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Report

Intracranial Aneurysms in Finnish Families: Confirmation of Linkage and Refinement of the Interval to Chromosome 19q13.3

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We recently reported a two-stage genomewide screen of 48 sib pairs affected with intracranial aneurysms (IAs) that revealed suggestive linkage to chromosome 19q13, with a LOD score of 2.58. The region supporting linkage spanned ~22 cM. Here, we report a follow-up study of the locus at 19q13, with a sample size expanded to 139 affected sib pairs, along with 83 other affected relative pairs (222 affected relative pairs in total). Suggestive linkage was observed in both independent sample sets, and linkage was significant in the combined set at 70 cM (LOD score 3.50; $P = .00006$) and at 80 cM (LOD score 3.93; $P = .00002$). Linkage was highly significant at 70 cM (LOD score 5.70; $P = .000001$) and at 80 cM (LOD score 3.99; $P = .00005$) when a covariate measuring the number of affected individuals in the nuclear family was included. To evaluate further the contribution to the linkage signal from families with more than two affected relatives, we performed model-based linkage analysis with a recessive model and a range of penetrances, and we obtained maximum linkage at 70 cM (LOD score 3.16; $P = .00007$) with a penetrance of 0.3. We then estimated location by using GENEFINDER. The most likely location for a gene predisposing to IAs in the Finnish population is in a region with a 95% confidence interval of 11.6 cM ($P = .00007$) centered 2.0 cM proximal to D19S246.

Intracranial berry aneurysms (IAs [MIM 105800]) are saccular outpouchings of the intracranial arteries, most commonly at arterial bifurcations, characterized by arterial wall remodeling. Cerebral arteries are especially vulnerable to aneurysm formation, since they lack an external elastic lamina. Most cases of ruptured IAs result in a subarachnoid hemorrhage, associated with high morbidity and mortality. We showed previously that ~10% of individuals with IA in the Finnish population have a family history of IA, and we estimated the sibling relative risk to be 9%–16% (Ronkainen et al. 1993, 1997). Our goal has been to recruit multiplex families with IA from the Finnish population (Wills et al. 2003) so that candidate regions for susceptibility genes could be investigated by use of linkage analysis. We recently reported the results (Olson et al. 2002*c*) of a genomewide scan that used a 10-cM intermarker interval (Phase I, stage 1) with an initial set of 48 affected sib pairs (ASPs). Additional markers in regions with LOD score >0.8 ($\alpha \approx 0.05$) were genotyped to either support or reject the linkage (Phase I, stage 2) (Olson et al. 2002*c*). A two-phase, two-stage study design was adopted to reduce the labor and cost of performing the genomewide scan (Guo and Elston 2001). The most promising signal was on chromosome 19q, which reached a maximum multipoint LOD score of 2.58 between markers D19S245 and D19S246, a region supported by a maximum LOD −1 of ~22 cM.

Here, we report a follow-up study (Phase II) of the 19q region with an expanded sample size and increased marker map–density. To date, we have collected 346 Finnish families with at least two affected individuals among first- and second-degree relatives (Wills et al. 2003). An individual was scored as "affected with IA" when at least

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one saccular aneurysm was treated surgically, was identified at autopsy, or—in two instances where surgical treatment was contraindicated—when an IA was identified only by magnetic resonance angiography (MRA). Families with nonsaccular aneurysms or predisposing conditions—such as polycystic kidney disease, Ehlers-Danlos syndrome, and Marfan syndrome (Ronkainen et al. 1997; Schievink 1997)—were excluded. There were 121 extended families who had two or more living relatives affected with IA—and who were therefore suitable for genetic studies—who consented to donate blood. These families had a total of 362 subjects with IA (both living and dead), of whom 272 had spontaneous ruptures, with 63 of the ruptures causing death, and 90 subjects had IAs detected by imaging. All but two of the subjects with IA detected by imaging had their IAs repaired surgically. From these families, we selected individuals with at least one ASP per family. For such families, we also genotyped available parents, additional siblings, and additional family members, if additional affected relatives were available.

