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Genomewide-Linkage and Haplotype-Association Studies Map Intracranial Aneurysm to Chromosome 7q11

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Rupture of intracranial aneurysms (IAs) causes subarachnoid hemorrhage, a devastating condition with high morbidity and mortality. Angiographic and autopsy studies show that IA is a common disorder, with a prevalence of 3%–6%. Although IA has a substantial genetic component, little attention has been given to the genetic determinants. We report here a genomewide linkage study of IA in 104 Japanese affected sib pairs in which positive evidence of linkage on chromosomes 5q22-31 (maximum LOD score [MLS] 2.24), 7q11 (MLS 3.22), and 14q22 (MLS 2.31) were found. The best evidence of linkage is detected at *D7S2472,* in the vicinity of the elastin gene (*ELN*), a candidate gene for IA. Fourteen distinct single-nucleotide polymorphisms (SNPs) were identified in *ELN,* and no obvious allelic association between IA and each SNP was observed. The haplotype between the intron-20/intron-23 polymorphism of *ELN* is strongly associated with IA ($P = 3.81 \times 10^{-6}$), and homozygous patients are at high risk ($P = .002$), with an odds ratio of 4.39. These findings suggest that a genetic locus for IA lies within or close to the *ELN* locus on chromosome 7.

Introduction

Large autopsy studies reveal that intracranial aneurysms (IAs [MIM 105800]) have a prevalence of 4.6% (Iwamoto et al. 1999), and angiographic studies indicate the prevalence of unruptured incidental IA among adults to be 2.7%–6.5% (Ujiie et al. 1993; Nakagawa and Hashi 1994). Rupture of an IA causes sudden subarachnoid hemorrhage (SAH), with high morbidity and mortality. For all ages, the annual incidence of SAH due to aneurysmal rupture is 18–23/100,000 (Inagawa et al. 1988, 1995); for individuals ⩾40 years old, it is 96/100,000 (Kiyohara et al. 1989). For patients with SAH, 8%–12% die before receiving medical attention (Phillips et al. 1980; Inagawa et al. 1995; Schievink et al. 1995*b*), 40%–60% die ⩽1 mo after onset of the disease (Sacco et al. 1984; Kiyohara et al. 1989; Inagawa et al. 1995), and more than a third of those who survive show major neurological deficits (Longstreth et al. 1993; Inagawa et al. 1995). Despite the improvements in medical and surgical care and in diagnostic methods during the past decades, aneurysmal SAH is still a major public health problem.

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Although genetic and environmental factors play equally important roles in the etiology of IA, recent progress in molecular genetics enables us to approach the genetic determinants directly. The risk of ruptured IA in first-degree relatives of patients with aneurysmal SAH is four times higher, and the relative risk in siblings is six times higher, than that in the general population (Schievink et al. 1995*a;* Ronkainen et al. 1997). A small fraction of IA is associated with heritable connective-tissue diseases such as polycystic kidney disease, Ehlers-Danlos syndrome type IV, and Marfan syndrome (Schievink 1997). Segregation analysis has been unable to define the inheritance pattern of IA (Schievink et al. 1994), possibly because of the complex etiology of the disease. We performed a genetic linkage study with Japanese nuclear families, to identify susceptible loci underlying IA, especially ruptured IA. Because IA has late onset and low penetrance, an occult phenotype may exist in a family, and complicated etiologies frequently are involved, so only affected sib pairs (ASPs) were used for the nonparametric linkage study. Difficulties in collection of ASPs were expected, because of the high mortality in ruptured IA. At all 1,100 hospitals in Japan that have been certified as training hospitals by the Japan Neurosurgical Society, we inquired regarding ASPs with IA. We were able to enroll 104 ASPs, comprising mainly patients with ruptured IA, at 94 of these hospitals. The subjects were examined in a genomewide linkage study. The best evidence of linkage was obtained on chromosome 7, near marker *D7S2472,* and the elastin gene (*ELN*), encoding a major

Table 1

Samples Used for Affected Sib Pair Linkage Analysis

Family Structure	No. of Families: SAH Positive	No. of Families: SAH Negative	No. of Families: Total	No. of Individuals	No. of Sib Pairs
Pairs	68	9	77	154	77
Trios	4	3	7	21	21
Quartets	1	0	1	4	6
Total	73	12	85	179	104

component of the blood-vessel wall, was found to lie very close to the marker. Since *ELN* is both a positional and functional candidate gene for IA, it was analyzed for allelic association, haplotype association, and linkage disequilibrium (LD).

Subjects and Methods

Subjects

Current samples comprise 85 nuclear families collected through neurosurgical services certified by the Japan Neurosurgical Society, and the number of possible ASPs was 104. The Ethical Committee of the Tokyo Women's Medical University approved the study, and all the participants (or their family members) gave written, informed consent. The families included at least two affected siblings, each of whom had an IA >5 mm, as ascertained by conventional angiography, three-dimensional computed tomography (CT) angiography, magnetic resonance (MR) angiography, or surgical findings. This collected sample comprised 179 individuals—51 males and 128 females. ASPs comprised 77 pairs, 7 trios, and 1 quartet of siblings with IA. In the 77 pairs, SAH (i.e., ruptured IA) was present in both siblings in 41 pairs, in one sibling in 27 pairs, and in neither sibling in 9 pairs; in the 7 trios, SAH was present in all three siblings in 3 trios, one sibling in 1 trio, and in none of the siblings in 3 trios; in the quartet, SAH was present in only one individual. Of the 85 families, 73 had at least one member with SAH (table 1). The detailed clinical features of these families have been reported elsewhere (Kasuya et al. 2000).

For the allelic-ssociation study, 172 patients with IA (70 men and 102 women; mean [SD] age 59.8 [10.5] years) and 192 controls (91 men and 101 women; mean [SD] age 59.0 [16.5] years) were enrolled. All subjects were of Japanese ethnicity. The 172 patients with IA include 78 probands in nuclear families, 9 patients with first-degree relatives with IA, and 85 patients without known family history of IA. The 192 controls were outpatients of Tokyo Women's Medical University Hospital who presented with headache and other neurological complaints. Selected controls had no history of SAH and were of ages similar to those of the patients with IA and, on conventional CT examination, showed no evidence of IA.

Genotyping

PCR amplifications were performed on the basis of standard protocols. Genotyping was performed, by a fluorescence-based semiautomated technique, on a DNA Sequencer model 377 (Applied Biosystems), with Linkage Mapping Set version 2 (Applied Biosystems). The marker alleles were assigned by GENESCAN and GENOTYPER software (Applied Biosystems). Heterozygosity of each microsatellite marker was determined on the basis of 64 unrelated healthy Japanese from various regions in Japan. Of the 400 markers in the set, 43 were not informative; the other 357, which, in 64 unrelated Japanese healthy subjects, had heterozygosities >60%, were analyzed. A set of 47 markers obtained from online information was added to the original set, to fill in gaps >20 cM (primer sequences of these additional markers are available on request). The average heterozygosity of the total of 404 markers was .756 in Japanese. The average interval between markers was 8.7 cM, and two gaps were >20 cM (maximum 26.8 cM).

Linkage Analysis

Because the mode of inheritance of IA is not known, we applied two different nonparametric linkage methods—the SIBPAL program from the S.A.G.E. package (version 3.1) (Elston et al. 1997) and the GENEHUNTER program (version 1.2) (Kruglyak et al. 1996). The SIBPAL program estimated the mean ratio (π) of alleles shared identical by descent (IBD) among ASPs, at each microsatellite marker. The π obtained was tested against the null hypothesis of no linkage ($\pi = .5$). The test statistic has a standard normal distribution under the null hypothesis, and, because the alternative hypothesis of linkage is given when IBD sharing is >50%, the test is one sided. Accordingly, accurate π values can be obtained by use of a one sided π-test as implemented in the SIBPAL program. Multipoint linkage analysis was performed by a maximum-likelihood method implemented in the GENEHUNTER program. Maximum LOD score (MLS) was calculated by the method of possible triangle constraints (Kruglyak et al. 1996). All sib pairs from sibships containing more than two affected individuals were counted, and the unweighted option was used.

