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SHORT COMMUNICATION

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Is there any evidence for linkage on chromosome 17cen in affected Japanese sib-pairs with an intracranial aneurysm?

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Abstract A linkage region on chromosome 17cen has previously been found in 29 Japanese families with a history of familial intracranial aneurysms (IA). To investigate whether there is evidence of linkage in affected Japanese sib-pairs we performed nonparametric and parametric linkage analysis of a total of 253 familial aneurysm cases including 111 affected sib-pairs (ASP). Ten microsatellite markers covering a 17.7 cM region were chosen, in accordance with previous work in which nominal *P* had been below 0.05. Statistical analysis was performed by use of Genehunter and Sibpal software. After calculation of the logarithm of the odds (LOD) and nonparametric linkage analysis (NPL) scores our study did not show any linkage in the region analyzed. Our conclusion did not change even after only ASP were analyzed. In contrast with a previous study examining multigenerational Japanese families with IA, most Japanese ASP may not have a genetic linkage to chromosome 17 cen.

Keywords Intracranial aneurysm · Sib-pair · Linkage analysis · Chromosome 17 cen

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Introduction

Several reports have recently been published on linkage studies that revealed different regions of suggestive linkage using familial intracranial aneurysm (IA) samples. Their cohorts consisted of affected sib-pairs (ASP) (Onda et al. 2001; Olson et al. 2002), several multigeneration pedigrees (Olson et al. 2002; Yamada et al. 2004), and large kindred (Roos et al. 2004; Nahed et al. 2005). Some of these findings have been confirmed by other studies (Farnham et al. 2004; van der Voet et al. 2004), but some could not be replicated (Yamada et al. 2003).

In a genome-wide scan for familial aneurysms of Japanese origin, Yamada et al. (2004) found suggestive linkage on chromosome 17 cen. In this work we sought to replicate genetic analysis of the 17cen region for a total of 253 familial IA cases consisting of sib-pairs from 106 pedigrees.

Materials and methods

The samples comprised 106 pedigrees collected through neurosurgical services certified by the Japanese Neurosurgical Society. 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 number of ASP was 111 from 90 pedigrees. The ASP comprised 80 pairs, 16 trios, seven groups of four siblings, and one of five, six, and seven siblings each. They all had a confirmed medical diagnosis of IA as described in our previous report (Onda et al. 2001): bigger than 5 mm, as diagnosed by conventional angiography, three-dimensional computed tomography angiography, magnetic resonance angiography, or surgical findings. The study included 16 further pedigrees with discordant sib-pairs, comprising 14 pairs, 1 trio, and 1 quartet. Total collected samples (including the discordant sib-pairs) consisted of 253

individuals—75 males and 178 females. All subjects were of Japanese ethnicity. The detailed clinical features of most of these families have been described elsewhere (Kasuya et al. 2000). The 253 genomic DNA samples were amplified by the polymerase chain reaction (PCR) using ten fluorescence-labeled (6-FAM, NED, VIC) and tailed primers. The PCR primers used to analyze the microsatellite markers around the chromosome 17cen region were chosen according to the fine-mapping findings described in the previous report (Yamada et al. 2004) (see Table 1; *D17S918*, *D17S1857*, and *AFMa126yd5* are not listed) and designed by accessing information from the UniSTS map (www.ncbi.nlm.nih.gov/genome/sts/). The size of the region with nominal $P < 0.05$ described in their publication was 17.7 cM.

Genomic DNA was isolated from whole blood cells, by use of a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany), or from buccal cells, by use of a BuccalAmp DNA Extraction Kit (Epicentre Technologies, Madison, WI, USA). PCR amplification was performed by use of standard procedures. Genotyping was performed on an Applied Biosystems (Foster City, CA, USA) model 3700 fluorescence-based, semi-automated DNA sequencer. The marker alleles were assigned by use of Genescan and Genotyper software (Applied Biosystems).

We used two different linkage methods to calculate a logarithm of likelihood ratio, which is represented as the parametric logarithm of odds (LOD) score and a nonparametric linkage analysis (NPL) score, and a nonparametric analysis (loglike LOD score) using the maximum likelihood allele sharing proportions (z-value) between ASP. The calculations were performed with the Genehunter program (Version 2.1) (Kruglyak et al. 1996). As the largest family study indicates a possible dominant model (Wills et al. 2003), we performed the parametric model-based analysis with the assumption of a dominant inheritance mode with a disease allele frequency of 0.01 and penetrances of the normal genotypes of 0.01 (van der Voet et al. 2004). The Sibpal program from the S.A.G.E. package (Version 3.1) (Elston et al. 1997) was used to estimate the mean ratio of alleles shared identical by descent (IBD) among ASP at each microsatellite marker. The value obtained was tested against the null hypothesis of no linkage ($\Pi = 0.5$). The statistic has a standard normal distribution under the null hypothesis; when IBD sharing is $>50\%$ the hypothesis of linkage is given and the test is then one-sided. To calculate the IBD value correctly we included the unaffected sibs from the discordant sib-pairs and determined their affected/unaffected status as unknown.

Results

We calculated multipoint LOD and NPL scores for individual families in a 17.7 cM region of chromosome 17 spanning from *D17S921* to *D17S1800*. We analyzed the data including markers that had heterozygosities larger than 0.7. Heterozygosity for the seven (out of ten) markers that had a value greater than 0.7 ranged from 0.71 to 0.82, with an average of $H_E = 0.747$ (Table 1).