Table 1 summarizes the families and numbers of genotyped affected-relative pairs (ARPs) included in Phase I and in Phase II of this study. The Phase I sample set consisted of 22 extended Finnish families comprising 24 sibships and 48 ASPs; here, an additional 16 ARPs were included from those families (for two of the extended families, samples from key connecting individuals were unavailable) (Olson et al. 2002*c*). The Phase II sample set consisted of 99 Finnish families comprising 65 sibships and 91 ASPs plus 67 other ARPs; a combined sample (Phase I plus Phase II) consisted of 139 ASPs and an additional 83 ARPs (222 ARPs in total). Phase I included samples from asymptomatic first-degree relatives screened for IA by MRA (Ronkainen et al. 1997); consequently, there was proportionally approximately double the number of IAs detected by imaging in Phase I compared with Phase II.

There were 35, 11, and 28 cases of IA characterized by rupture and survival, rupture causing death, and detection by imaging, respectively, in Phase I, compared with 174, 52, and 62 in Phase II ($P < .015$ with the Pearson χ^2 test). Inclusion in ARP methods of cases of asymptomatic IA detected by imaging is expected to improve the power to detect linkage to predisposition to IA, although it will result in erroneously calling individuals affected if the phenotype studied is ruptured IA. Phase I probands were all recruited through the University Hospital of Kuopio in eastern Finland, whereas Phase II probands were recruited through either the University Hospital of Kuopio or the University Hospital of Helsinki in southern Finland. Eastern Finland is a younger population that underwent a rapid expansion from a small number of founders, whereas coastal southern Finland has been stably populated for millennia (Norio 2003; Varilo et al. 2003). Families from both sites were ascertained from registries of patients who underwent surgical treatment for IA; the proportion of cases of IA detected by imaging was the

Table 1

Number of Affected Pairs per Family in IA Data Sets

No. of Affected			No. of Families in Data Set			No. of Pairs,
Sib Pairs	Half-Sib Pairs	Other[b]	Phase I	Phase II	Combined	Combined[a]
		1		29	29	29
		2		1	1	2
		3		3	3	9
	1			1	1	1
1			10	48	58	58
1		1	1	4	5	10
1		2	3	3	6	18
1		4		1	1	5
1	2			2	2	6
1	5		1		1	6
2		8		1	1	10
3			3	3	6	18
3		3	1		1	6
4			1		1	4
6			1	2	3	18
10				1	1	10
11		1	1		1	12
Total			22	99	121	222

[a] The number of affected pairs computed as the sum of all pair types multiplied by the number of families.

[b] Includes non-sib and non-half-sib pairs, such as avuncular (aunt/uncle to niece/nephew), grandparental, and cousin pairs.

Table 2

Results of Standard and Covariate Linkage Analyses for IA Data Sets

Location (cM) and Model	Phase I (48 ASPs, 64 ARPs)				Phase II (91 ASPs, 158 ARPs)				Combined (139 ASPs, 222 ARPs)			
	LOD	P[a]	β[b]	γ[c]	LOD	P[a]	β[b]	γ[c]	LOD	P[a]	β[b]	γ[c]
60:[d]												
Baseline (2-parameter)	2.95	.0002	.00	1.14	.34	.145	.00	.32	2.37	.0008	.00	.64
Baseline (1-parameter)	2.25	.0006	.47		.17	.188	.07		1.62	.0032	.20	
Sex	2.41	.0024	.49	−.24	1.02	.063	.10	.22	1.91	.0077	.21	.13
N_{aff}	3.13	.0004	.69	.64	.34	.334	.04	.06	2.39	.0025	.15	.16
70:[e]												
Baseline (2-parameter)	2.43	.0007	.00	1.12	1.35	.0102	.00	.63	3.50	.00006	.00	.80
Baseline (1-parameter)	1.22	.0089	.30		1.06	.0136	.21		2.26	.0006	.25	
Sex	1.26	.0355	.29	−.08	1.45	.0226	.22	.18	2.38	.0026	.25	.08
N_{aff}	2.55	.0017	.47	.51	3.21	.0004	.16	.42	5.70	.000001	.23	.43
80:[f]												
Baseline (2-parameter)	3.07	.0002	.00	1.31	1.40	.0090	.00	.63	3.93	.00002	.00	.85
Baseline (1-parameter)	1.82	.0019	.43		.88	.022	.18		2.48	.00036	.26	
Sex	1.86	.0086	.45	.11	1.12	.0495	.17	.14	2.63	.0014	.26	.11
N_{aff}	2.03	.0058	.47	.21	2.03	.0058	.08	.23	3.99	.00005	.20	.24