Physical Map of the ELN *Locus*

A physical map of the *ELN* locus was constructed on the basis of both the GenBank database (accession numbers AC005089, AC005056, U93037, U63721, U62292, U62293, AC005057, AF045555, AC005081,

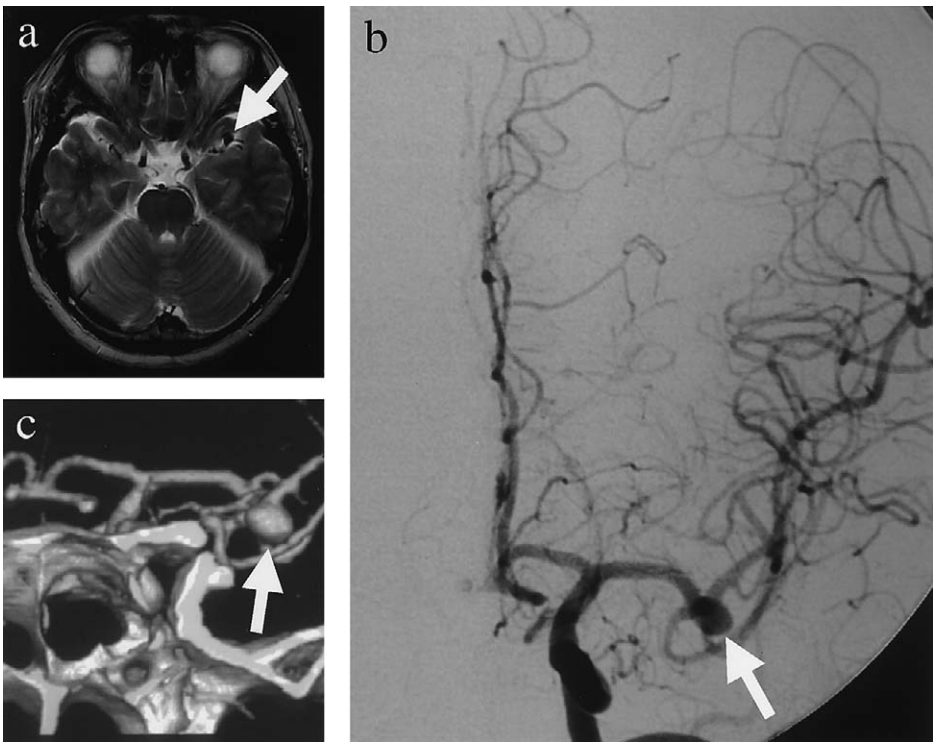

Figure 1 Radiographic examinations for IA. A 51-year-old male patient with a complaint of vertigo received MR imaging, which showed a flow void, an indication of aneurysm, in the left middle cerebral artery (*a, arrow*). Cerebral angiography (*b, arrow*) and three-dimensional CT angiography (*c, arrow*) further confirmed IA.

and AC005015.2) and the physical map of the microdeletion in Williams-Beuren syndrome (WBS) (Peoples et al. 2000). Eight polymorphic dinucleotide or tetranucleotide repeats in the vicinity were discovered, and linkage and allelic association studies were performed.

Single-Nucleotide Polymorphisms (SNPs) of ELN

A total of 16 IA probands and 8 controls were screened to identify SNPs of *ELN*. Direct sequencing was performed on PCR-amplified segments spanning all 34 exons, acceptor, donor, and branch-point sequences in the introns, 1.0 kb of putative promoter sequence, and 1.2 kb of the 3′UTR sequence. A total of 42 primer sets were designed on the basis of the human *ELN* cDNA and genomic sequences (accession numbers M36860 and AC005056, respectively), obtained from the GenBank database. Primer sequences and PCR conditions are available from the authors on request.

Allelic Association, Haplotype Analysis, and LD

Allelic association with IA was evaluated by χ^2 test statistic, for each SNP; the odds ratio and 95% confidence interval (95%CI) also were calculated for each SNP. Because the gametic phase was unknown, the haplotype frequencies were calculated from two-locus genotype data by the maximum-likelihood estimates, by use of the ARLEQUIN program. The haplotype frequencies of ELN were compared in patients with IA versus controls and were evaluated by the contingency table of χ^2 test statistics. The extent of pairwise LD (D) was evaluated as $D = p_{11}p_{22} - p_{12}p_{21}$, where p_{11}, p_{22}, p_{12}, and p_{21} are the frequencies of haplotypes of A_1B_1, A_2B_2, A_1B_2, and A_2B_1, respectively, at loci A and B. p_1, p_2, q_1, and q_2 are the frequencies of alleles A_1, A_2, B_1, and B_2, respectively and two LD measures are applied: first, coefficient D' is given by D/D_{max}, where either D_{max} is the smaller of p_1q_2 and p_2q_1 when $D > 0$ or D_{max} is the smaller of p_1q_1 and p_2q_2 when $D < 0$ (Lewontin 1964); second, r^2 is given by $D^2/(p_1p_2q_1q_2)$ (Hill and Robertson 1968).

Results

Radiographic Examinations of IA

Modern diagnostic techniques allow the detection of many potentially dangerous conditions before symptoms occur. Most patients with IA are asymptomatic, however, until sudden rupture and life-threatening SAH. IA could be diagnosed by various radiographic methods, such as cerebral angiography, three-dimensional CT angiography, and MR angiography. Figure 1 shows MR

Table 2

Single-Point Linkage Analysis by SIBPAL

Chromosome and Marker	Distance (cM)	Heterozygosity	No. of Pairs	IBD	*t*	*P*
1:						
D1S468	6.2	.71	83	.52	.73	.234
D1S214	16.4	.72	83	.51	.47	.320
D1S450	22.9	.85	81	.49	−.41	.659
D1S2667	26.9	.82	82	.47	−.9	.815
D1S507	36.2	.83	80	.44	−2.07	.979
D1S199	47.7	.77	83	.48	−.6	.726
D1S234	56.6	.81	81	.49	−.35	.638
D1S496	65.6	.87	70	.48	−.54	.706
D1S2797	77.6	.78	83	.55	1.81	.037*
D1S2700	89.3	.83	82	.52	.51	.304
D1S230	97.4	.61	83	.49	−.3	.619
D1S2841	108.8	.83	83	.50	.11	.456
D1S207	117.6	.83	81	.49	−.31	.622
D1S2868	129.9	.61	80	.55	2.05	.022*
D1S206	137.6	.78	83	.45	−1.88	.968
D1S2726	149	.72	83	.49	−.28	.612
D1S252	155.1	.80	82	.50	.01	.496
D1S498	160.7	.73	82	.51	.21	.418
D1S484	173.9	.68	82	.47	−1.08	.859
D1S2878	181.7	.84	83	.47	−.84	.798
D1S196	186.4	.72	83	.53	1.22	.114
D1S218	196.5	.82	83	.50	−.13	.553
D1S238	206.7	.81	82	.52	.6	.274
D1S413	216.5	.63	82	.50	.12	.453
D1S249	225.1	.67	83	.52	.54	.296
D1S425	235.3	.61	82	.51	.49	.314
D1S213	246.2	.85	81	.50	.04	.483
D1S2800	256.1	.71	83	.50	.12	.452
D1S2785	269.7	.85	82	.50	−.11	.542
D1S2842	277.3	.72	81	.47	−.93	.822
D1S2836	290.1	.68	83	.44	−1.98	.975
2:						
D2S319	6	.72	82	.47	−1.00	.839
D2S2211	14	.64	83	.51	.33	.371
D2S162	21.3	.79	83	.48	−.69	.755
D2S168	28.6	.80	82	.49	−.43	.664
D2S305	40.7	.77	81	.51	.37	.358
D2S165	50.7	.86	81	.46	−1.02	.845
D2S367	58.3	.87	83	.50	−.15	.558
D2S2259	67.4	.62	83	.49	−.25	.598
D2S391	73.8	.71	82	.48	−.59	.720
D2S337	84.1	.84	82	.43	−2.24	.986
D2S2368	89.2	.83	83	.44	−2.22	.986
D2S286	98.4	.74	81	.47	−.86	.803
D2S2333	107.7	.84	83	.52	.77	.223
D2S2216	115.3	.72	82	.48	−.84	.799
D2S160	127.4	.71	83	.54	1.73	.044*
D2S347	135.7	.61	81	.45	−1.8	.962
D2S112	145.8	.60	83	.47	−1.18	.880
D2S151	156.4	.77	82	.44	−2.29	.988
D2S142	166.3	.73	82	.44	−2.08	.980
D2S2330	175.5	.84	83	.49	−.20	.580
D2S335	182.5	.84	83	.49	−.30	.617
D2S364	192.9	.78	82	.45	−1.66	.949
D2S117	201.4	.88	83	.47	−.79	.784
D2S325	210.9	.79	82	.45	−1.67	.951
D2S164	222	.65	80	.45	−1.85	.966
D2S126	228.8	.80	82	.48	−.60	.725
D2S396	240.2	.85	82	.50	−.16	.562
D2S206	248.3	.80	82	.54	1.35	.091
D2S338	258.7	.81	83	.51	.38	.353
D2S125	269.5	.81	83	.52	.74	.232
3:						
D3S1297	2.5	.76	83	.54	1.47	.073
D3S1304	16.5	.79	82	.50	−.17	.569
D3S1263	30.4	.89	83	.47	−1.08	.858
D3S2338	36.3	.73	82	.48	−.63	.736
D3S1266	46.9	.66	83	.48	−.85	.801
D3S1277	56.1	.69	80	.47	−1.22	.887
D3S1289	69.1	.81	81	.48	−.49	.686
D3S1300	79	.81	80	.55	1.42	.080
D3S1285	91	.76	83	.48	−.61	.728
D3S1566	97.2	.84	80	.46	−1.26	.895
D3S3681	108.8	.82	79	.52	.73	.235
D3S1271	117.7	.60	83	.53	1.42	.080
D3S1278	131.8	.70	83	.51	.33	.373
D3S1267	141.1	.65	83	.47	−1.08	.859
D3S1292	148.7	.89	83	.51	.42	.338
D3S1569	162	.79	83	.45	−1.84	.965
D3S1279	173	.62	83	.45	−1.98	.975
D3S1614	183.1	.67	83	.54	1.63	.053
D3S1565	193	.80	83	.52	.66	.257
D3S1262	207.2	.72	83	.52	.65	.259
D3S1580	213.7	.84	82	.53	.95	.172

