the high-risk haplotype already helped to precise all clinical features as well as the natural clinical and neuroimaging history of this disorder in homogenous families (Chabriat et al. 1995). This is of particular importance because of the recent identification of this condition. In brief, most patients >40 years of age suffer of recurrent strokes (84%) leading, within 10–20 years, to a subcortical dementia. Migraine is also a frequent symptom of this disorder (23%) and often precedes the occurrence of strokes by several years. In some patients, the previous symptoms may be associated with mood disorders (21%). A constant feature is the presence of a leukoencephalopathy on cerebral MRI, an abnormality that is most likely the first to be detectable. The diagnostic marker provided by the indirect genotypic diagnosis will also allow the selection of homogenous groups of patients for therapeutic trials. Indirect genetic testing is now possible in families and may be used as a presymptomatic diagnosis test, with respect of all ethical rules in this severe condition.

It is interesting that two other autosomal dominant diseases, FHM and HPCA, have been mapped recently on 19p in close vicinity to CADASIL, raising the question of the allelism of these three disorders (Joutel et al. 1993; Kramer et al. 1995; Tean Teh et al. 1995; Vahedi et al 1995, Von Brederlow et al. 1995). The data reported herein strongly suggest that CADASIL and HPCA are not allelic disorders, since the HPCA gene resides distal to D19S226, whereas the CADASIL locus is centromeric to this marker. With regard to FHM, it is well established that 50% of the FHM families map on 19p (Joutel et al. 1994; Ophoff et al. 1994). The most likely interval is bracketed by D19S413 and D19S199 overlapping, therefore, the CADASIL interval reported here (Joutel et al. 1994). However Ophoff et al. (1994) reported an affected recombinant at D19S226 that favored the location of the FHM gene distal to this marker. We suggest that the analysis of additional families is required before any firm conclusion can be drawn for FHM.

In addition, these data will facilitate the genetic screening of other familial cerebral arteriopathies sharing clinical and neuroimaging features with CADASIL and suspected to be due to the alteration of the same gene, such as cerebrovascular disease with thin skin, alopecia, and disk disease, an autosomal recessive disorder initially described by Yamamura et al. (1987) or retinal cerebral arteriopathy, an autosomal dominant vasculopathy for which linkage analysis with 19p markers is under way (Jen et al. 1995).

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