Note.—Significant ($P < .05$) covariate effects are underlined.

[a] P value for entire model (overall linkage).

[b] Parameter estimate β, average linkage in the sample.

[c] Parameter estimate γ, change in linkage as a function of the covariate.

[d] Between markers D19S587 and D19S876.

[e] Between markers D19S178 and D19S545.

[f] Between markers D19S246 and D19S601.

only detectable phenotypic difference between the subpopulations. Nonetheless, there may have been additional undetected heterogeneity due to the population structure. Informed consent was obtained from all the research participants; the study was approved by the ethics committees at the Universities of Kuopio and Helsinki, as well as by the institutional review board of the Wayne State University School of Medicine.

Research participants were genotyped for 18 microsatellite markers spanning a 52-cM region on chromosome 19 that included the previous linkage signal. Microsatellite markers from a map generated by Marshfield Genetics (Center for Medical Genetics) were selected in order to obtain an ~3-cM map distribution and an average 80% heterozygosity, on the basis of family data from CEPH. Genomic DNA was isolated from blood by use of a DNA isolation kit (Puregene [Gentra Systems]). Template DNA and positive and negative controls were generated by linear whole-genome amplification with the primer-extension preamplification protocol (Zhang et al. 1992; Kuivaniemi et al. 2002). Primers for the microsatellites were ordered from Research Genetics, and genotyping was performed as described elsewhere (Olson et al. 2002*c*). Alleles were assigned on the basis of size in comparison with CEPH controls (1331-1 and 1331-2) and were stored in a custom Oracle database. Microsatellite markers were ordered according to version 10 of the Marshfield genetic map (Center for Medical Genetics). Markers genetically at the same locus were ordered according to the National Center for Biotechnology Information (NCBI) build 29 physical map. Family relationships within each pedigree, for families with genome scan data—that is, the Phase I samples—were tested using a genomewide test for genetic relationships (Olson 1999*b*). One non-sib pair was detected in one of the sibships in a family (family 21) and was removed from ASP analysis (Olson et al. 2002*c*) but was included as a half-sib pair in ARP analyses. Data were checked for possible genotyping errors, for all families, by use of the program MARKERINFO (S.A.G.E. 2003). The laboratory genotyping error rate was ~1%. No families were excluded. Multipoint identity-by-descent (IBD) estimates were obtained for all ARPs through use of the program GENIBD (S.A.G.E. 2003).

Our linkage design tests allele sharing by robust, model-free ARP analysis (Olson et al. 2002*a*, 2002*b*) to address the complex nature of IA (Olson et al. 2002*c*). For ease of comparison of these results with our previously published data, baseline LOD scores were computed, both by a model-free, two-parameter linkage method (Risch 1990) and by a one-parameter modification that allows for the inclusion of covariates as a surrogate measure of linkage heterogeneity. Specifically, we fitted the one-parameter modification (Goddard et al. 2001) of the conditional-logistic parameterization of the ASP LOD score method introduced by Olson (1999*a*). This method allows for the inclusion of continuous, as well as dichotomous, covariates that were incorporated