(*continued*)

Table 2 (*Continued*)

Chromosome and Marker	Distance (cM)	Heterozygosity	No. of Pairs	IBD	*t*	*P*
D3S1601	220.4	.79	83	.53	1.04	.150
D3S1311	230.7	.73	82	.55	2.22	.014*
4:						
D4S412	3.7	.68	83	.47	−.94	.824
D4S2935	12.2	.64	82	.50	.06	.476
D4S3036	23.1	.78	82	.50	−.09	.534
D4S419	32.6	.69	83	.53	1.11	.134
D4S391	43.2	.78	83	.53	1.23	.112
D4S405	56.7	.75	83	.49	−.21	.582
D4S1592	68.4	.78	83	.50	−.15	.558
D4S392	77.9	.82	82	.54	1.16	.125
D4S2964	87.1	.70	83	.50	−.16	.565
D4S1534	93.5	.80	83	.52	.56	.287
D4S414	99.2	.75	83	.52	.62	.268
D4S1572	106.3	.83	83	.52	.72	.236
D4S406	115.8	.70	81	.51	.31	.379
D4S402	123.5	.84	81	.54	1.16	.124
D4S3039	131.9	.82	81	.53	.89	.189
D4S424	143.8	.76	83	.52	.69	.247
D4S413	157.9	.62	83	.54	1.57	.060
D4S2979	170.9	.65	81	.50	−.17	.567
D4S2991	179.6	.81	82	.55	1.77	.040*
D4S415	185	.73	82	.52	.62	.269
D4S1535	198.5	.76	81	.52	.85	.198
D4S426	211	.72	83	.48	−.62	.733
5:						
D5S1981	.6	.76	83	.51	.47	.321
D5S406	10.7	.73	82	.51	.43	.333
D5S630	18.6	.90	83	.52	.46	.322
D5S416	27.9	.64	81	.50	.07	.473
D5S419	39.5	.87	80	.47	−1.02	.846
D5S426	51.6	.78	82	.48	−.75	.772
D5S418	58.1	.78	81	.54	1.19	.12
D5S407	65	.86	83	.52	.48	.316
D5S647	74.7	.82	83	.54	1.25	.108
D5S424	82.8	.68	82	.52	.86	.196
D5S641	92.3	.81	82	.51	.33	.371
D5S428	95.4	.68	81	.57	3.09	.001**
D5S644	104.5	.83	81	.56	2.14	.018*
D5S433	112.2	.75	83	.53	1.08	.141
D5S2027	118.9	.59	82	.52	.79	.216
D5S471	129.6	.71	83	.53	1.40	.083
D5S2115	138.6	.74	82	.54	1.45	.075
D5S436	147.2	.75	83	.52	.74	.231
D5S410	156	.57	83	.50	.01	.496
D5S422	163.9	.81	82	.50	−.11	.545
D5S400	174.3	.88	82	.50	.01	.496
D5S1960	179.1	.73	64	.48	−.59	.721
D5S408	195.8	.68	81	.49	−.24	.596
6:						
D6S1574	8.7	.73	81	.50	−.12	.549
D6S309	13.6	.76	80	.52	.84	.200
D6S470	17.7	.72	82	.47	−1.08	.859
D6S289	29.6	.81	83	.50	.11	.458
D6S422	35.7	.67	83	.52	.77	.223
D6S276	44.9	.73	83	.53	1.05	.148
D6S1610	53.9	.78	83	.51	.42	.339
D6S1575	60.7	.84	83	.54	1.32	.095
D6S452	72.2	.85	80	.47	−1.01	.843
D6S257	80	.88	83	.51	.30	.384
D6S460	90	.80	82	.50	.09	.462
D6S300	103.5	.71	82	.53	.96	.170
D6S434	109.2	.78	83	.53	1.00	.161
D6S287	122	.68	81	.51	.55	.291
D6S262	129.8	.77	83	.52	.48	.317
D6S292	138.2	.85	82	.49	−.25	.599
D6S308	145.5	.65	83	.50	.14	.444
D6S441	155.3	.79	81	.50	.08	.469
D6S305	166.6	.82	80	.48	−.47	.682
D6S1719	177.9	.77	79	.47	−.89	.812
D6S281	201.1	.81	83	.46	−1.18	.880
7:						
D7S531	4.8	.77	81	.53	1.14	.130
D7S517	7.8	.79	83	.54	1.55	.063
D7S513	17.7	.9	83	.53	.89	.189
D7S507	29.1	.82	83	.50	−.09	.538
D7S493	35	.73	83	.49	−.29	.615
D7S516	42.1	.76	83	.49	−.24	.596
D7S484	55.6	.79	82	.51	.34	.367
D7S510	60.5	.82	83	.55	1.87	.032*
D7S519	70.5	.74	83	.53	1.32	.095
D7S502	79.6	.85	82	.54	1.13	.131
D7S669	90.9	.83	83	.56	2.06	.021*
D7S630	98.7	.77	82	.55	1.77	.040*
D7S657	105.2	.77	83	.54	1.52	.066
D7S515	112.9	.75	83	.56	2.24	.014*
D7S486	125.3	.76	83	.50	.05	.478
D7S530	136.4	.72	81	.48	−.59	.722

(*continued*)

Table 2 (*Continued*)