Table 3

The Covariate Sex for Affected Subjects in the Phase I and Phase II Samples

Sex	No. (%) of Affected Subjects		
	Phase I	Phase II	Total
Males	32 (43)	122 (46)	154 (45)
Females	42 (57)	146 (54)	188 (55)
Total	74	268	342

into the analysis by setting $\lambda_1(x) = \exp(\beta + \sum_{k=1}^{K} \gamma_k x_k)$. Parameterization is in terms of offspring recurrence risk ratio (λ_1), conditional on K covariates x_k. "Average" linkage in the sample is measured by the parameter β; the change in linkage as a function of the covariates is measured by γ_k. All covariates were centered on their sample mean before inclusion in the linkage model, to simplify specification of constraints on parameter estimates, improve numerical stability, and so that β reflects average allele sharing. In general, values of β and γ_k depend on the choice of coding scheme of the covariates; here, a linear transformation of the covariate does not change either the LOD score or the estimates of covariate-specific recurrence risk ratios. What is more important is that conclusions about the existence of locus heterogeneity and the extent or nature of locus heterogeneity do not depend on the estimated value of β (which may equal 0). To reduce the number of additional parameters needed for each covariate from two to one (Goddard et al. 2001), the MZ twin relative risk $\lambda_2(x)$ was constrained as $\lambda_2(x) = 3.634\lambda_1(x_k) - 2.634$. The restriction is a reparameterization of the Whittemore and Tu (1998) minmax constraint. The minmax one-parameter ASP LOD score was shown by Whittemore and Tu (1998) to be more robust against misspecifications than other one-parameter models, and it performed as well as or better than the constrained likelihood test that is based on the two-parameter model by Risch (1990). The one-parameter modification of the conditional-logistic parameterization model is implemented in the program LODPAL (S.A.G.E. 2003). *P* values were obtained by asymptotic distributions of likelihood ratio tests (Goddard et al. 2001). LOD scores (Nyholt 2000) are reported as the likelihood ratio statistics divided by 4.605 (i.e., $2\log_e 10$); critical values were obtained as described by Self and Liang (1987), to allow testing of both the significance of the contribution of a covariate and the overall evidence for linkage. The computer program GENEFINDER (Liang et al. 2001; Chiu et al. 2002; Glidden et al. 2003; Department of Biostatistics Web site, Johns Hopkins University) was used to obtain 95% CIs for the location of the gene on chromosome 19. Multipoint model-based linkage analysis was performed using MLOD (S.A.G.E. 2003).

Results of the model-free linkage analyses are summarized in table 2. Through use of the two-parameter model, increased IBD sharing was reflected by LOD scores of 3.07 ($P = .0002$), 1.40 ($P = .0090$), and 3.93 ($P = .00002$) in the Phase I, Phase II, and combined samples, respectively, at 80 cM. The one-parameter model gave lower LOD scores, indicating that the mode of inheritance in our sample was substantially different from the minmax model, which assumes a genetic mode of inheritance approximately halfway between dominant and recessive. Model parameters in the two-parameter case suggest a recessive model of inheritance. However, since estimation of the mode of inheritance for a complex disease lacks precision, and because the two-parameter model is not suitable for covariate analysis (Olson 2002), we chose to retain the minmax mode of inheritance in our covariate analyses to avoid additional multiple testing. Covariate effects were considered significant when the LOD score for the covariate model was 0.83 LOD units larger than the one-parameter baseline LOD, corresponding to the $\alpha = 0.05$ level of significance.

Covariates chosen were the number of females in the ARP (sex) and the number of first-degree affected relatives, living and deceased, in the nuclear family containing the ASP (N_{aff}). Sex was chosen as a covariate, since previous studies found that females have an increased risk of IA (Sekhar and Heros 1981; Østergaard 1989; Kissela et al. 2002). The covariate distributions in the two subpopulations were similar (tables 3 and 4). Inclusion of sex as a covariate did not significantly increase the LOD score in either sample (except the Phase II sample at 60 cM), which suggested that the subtype of IA due to this locus does not present a sex ratio that differs from that of the overall sample. The covariate N_{aff}, on the other hand, significantly increased the LOD scores (fig. 1; table 2). The highest LOD scores (and corresponding *P* values for overall linkage) were seen at 70 cM in the Phase I, Phase II, and combined sample sets: LOD score

Table 4

The Covariate "Number of Affected First-Degree Relatives" for the Phase I and Phase II Samples

No. of First-Degree Affected Relatives	No. (%) of Families		
	Phase I	Phase II	Total
0	0	27 (27)	27 (22)
2	10 (42)	46 (47)	56 (46)
3	12 (50)	20 (20)	32 (26)
4	1 (4)	2 (2)	3 (3)
5	1 (4)	1 (1)	2 (2)
6	0	0	0
7	0	1 (1)	1 (1)
Total	24	97	121

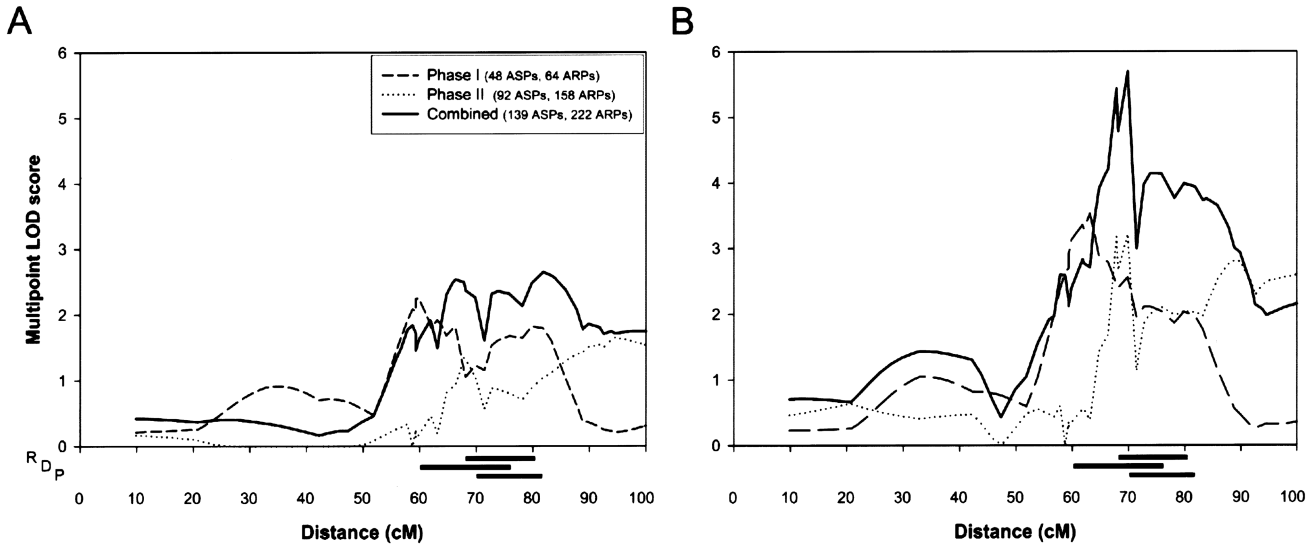

Figure 1 Multipoint LOD score analyses for chromosome 19. *A,* One-parameter baseline linkage analysis that allows for the inclusion of covariates. Note that the mode of inheritance is not optimal, which resulted in a decrease of LOD scores compared with the two-parameter baseline (table 2). *B,* One-parameter linkage analysis with N_{aff} as a covariate. The covariate significantly increased the LOD scores, which reached maximum values at 70 cM (LOD score 5.70) and at 80 cM (LOD score 3.99). The horizontal bars below each plot indicate the GENEFINDER (Department of Biostatistics Web site, Johns Hopkins University) 95% CIs (table 5) for the recessive (R), dominant (D), and partial-penetrance (P) models, respectively.

2.55 ($P = .0017$), LOD score 3.21 ($P = .0004$), and LOD score 5.70 ($P = .000001$), respectively. These results suggest that families with larger numbers of affected individuals are more likely to have linkage to this region. We recognized, however, that significance of this covariate might simply reflect the possibility that pairs from larger families often provide better information about allele sharing and thus may provide more power to detect linkage. We therefore tested a measure of marker informativeness (Kruglyak and Lander 1995; Kruglyak et al. 1996) as a covariate, but we observed little, if any, increases in LOD scores from the baseline model (results not shown), suggesting that evidence of stronger linkage in pairs from larger families is not explained by marker informativeness.