Chromosome and Marker	Distance (cM)	Heterozygosity	No. of Pairs	IBD	*t*	*P*
D7S640	139.7	.85	83	.48	−.59	.722
D7S684	149.6	.78	83	.45	−1.68	.952
D7S661	157.5	.84	83	.47	−.95	.828
D7S636	165	.93	82	.47	−.70	.757
D7S798	171.3	.75	80	.52	.62	.270
D7S2465	182.1	.77	81	.53	.83	.204
8:						
D8S264	.7	.83	83	.52	.65	.260
D8S277	8.4	.81	80	.48	−.58	.717
D8S550	20.4	.72	83	.48	−.62	.731
D8S1731	30.7	.70	82	.49	−.20	.581
D8S258	40.3	.68	83	.51	.51	.304
D8S177 1	49.6	.68	80	.45	−1.70	.953
D8S505	60	.77	83	.46	−1.31	.904
D8S285	70.6	.70	83	.46	−1.31	.904
D8S260	78.8	.76	83	.50	.04	.482
D8S543	86.7	.73	80	.52	.93	.177
D8S1705	94.3	.75	83	.55	1.60	.057
D8S270	102.1	.70	82	.52	.87	.194
D8S514	128.9	.77	83	.50	.18	.428
D8S284	142.7	.80	81	.51	.17	.432
D8S272	152.5	.80	83	.50	−.06	.525
9:						
D9S288	8.8	.81	83	.57	2.34	.011*
D9S286	16.8	.75	81	.53	1.13	.131
D9S285	27.9	.62	83	.49	−.39	.650
D9S157	31.8	.83	83	.52	.50	.309
D9S265	42	.63	83	.53	1.35	.090
D9S1678	50.3	.75	79	.51	.34	.368
D9S1817	57.9	.86	83	.51	.47	.320
D9S166	65	.75	82	.53	1.19	.118
D9S175	68.8	.62	82	.50	.13	.447
D9S167	82.4	.84	83	.55	1.50	.069
D9S283	93.2	.73	81	.51	.42	.338
D9S287	103.3	.64	82	.52	.99	.162
D9S1690	106.5	.78	83	.52	.58	.281
D9S1677	117.8	.87	82	.53	1.00	.160
D9S1776	124.2	.76	83	.51	.22	.413
D9S1682	132.9	.64	78	.51	.52	.301
D9S290	141.1	.66	83	.46	−1.44	.923
D9S164	148.1	.79	80	.49	−.39	.651
D9S1826	160.2	.82	82	.51	.35	.364
D9S158	163	.72	82	.54	1.49	.071
10:						
D10S249	0	.82	82	.49	−.21	.583
D10S552	13	.76	83	.55	1.50	.069
D10S189	17.3	.72	83	.55	1.62	.055
D10S570	32.1	.73	83	.49	−.39	.651
D10S1653	38.8	.75	83	.48	−.61	.730
D10S548	43.4	.59	82	.48	−.90	.814
D10S197	50.5	.72	83	.51	.32	.376
D10S208	60.2	.79	83	.52	.57	.286
D10S196	72.5	.70	80	.44	−2.39	.990
D10S1652	83.3	.70	79	.53	1.19	.119
D10S537	93.8	.82	82	.51	.38	.352
D10S1686	109.2	.66	82	.45	−1.75	.958
D10S185	123.3	.78	83	.52	.66	.256
D10S192	131.2	.84	82	.49	−.29	.612
D10S1269	140.2	.64	81	.50	−.04	.515
D10S1693	146.1	.82	77	.44	−1.96	.973
D10S587	156.6	.82	83	.45	−1.52	.934
D10S217	167.2	.82	81	.49	−.25	.600
D10S1651	178.3	.64	80	.47	−.99	.838
D10S1711	180.5	.61	80	.46	−1.47	.928
11:						
D11S4046	3.9	.85	83	.51	.50	.310
D11S1338	14.9	.62	83	.53	1.21	.114
D11S902	24.7	.84	80	.54	1.14	.129
D11S904	37	.71	83	.50	.10	.460
D11S935	49.6	.72	82	.50	−.14	.555
D11S905	55.7	.81	83	.47	−1.01	.843
D11S4191	63.4	.88	83	.54	1.22	.113
D11S987	67.5	.84	83	.55	1.75	.042*
D11S1314	77.5	.79	82	.54	1.30	.099
D11S937	84.6	.76	83	.53	1.20	.118
D11S901	89.8	.68	83	.51	.30	.381
D11S4175	96.3	.84	82	.50	−.05	.518
D11S1339	104.8	.70	82	.52	.61	.272
D11S4111	112.9	.80	82	.49	−.16	.564
D11S925	123.5	.81	83	.54	1.11	.136
D11S4151	132.9	.61	80	.54	1.58	.060
D11S910	145.6	.72	82	.55	2.02	.023*
D11S4125	152.8	.74	81	.51	.44	.332
12:						
D12S352	0	.68	83	.46	−1.74	.957
D12S99	13.9	.81	82	.46	−1.36	.911

(*continued*)

Table 2 (*Continued*)

Chromosome and Marker	Distance (cM)	Heterozygosity	No. of Pairs	IBD	*t*	*P*
D12S336	21	.74	83	.50	−.09	.535
D12S364	31.7	.81	82	.46	−1.45	.924
D12S310	36.1	.64	80	.52	.87	.193
D12S1617	45.1	.84	83	.48	−.62	.731
D12S345	54.4	.84	83	.49	−.30	.619
D12S85	62.7	.80	82	.45	−1.41	.919
D12S368	67.3	.66	82	.49	−.56	.713
D12S83	76.5	.81	83	.51	.48	.316
D12S326	87.6	.61	80	.47	−1.08	.859
D12S351	97.1	.74	83	.53	.92	.181
D12S346	106.1	.73	81	.48	−.60	.726
D12S78	113.3	.79	81	.53	.92	.180
D12S79	126.1	.80	82	.49	−.20	.581
D12S86	135.1	.68	83	.50	.05	.480
D12S32 4	148.3	.64	83	.47	−1.16	.876
D12S36v7	160.9	.71	82	.49	−.39	.649
D12S1723	165.7	.79	83	.50	−.04	.515
13:						
D13S175	7.4	.67	83	.49	−.24	.594
D13S217	19.1	.67	82	.52	.75	.229
D13S171	27.3	.65	82	.51	.25	.401
D13S218	35.3	.60	82	.49	−.28	.611
D13S263	40.4	.81	82	.52	.64	.262
D13S153	47.5	.89	83	.56	1.84	.034*
D13S156	57.3	.82	83	.54	1.4	.083
D13S170	65.4	.83	82	.54	1.21	.114
D13S265	70.6	.66	83	.53	1.09	.138
D13S159	81.5	.73	82	.50	−.06	.524
D13S158	86.9	.73	83	.51	.41	.342
D13S173	95.9	.63	83	.53	.89	.188
D13S1265	101.7	.83	82	.51	.27	.396
D13S285	112.8	.85	78	.51	.38	.353
14:						
D14S261	0	.59	82	.51	.22	.413
D14S283	7.5	.8	83	.52	.83	.203
D14S275	21.9	.6	83	.51	.52	.303
D14S70	32.9	.67	83	.45	−1.77	.960
D14S288	39.1	.87	83	.53	.90	.187
D14S276	47	.77	83	.54	1.35	.090
D14S63	59	.76	83	.53	1.01	.158
D14S258	65.8	.64	83	.55	1.86	.034*
D14S74	76.4	.8	83	.58	2.82	.003**
D14S68	86.3	.83	83	.52	.55	.292
D14S280	95.5	.68	83	.48	−.58	.719
D14S65	108.1	.71	83	.52	.65	.258
D14S985	117.1	.72	83	.53	1.20	.117
D14S292	124.2	.71	83	.51	.33	.371
15:						
D15S128	6.1	.85	82	.50	−.14	.556
D15S1002	14.5	.76	83	.50	−.07	.528
D15S1048	19.1	.66	81	.50	−.11	.543
D15S1007	25.9	.82	83	.52	.48	.317
D15S1042	32.3	.78	81	.49	−.25	.599
D15S994	40	.76	83	.49	−.21	.582
D15S978	45.5	.74	81	.50	−.10	.540
D15S117	50.8	.74	82	.50	−.01	.503
D15S153	62.1	.79	83	.51	.38	.352
D15S131	70.7	.75	82	.49	−.37	.645
D15S205	77.4	.88	83	.53	.96	.171
D15S127	84.8	.83	81	.47	−1.06	.853
D15S1004	95.7	.62	81	.48	−.93	.822
D15S120	109.6	.79	83	.47	−.97	.832
16:						
D16S423	8.4	.85	75	.51	.22	.413
D16S404	16.7	.69	82	.51	.30	.383
D16S3075	21.8	.8	81	.49	−.17	.566
D16S3017	31.1	.73	79	.47	−.94	.824
D16S3046	39.3	.65	83	.47	−1.30	.902
D16S3068	46.6	.73	83	.54	1.36	.089
D16S3136	60	.65	82	.48	−.70	.758
D16S415	65.6	.69	81	.48	−.61	.727
D16S503	81.8	.66	76	.51	.37	.355
D16S515	90.2	.87	77	.48	−.59	.723
D16S516	98.3	.72	75	.48	−.61	.729
D16S3091	109.1	.83	83	.50	.02	.491
D16S520	123.3	.8	80	.52	.68	.248
17:						
D17S849	.6	.74	83	.48	−.70	.758
D17S831	6.6	.85	78	.48	−.57	.714
D17S938	14.8	.82	81	.48	−.76	.774
D17S1852	23.2	.8	83	.49	−.25	.598
D17S947	32.8	.85	80	.46	−1.23	.888
D17S921	37.3	.73	80	.53	1.11	.135
D17S925	49.5	.71	80	.55	1.96	.027*
D17S1872	58.3	.90	79	.47	−.89	.811
D17S1868	65.1	.78	76	.52	.56	.290