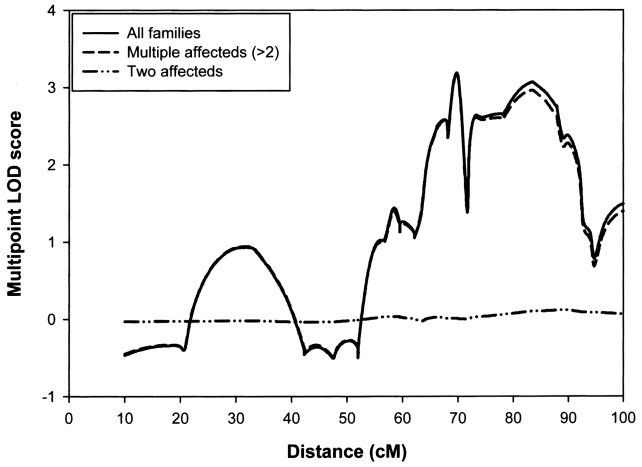

Figure 2 Model-based multipoint LOD score for chromosome 19. The plot shows the results from a recessive model, with disease allele frequency of 0.01, disease penetrance of 0.3, and a phenocopy penetrance of 0.01. Note that the solid line (all families) and the dashed line (families with more than two affected individuals) very nearly overlap; therefore, the dashed line is visible only in a few places.

The model-free linkage results suggested that ARPs from families with more affected individuals are more likely to show linkage and that the mode of inheritance is most likely recessive. To verify this, we followed up with multipoint model-based analysis (MLOD) (S.A.G.E. 2003), under the assumption of a recessive model with a disease allele frequency of 0.01 and the penetrances of the normal genotypes (i.e., phenocopy penetrance for sporadic cases) of 0.01. We then varied the penetrance of the disease genotype from 0.1 to 0.8 in increments of 0.1. This analysis included 15 additional multicase families with types of ARPs that are not analyzed by LODPAL (such as offspring of half-siblings or parent-offspring pairs) but can be included in model-based analyses that allow for arbitrary pedigree structures. The largest LOD scores, obtained using a penetrance of 0.3, are shown in figure 2 for the total sample of 136 families (LOD score 3.16; $P = .00007$), for 58 families with more than two affected individuals (LOD score 3.15; $P = .00007$), and for 78 families with two affected individuals (LOD score

0.10; $P = .25$). Varying the disease allele frequency and phenocopy penetrances did not improve the LOD scores. In parallel with our model-free results, families with more than two affected individuals contributed disproportionately to the linkage signal.

We tentatively interpret these results as supporting that there may be two IA susceptibility loci in close proximity on chromosome 19q, but location estimates of susceptibility loci by nonparametric analyses for small samples of complex diseases have been demonstrated to yield substantial variation (Roberts et al. 1999). We therefore obtained 95% CIs for the location of the gene on chromosome 19 under different genetic models (table 5; fig. 1). For models with complete penetrance and no phenocopies, the recessive model was a better fit ($Z = 3.43$; $P = .00031$) than the dominant model ($Z = 2.26$; $P = .012$). Here, Z is an asymptotically normally distributed linkage statistic at the location of maximum estimated sharing, as estimated by the program GENEFINDER (Department of Biostatistics Web site, Johns Hopkins University). For models with incomplete penetrance and phenocopies, recessive and dominant models gave identical results ($Z = 3.80$; $P = .000073$). Additional analyses that included the number of affected individuals as a covariate and/or used lower penetrance values did not further narrow the CI. The latter models defined a candidate region with CIs of 11.6 cM, centered ~2 cM proximal to D19S246. This CI included linkage peaks at both 70 cM (D19S545) and 80 cM (D19S246) (fig. 1). The 95% CIs obtained for both recessive models and the dominant model with incomplete penetrance overlap at markers D19S545, D19S606, and D19S246, with D19S545–D19S606 being the most likely interval.