(*continued*)

Table 2 (*Continued*)

Chromosome and Marker	Distance (cM)	Heterozygosity	No. of Pairs	IBD	*t*	*P*
D17S787	75.7	.83	83	.46	−1.25	.892
D17S948	84.1	.70	80	.48	−.62	.732
D17S949	94.9	.80	82	.51	.40	.344
D17S785	104.7	.70	83	.47	−.98	.836
D17S784	117.7	.60	83	.51	.41	.340
D17S928	128.7	.83	82	.52	.46	.325
18:						
D18S59	.1	.80	76	.55	1.68	.049*
D18S63	7.9	.71	80	.47	−1.25	.892
D18S452	17.7	.81	82	.50	.04	.485
D18S1153	34.7	.81	83	.50	−.14	.554
D18S53	40.4	.82	78	.48	−.68	.751
D18S478	52.3	.65	83	.50	−.14	.554
D18S1102	61.7	.68	82	.44	−2.29	.988
D18S474	71.3	.72	76	.49	−.39	.651
D18S64	83	.82	83	.50	.06	.476
D18S68	94.4	.72	78	.50	−.16	.563
D18S61	102.8	.82	82	.54	1.45	.076
D18S1161	112	.74	82	.54	1.41	.082
D18S462	118	.72	81	.50	.02	.494
D18S70	123.8	.75	82	.50	−.07	.528
19:						
D19S209	10.8	.82	83	.48	−.56	.712
D19S894	15.4	.81	74	.49	−.26	.603
D19S884	26	.84	83	.47	−1.13	.868
D19S221	35.5	.81	81	.45	−1.69	.952
D19S226	41.7	.86	82	.45	−1.42	.920
D19S414	53.2	.60	79	.47	−1.19	.882
D19S220	61.4	.87	80	.49	−.35	.637
D19S420	66	.79	82	.51	.21	.419
D19S902	76.2	.79	83	.51	.33	.370
D19S921	91.7	.84	75	.52	.50	.309
D19S418	97.5	.65	83	.49	−.4	.655
D19S210	104.9	.67	83	.51	.30	.382
20:						
D20S117	2.9	.82	83	.52	.50	.310
D20S889	11	.78	83	.50	.13	.447
D20S192	18.5	.76	81	.46	−1.24	.891
D20S186	33.2	.88	83	.48	−.42	.661
D20S112	39.3	.73	83	.51	.26	.399
D20S195	50.2	.74	81	.52	.92	.180
D20S107	54.9	.71	82	.48	−.69	.755
D20S119	61	.79	83	.47	−.97	.833
D20S178	65.5	.77	82	.48	−.69	.755
D20S196	74.5	.81	83	.45	−1.69	.953
D20S100	83.4	.71	83	.47	−1.28	.899
D20S171	94.4	.71	82	.47	−.98	.836
D20S173	96.5	.61	83	.53	1.11	.136
21:						
D21S1256	8.6	.82	83	.48	−.45	.671
D21S1914	23	.81	83	.48	−.50	.690
D21S263	31.4	.82	81	.54	1.14	.129
D21S1252	38.7	.82	83	.52	.70	.243
D21S266	49.9	.82	82	.49	−.38	.649
22:						
D22S420	0	.70	82	.47	−1.19	.881
D22S446	9	.65	82	.46	−1.55	.938
D22S315	16.2	.80	83	.46	−1.21	.884
D22S280	25.9	.79	83	.49	−.42	.662
D22S283	33.4	.75	82	.51	.50	.308
D22S423	40.2	.83	82	.49	−.20	.581
D22S274	45.5	.84	79	.51	.29	.386

* $P < .05$.
** $P < .01$.

imaging (fig. 1*a*), cerebral angiography (fig. 1*b*), and three-dimensional CT angiography (fig. 1*c*) of a typical patient with IA who has a saccular aneurysm in the left middle cerebral artery.

Genomewide Linkage Studies

The sample for linkage study comprised 85 Japanese nuclear families, and the maximum number of ASPs with IA was 104 (table 1). Because the quantity of DNA available from 25 participants was insufficient for genotyping of the 404 microsatellite markers for genomewide scan, 154 of the 179 individuals comprised by the 83 ASPs were genotyped. The statistical probability of linkage between each marker and IA was tested by SIBPAL (table 2). Regions of the genome were considered to have suggestive evidence of linkage in the first data set either if an individual marker attained statistical significance $P < .01$ or if two or more adjacent markers each attained statistical significance at $P < .05$. By these criteria, suggestive evidence of linkage to IA obtains for markers within three distinct chromosomes: chromosome 5 (markers *D5S428* and *D5S644*), chromosome 7 (markers

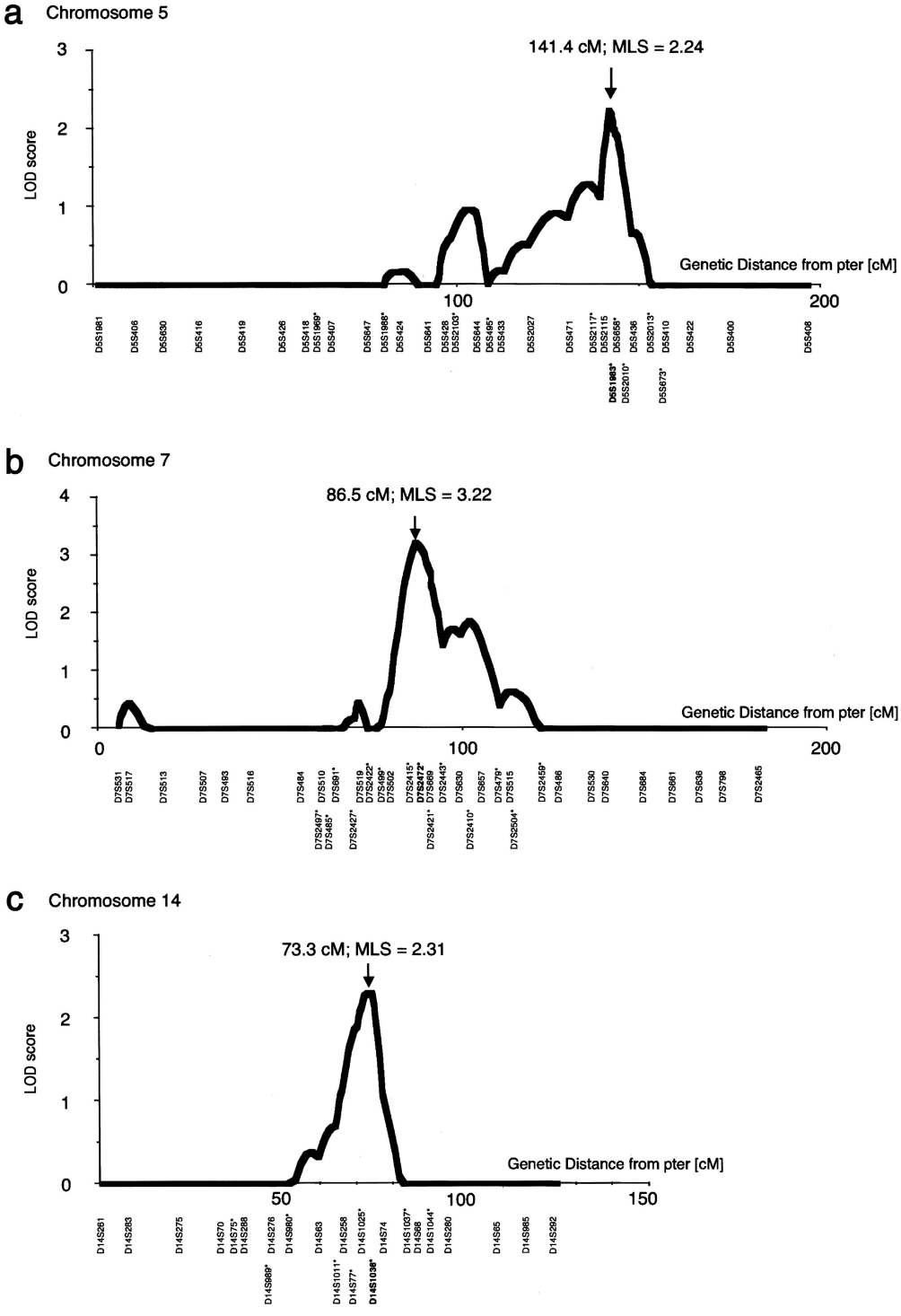

Figure 2 Results of multipoint linkage analyses for high-resolution mapping on chromosomes 5 (*a*), 7 (*b*), and 14 (*c*) in 104 Japanese sib pairs with IA. Positions of the MLS are indicated by arrows: MLS = 2.24, 3.22, and 2.31, and distances from the p-terminal end (pter) were 141.4, 86.5, and 73.3 cM, on chromosome 5, 7, and 14, respectively. The borders (defined by LOD >1.0) of positive linkage lie between markers *D5S471* and *D5S2010, D7S2415* and *D7S657,* and *D14S258* and *D14S74.* Additional markers used for high-resolution mapping are indicated by asterisks (*).

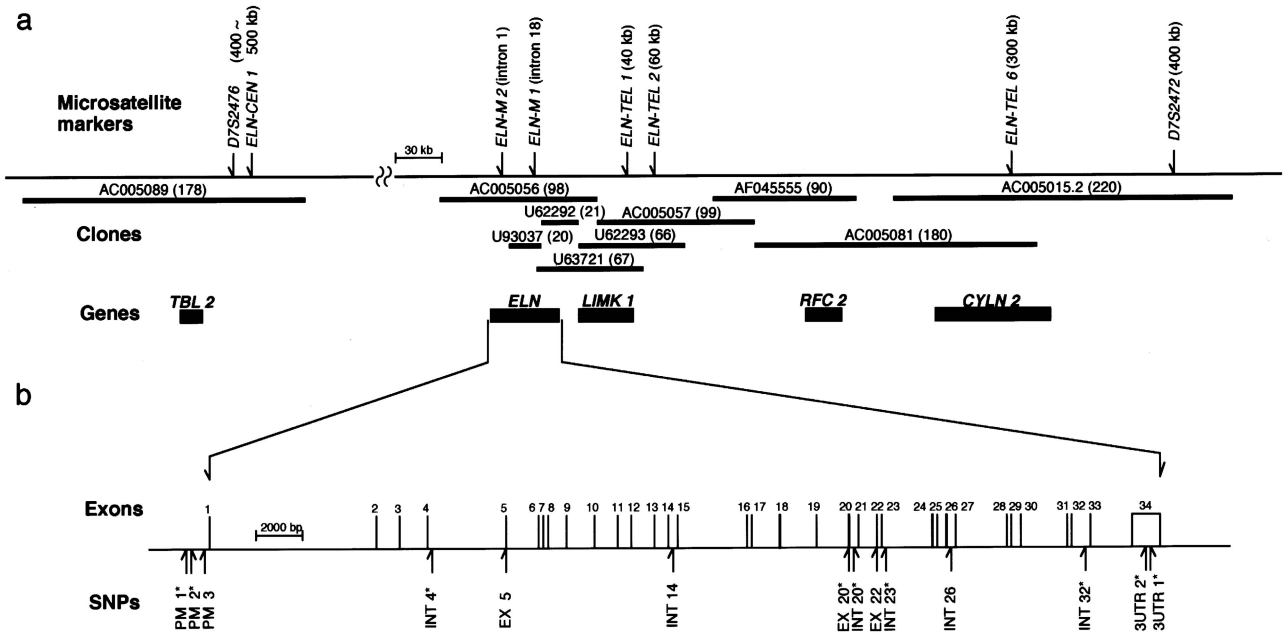

Figure 3 Physical map of *ELN* locus. *a,* Contigs and microsatellite-marker locations at locus. The thicker lines denote clones, which have been registered in the GenBank database, in the *ELN* locus; and the numbers in parentheses are the length (in kb) of the clones. The vertical arrows above the thinner lines indicate positions of eight microsatellite markers at the locus; and distances from *ELN* are in parentheses. Blackened rectangles indicate positions of known genes lying near *ELN: TBL2* = transducin β-like 2; *LIMK1* = LIM domain kinase 1; *RFC2* = replication factor C 2; *CYLN2* = cytoplasmic linker 2. *b,* Expanded view of 43-kb segment of *ELN.* The exon-intron organization of *ELN* and the positions of 14 distinct SNPs are indicated. Nine SNPs, indicated by asterisks (*), were used for pairwise haplotype-association study.

D7S669 and *D7S630*), and chromosome 14 (markers *D14S258* and *D14S74*) (table 2). These chromosomes were further tested by multipoint linkage analysis using GENEHUNTER. Multipoint analyses of the three regions showed at least nominal evidence for linkage (defined by MLS >1.0; data not shown). Linkage analysis of chromosome X by GENEHUNTER indicated no evidence of linkage (data not shown).

Next, all individuals in the 104 ASPs were genotyped by addition of microsatellite markers covering the candidate regions, on chromosomes 5q, 7q, and 14q, that showed putatively positive evidence of linkage; 10 markers on chromosome 5 (*D5S1969, D5S1988, D5S2103, D5S495, D5S2117, D5S1983, D5S658, D5S2010, D5S2013,* and *D5S673*), 14 markers on chromosome 7 (*D7S2497, D7S485, D7S691, D7S2427, D7S2422, D7S499, D7S2415, D7S2472, D7S2421, D7S2443, D7S2410, D7S479, D7S2504,* and *D7S2459*), and 9 markers on chromosome 14 (*D14S75, D14S989, D14S980, D14S1011, D14S77, D14S1025, D14S1036, D14S1037,* and *D14S1044*) were added, for high-resolution mapping (see The Whitehead Institute for Biomedical Research/MIT Center for Genome Research web site). Multipoint linkage analyses by GENEHUNTER revealed evidence of linkage to loci on chromosomes 5q22-31 (MLS 2.24, $P = .00149$), 7q11 (MLS 3.22, $P = .00046$), and 14q22 (MLS 2.31, $P = .00120$) (fig. 2); the MLSs were near markers *D5S1983, D7S2472,* and *D14S1036,* respectively. 1-LOD support intervals lay between *D5S471* and *D5S2010,* between *D7S2415* and *D7S657,* and between *D14S258* and *D14S74,* comprising regions of ~14, ~21, and ~11 cM, respectively.