Our results suggest that the most likely location for a susceptibility gene to IA is flanked by markers D19S545 and D19S246, the region that includes marker D19S606. There are currently 135 genes annotated in that region, of which 33 are predicted genes with an unknown function. If we consider the physiological and biological information on the 102 characterized genes, some plausible candidates include: (1) the D site of the albumin promoter (albumin D-box)–binding protein (*DBP* [MIM 124097; HUGO]), which is induced by angiotensin II in vascular smooth-muscle cells (Nonaka et al. 2001); (2) the histidine-rich calcium-binding protein (*HRC* [MIM 142705; HUGO]), which plays a role in the sarcoplasmic reticulum function and could thereby influence cerebral artery diameter (Pathak et al. 1992; Wellman et al. 2002); (3) the nitric oxide synthase–interacting protein (*NOSIP* [LocusLink locus ID 51070; HUGO]), a modulator of endothelial nitric oxide synthase activity (Dedio et al. 2001); and (4) the tumor upregulated CARD-containing antagonist of caspase nine (*CARD8* [LocusLink locus ID 22900; HUGO]), involved in cell survival (Razmara et al. 2002).

In conclusion, our follow-up studies on chromosome 19q13 strongly support our hypothesis that this locus harbors susceptibility genes for IA. Our results showed that ARPs from families with larger numbers of affected individuals provided the most linkage evidence, whereas inclusion of sex as a covariate did not significantly increase the LOD score. On the basis of our linkage analyses and further refinement of the candidate region with 95% CIs, we conclude that the most likely location for a gene predisposing to IA in our Finnish sample is a 6.6-cM region on chromosome 19q13.3 between markers D19S545 and D19S246. Future directions for research include high-resolution fine mapping of the 95% CI, prioritization of potential candidate genes by gene-expression analyses in IA tissues, and association studies of candidate genes in the interval. The locus described here is, however, most probably not the only locus predisposing families to IA. That fact was demonstrated by a linkage study in the Japanese population (Onda et al. 2001), which found different loci from those in the Finns. The major Japanese locus could, however, not be replicated in a highly selected sample of Japanese families with IA (Yamada et al. 2003), suggesting within-population heterogeneity. Such heterogeneity is not surprising, since (sub)population isolates can have different population histories and thus harbor different disease susceptibility loci. Replication studies in a variety of populations would give insight into the global representation of the 19q13.3 IA locus confirmed here.

Table 5

Predictions of the Candidate Gene Location for IA with 95% CI

Genetic Model and Penetrances	Z (P)	95% CI (cM)	Midpoint Position in cM (Nearest Marker)
Recessive:			
0, 0, and 1	3.43 (.00031)	68.1–80.4	74.2 (D19S606)
.2, .2, and .7	3.80 (.000073)	70.1–81.7	75.9 (D19S246)
Dominant:			
0, 1, and 1	2.26 (.012)	60.1–76.1	68.1 (D19S718)
.2, .7, and .7	3.80 (.000073)	70.1–81.7	75.9 (D19S246)

NOTE.—Results were generated using the program GENEFINDER (Department of Biostatistics Web site, Johns Hopkins University). Allele frequency = 0.01.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/ (for genetic distance between markers, map version 10)

Department of Biostatistics, Johns Hopkins University, http://www.biostat.jhsph.edu/biostat/research/genefinder.shtml (for the GENEFINDER program)

Foundation Jean Dausset—CEPH, http://www.cephb.fr/cephdb/ (for positive control genotype data and marker heterozygosity estimates)

HUGO Gene Nomenclature Committee, http://www.gene.ucl.ac.uk/nomenclature/ (for approved gene symbols)

LocusLink, http://www.ncbi.nlm.nih.gov/LocusLink/ (for *NOSIP* [locus ID 51070] and *CARD8* [locus ID 22900])

National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?org=hum=19 (for map view build 34; identification of candidate genes in locus of interest)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for IA, *DBP,* and *HRC*)

Statistical Analysis for Genetic Epidemiology (S.A.G.E.), http://darwin.cwru.edu/sage/index.php (release 4.3; for MARKERINFO, GENIBD, LODPAL, MLOD programs)

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