Physical Map of the ELN *Locus*

In the search for candidate genes in the linkage regions, the candidate gene *ELN* was found, 400 kb from marker *D7S2472* on chromosome 7. Elastin is the predominant protein of mature elastic fibers in arterial walls. The *ELN* locus on 7q11.23 has been extensively analyzed in studies of WBS (Peoples et al. 2000) and supravalvular aortic stenosis (Curran et al. 1993; Li et al. 1997; Tassabehji et al. 1997). A physical map of the *ELN* locus was reconstructed on the basis of the GenBank database and a physical map of microdeletion in WBS (Peoples et al. 2000). A clone (accession number AC005056) contained the full length of *ELN,* and the contig was extended to marker *D7S2472,* telomeric to *ELN.* Another clone (accession number AC005089) was closest to *ELN* on the centromeric side; the sequences between *ELN* and the latter clone have not been registered in the GenBank database. Eight polymorphic dinucleotide or tetranucleo-

Table 3

Linkage Analysis and Association Study Using Microsatellite Markers at the *ELN* Locus

			Linkage Analysis			Association Study		
Microsatellite Marker	Heterozygosity	Distance from *ELN*	IBD	*t*	*P*	No. of Alleles (CTR/IA)[a]	χ^2 (df)	*P*
D7S2476	.42	400–500 kb (centromere)	.5367	2.021	.0232*	384/328	13.52 (12)	.3327
ELN-CEN1	.12	400–500 kb (centromere)	.5018	.138	.4453	382/322	4.79 (3)	.1881
ELN-M2	.78	Intron 1 (*ELN*)	.5413	1.499	.0687	378/320	19.22 (8)	.0137*
ELN-M1	.59	Intron 18 (*ELN*)	.5051	.228	.4100	384/328	3.43 (5)	.6338
ELN-TEL1	.87	40 kb (telomere)	.5647	2.061	.0212*	382/322	20.78 (14)	.1075
ELN-TEL2	.76	60 kb (telomere)	.5302	1.209	.1150	382/326	9.20 (10)	.5132
ELN-TEL6	.77	300 kb (telomere)	.5363	1.264	.1049	374/320	8.39 (9)	.4952
D7S2472	.74	400 kb (telomere)	.6084	4.426	.000029**	374/328	8.80 (10)	.5511

[a] CTR = controls; IA = patients with IA.
* $P < .05$.
** $P < .01$.

tide repeats identified in the *ELN* locus were distributed as follows: *D7S2476* and *ELN-CEN1*, both at 400–500 kb from *ELN, ELN-M2* at intron 1 of *ELN* (Urban et al. 1997), *ELN-M1* at intron 18 of *ELN* (Foster et al. 1993), *ELN-TEL1* at 40 kb, *ELN-TEL2* at 60 kb, *ELN-TEL6* at 300 kb, and *D7S2472* at 400 kb, respectively (fig. 3). All eight markers were tested for linkage, by SIBPAL. Although evidence of linkage was not strong, all the markers showed means >.5, for alleles sharing linkage, throughout the region (table 3). The allelic frequencies of the eight markers were compared in patients with IA versus controls. A weak allelic association was detected in the allele-frequency distribution of the marker *ELN-M2* ($\chi^2 = 19.22$, df = 8, $P = .0137$) (table 3). Considered together, these findings indicate that *ELN* is a primary candidate gene for IA.

SNPs in ELN

ELN was extensively screened for SNPs. Systematic direct sequencing was performed on all 34 exons; acceptor, donor, and branch-point sequences of all introns; 1.0 kb of the putative promoter sequence (Kahari et al. 1990); and 1.2 kb of 3′UTR sequence. Fourteen distinct SNPs, including two previously published polymorphisms (Tromp et al. 1991; Urban et al. 1999), were identified (fig. 3 and table 4). Three of the SNPs occur in the coding regions: EX5, a C→T substitution at exon 5 (+16), and EX20, a G→A substitution at exon 20 (+114), resulted in amino acid substitutions A71V and G422S, respectively, whereas EX22, a G→A substitution at exon 22 (+23), was a silent substitution. Allelic frequencies of the 14 SNPs were compared in patients versus controls (table 4). Allelic-frequency differences between cases and controls were found for two SNPs; but they did not reach statistical significance ($\chi^2 = 3.39$, df = 1, and $P = .067$, for INT20; and $\chi^2 = 2.97$, df = 1, and $P = .085$, for 3UTR1) (table 4). All of the SNP frequencies of controls were in Hardy-Weinberg equilibrium.

Pairwise Haplotype Association Study of IA

Thirty-six pairwise haplotype combinations were constructed from nine SNPs (i.e., PM1, PM2, INT4, EX20, INT20, INT23, INT32, 3UTR1, and 3UTR2; table 4) that showed relatively high allelic frequencies (≥.05). The pairwise haplotype frequencies were calculated by a maximum-likelihood estimation using theARLEQUIN program, and the haplotype frequencies were compared in the 172 patients with IA versus the 192 controls (fig. 4); most often, two SNPs of any combination created four haplotypes, reflecting weak LD throughout the gene. The haplotype frequencies of all pairwise combinations then were compared in a global test with 3 df. The best evidence of haplotype association was observed for INT20/INT23 ($\chi^2 = 27.90$, df = 3, $P = 3.81 \times 10^{-6}$). An Mm haplotype (major allele [i.e., M] for INT20 and minor allele [i.e., m] for INT23) was more prevalent in patients with IA than in controls ($\chi^2 = 11.17$, df = 1, $P = .0008$), with an odds ratio of 1.85 (95%CI 1.53–2.65). Subjects who were homozygous for the Mm haplotype and whose haplotype was unambiguously determined appeared more frequently among patients with IA than among controls (10.7% vs. 2.7%; $\chi^2 = 9.52$, df = 1, $P = .002$), with an odds ratio of 4.39 (95%CI 2.62–12.11). Significant associations also were detected in the haplotype-frequency distribution of two combinations: PM2/INT23 ($\chi^2 = 14.85$, df = 3, $P = 1.94 \times 10^{-3}$), and INT4/INT23 ($\chi^2 = 24.10$, df = 3, $P = 2.39 \times 10^{-4}$) (fig. 4).

Pairwise LD of ELN

The extent of pairwise LD in the 36 pairs of combinations was investigated in complete detail, by two LD measures—D' and r^2—in 192 controls. LD is generally a measure of distance between SNPs. However, in *ELN,* the distribution of LD is highly irregular, and generally weak degrees were observed between the SNPs (fig. 5*a*). Among

Table 4

Polymorphisms in *ELN,* and Association Study of Patients with IA and of Controls

SNP Name	Location (Position [a])	Change		Allele Frequency[b]			
		M→m[c]	Amino Acid	Controls	Patients with IA	χ^2 [d]	*P*
PM 1	Promoter (−1042)	C→T		.202 (77/382)	.208 (69/332)	.043	.836
PM 2	Promoter (−972)	G→A		.178 (68/382)	.145 (48/332)	1.459	.227
PM 3	Promoter (−38)	C→T		.021 (4/188)	.040 (7/174)	1.102	.294
INT 4	Intron 4 (+71)	G→A		.201 (76/378)	.178 (60/338)	.643	.423
EX 5	Exon 5 (+16)	C→T	Ala→Val	.021 (4/188)	.029 (5/174)	.207	.649
INT14	Intron 14 (−28)	G→A		.016 (3/186)	.035 (6/170)	1.324	.250
EX 20	Exon 20 (+114)	G→A	Gly→Ser	.189 (71/376)	.210 (71/338)	.503	.478
INT 20	Intron 20 (+17)	T→C		.269 (101/376)	.210 (71/338)	3.388	.067
EX 22	Exon 22 (+23)	G→A	Leu→Leu	.011 (4/376)	.018 (6/326)	.750	.387
INT 23	Intron 23 (+24)	T→C		.294 (113/384)	.308 (106/344)	.166	.684
INT 26	Intron 26 (−20)	C→T		.016 (3/192)	.006 (1/174)	.824	.364
INT 32	Intron 32 (−34)	C→T		.052 (20/384)	.056 (19/340)	.051	.821
3UTR 1	3′UTR (+502)	A insertion		.102 (39/382)	.144 (49/340)	2.968	.085
3UTR 2	3′UTR (+659)	G→C		.050 (19/382)	.060 (20/336)	.153	.696

[a] No. of nucleotides from nearest start of promoter, intron, exon, or 3′UTR.
[b] CTR = controls; IA = patients with IA.
[c] M = major, common allele; m = minor, less common allele.
[d] df = 1.

these, LD was conserved at PM1/EX20, PM2/INT4, PM2/INT20, INT4/INT20, INT32/3UTR1, INT32/3UTR2, and 3UTR1/3UTR2 ($D' > .7$ and $r^2 > .3$). A very weak LD was indicated between INT23 and other SNPs. A similar LD was observed for patients with IA. According to the extent of LD, the five putative ancestral haplotype groups could be classified: H0 = no polymorphism in the allele; H1= polymorphisms at PM1 and EX20; H2 = polymorphisms at PM2, INT4, and INT20; H3 = polymorphism at INT23; and H4 = polymorphisms at INT32, 3UTR1, and 3UTR2 (fig. 5*b*). Because Mm haplotypes at PM2/INT23, INT4/INT23, and INT20/INT23 were significantly more frequent in patients with IA than in controls (fig. 4), it may well be that the ancestral H3 haplotype puts an individual at risk for IA. A recombinant haplotype of either H2 and H3 or INT20 and INT23 was more common in controls than in patients with IA.

Discussion

In the present study, we have identified, by genomewide linkage study and the candidate-gene approach, a molecular determinant of IA, a determinant that suggests both the pathogenesis of the disease and also novel therapeutic targets. Evidence of linkage throughout the genome in 83 Japanese ASPs was established first by SIBPAL analysis, in which excess allele sharing was found. Evidence of linkage was observed at markers on chromosomes 5q, 7q, and 14q. Dense mapping with all 104 ASPs was performed in these regions by multipoint analysis with GENEHUNTER. The best evidence of linkage was observed on chromosome 7q11, near marker *D7S2472*. In the three chromosomal regions, the candidate genes for either vascular components or vascular formation are those for lysyl oxidase (*LOX*), fibrillin 2 (*FBN2*), and fibroblast growth factor 1 (*FGF1*), all on chromosome 5; *ELN* and the genes for KREV interaction trapped 1 (*KIRT1*) and collagen type 1 $\alpha 2$ (*COL1A2*), all on chromosome 7; and that for latent transforming growth factor β–binding protein 2 (*LTBP2*), on chromosome 14.

Defects relating to the medial muscle layer at the branch points of intracranial major arteries, favorable sites for IA, are observed frequently, and external elastic lamina is absent in cerebral arteries. Internal elastic lamina is the major structural support in the cerebral vessels (Glynn 1940). It is interesting to note that the several genes associated with elastic-fiber production—that is, *LOX, FBN2,* and *ELN*—lie within the linkage regions. Pathology examination of IA often reveals a defect in the internal elastic lamina of IA lesions (Carmichael 1945, 1950; Stebens 1963). Animal models of IA have been established by treatment with either elastase (Anidjar et al. 1990; Miskolczi et al. 1998) or β-aminopropionitrile, which inhibit cross-linking reactions between elastin molecules (Hashimoto et al. 1978). Defects in or degeneration of the internal elastic lamina, therefore, might play an important role in the etiology of IA.

In this study, *ELN* was extensively screened for the presence of molecular variants, since (*a*) it gene lies very close to the marker—*D7S2472*—that showed the best evidence for linkage and (*b*) elastin constitutes the predominant protein in elastic fibers. We identified 14 SNPs in *ELN,* and the allelic frequencies in patients with IA were compared with those in controls. Although there appeared to be no allelic association with any SNP, increasing the sample size led to statistical significance, at

Figure 4 Association study using pairwise haplotype frequencies in patients with IA versus those in controls. *P* values <.01 are indicated by double asterisks (**).

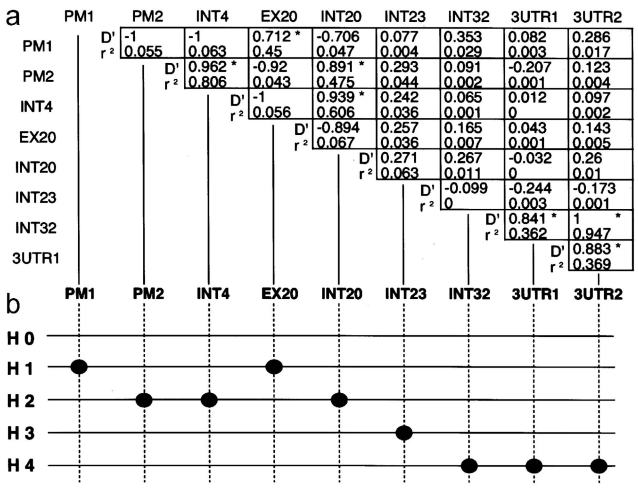

Figure 5 Pairwise LD between SNPs in *ELN*. *a*, Extent of pairwise LD of *ELN*, measured by two distinct formulas. The upper and lower numerals in each box indicate coefficient D' and r^2, respectively. Formulas for D' and r^2 are described in the text. Pairwise combinations with relatively high LD measures (defined by $D' > .7$ and $r^2 > .3$) in 192 controls are indicated, by boxes containing an asterisk (*). *b*, Illustration of five ancestral haplotype groups. Black dots represent the SNPs at each site. H0 = no polymorphism in the allele; H1 = polymorphisms at PM1 and EX20; H2 = polymorphisms at PM2, INT4, and INT20; H3 = one polymorphism, at INT23; H4 = polymorphisms at INT32, 3UTR1, and 3UTR2.

the intron 20 polymorphism. Pairwise haplotype analysis was performed with combinations of nine SNPs (fig. 4). Haplotype analysis can reveal the degree of predisposition of a specific allele to a disease and is especially useful when causal variants have not been identified. The Mm haplotype at INT20/INT23 was observed more frequently in patients with IA than in controls and indicates risk for IA among Japanese ($\chi^2 = 27.90$, df = 3, $P = 3.81 \times 10^{-6}$) (fig. 4). The functional role of the *ELN* haplotype in pathophysiology is not known. Similarly, a recent report has shown that a specific haplotype combination of calpain-10 is a risk factor for type II diabetes in a Mexican American population (Horikawa et al. 2000). That study suggests that heterozygosity for two different, common haplotypes may be necessary for the development of diabetes. Analyses of all the possible pairwise LD revealed weak LD throughout *ELN*, and the pairwise LD between INT23 and others in the vicinity was especially weak (fig. 5). *ELN* is highly rich in *Alu* repeats, with possibly ⩾30 *Alu* sequences within the 43-kb region (data not shown). *Alu* repeats may be associated with genome instability (Calabretta et al. 1982), which could partly explain the low LD observed in *ELN*. Indeed, loss of the exons in primates may be due to an *Alu*-mediated recombination event that might confer an evolutionary advantage in elastic tissue (Szabo et al. 1999). It is curious that poor LD was found between INT20 and INT23, whereas a strong association was observed in the haplotype created by the two SNPs. In general, with phase-unknown samples, the haplotype may not be precisely defined at low LD; however, individuals who were homozygous for the Mm haplotype and whose haplotype was unambiguously determined were remarkably more common among the patients with IA patients than among controls (10.7% vs. 2.7%; $\chi^2 = 9.52$, df = 1, $P = .002$), with an odds ratio of 4.39. The Mm haplotype, therefore, is associated with IA, and a disease-causing variant should lie either on the allele within *ELN* or, possibly, in a nearby gene.

We have mapped three chromosomal loci for IA and have identified a candidate gene, *ELN*, on the basis of its chromosomal position and function. We have determined that the Mm haplotype at INT20/INT23 indicates risk for the disease in the Japanese. Long-term investigation including replication studies in distinct ethnic groups, as well as functional studies using biochemical and cellular biological techniques, will be necessary to clarify the mechanism of the relationship between the genetic variation and the disease.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

ARLEQUIN, http://anthro.unige.ch/arlequin (for software for population genetics data analysis)

GenBank Overview, http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html (for *ELN* locus [accession numbers AC005089, AC005056, U93037, U63721, U62292, U62293, AC005057, AF045555, AC005081, AC005015.2, and M36860])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for IA [MIM 105800])

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, The, http://www-genome.wi.mit.edu/ (for markers *D5S1969*, *D5S1988*, *D5S2103*, *D5S495*, *D5S2117*, *D5S1983*, *D5S658*, *D5S2010*, *D5S2013*, *D5S673*, *D7S2497*, *D7S485*, *D7S691*, *D7S2427*, *D7S2422*, *D7S499*, *D7S2415*, *D7S2472*, *D7S2421*, *D7S2443*, *D7S2410*, *D7S479*, *D7S2504*, *D7S2459*, *D14S75*, *D14S989*, *D14S980*, *D14S1011*, *D14S77*, *D14S1025*, *D14S1036*, *D14S1037*, and *D14S1044*)

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