Under the dominant model the maximum observed multipoint parametric LOD score was −12.74 at *D17S921*. The maximum NPL score (MNS) calculated was −0.91 with a nominal $P = 0.84$ at *D17S1294*. No regions of potential interest (nominal $P < 0.05$) by multipoint NPL scores were observed (Table 1, Fig. 1). Nonparametric analysis of ASP only yielded loglike LOD scores of 0 for all marker sites. The linkage analyses including the uninformative markers (*D17S918*, *D17S1857*, *AFMa126yd5* that had heterozygosities of 0.5, 0.48, and 0.53, respectively) did not change the major portion of results (data not shown).

In summary, these results cannot confirm a linkage to the chromosome 17cen region in these 253 samples of 106 pedigrees.

Discussion

To address the complexity of issues such as low sibling relative risks, because of the high population prevalence [2.7–6.5% (Ujiie et al. 1993; Nakagawa and Hashi 1994)], the presence of multiple genetic loci, and incomplete penetrance of genetic loci, we used a model-free allele-sharing analysis. We could not identify any evidence of linkage to the chromosome 17cen region by multipoint analysis with the Genehunter software and by singlepoint analysis using Sibpal.

Table 1 Microsatellite markers and linkage analysis results

Position kb (cM)	Marker/gene	LOD	Heterozygosity	NPL(all)	*P* value (GH)	IBD	*t*	*P* (Sibpal)
14461 (38 cM)	D17S921	−12.74	0.71	−1.144	0.89	0.46	−1.97	0.974
17465 (45.9 cM)	D17S2196	−20.22	0.72	−1.32	0.93	0.49	−0.342	0.633
20924 (48.2 cM)	D17S1871	−25.32	0.72	−1.292	0.92	0.49	−0.506	0.693
21493 (51.0 cM)	D17S783	−26.16	0.75	−1.236	0.91	0.51	0.509	0.306
26806 (52.4 cM)	D17S1824	−24.72	0.82	−1.133	0.89	0.49	−0.397	0.654
28528 (54.1 cM)	D17S1294	−21.84	0.77	−0.914	0.84	0.51	0.195	0.423
30082 (55.7 cM)	D17S1800	−22.05	0.74	−1.078	0.88	0.46	−1.432	0.922

GH values were calculated using Genehunter software; Sibpal values were calculated using Sibpal software; IBD, identity by descent; LOD, logarithm of odds score; NPL, nonparametric LOD score

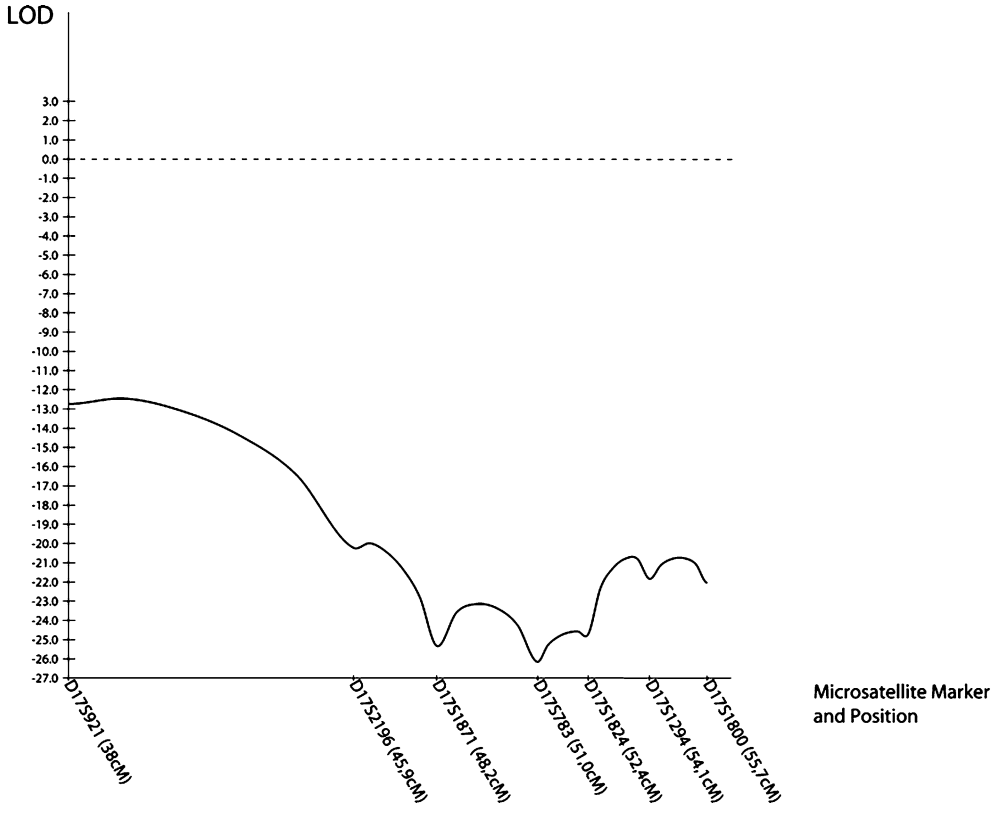

Fig. 1 Multipoint logarithm of odds (*LOD*) score of a 17.7 cM-sized region on chromosome 17

Genome-wide IA linkage studies of different ethnicity which have been published so far show various regions with a suggestive linkage, emphasizing the genetic heterogeneity (Onda et al. 2001; Olson et al. 2002). In two sib-pair analysis studies tentative linkage was found on chromosome 7q near the telomere in a Finnish cohort (Olson et al. 2002) but that did not overlap a region near the centromere, which was found in our linkage analysis of Japanese sib-pairs (Onda et al. 2001). The findings of Olson et al. were later reproduced with an expanded sample size (including the same initial group) and increased marker map-density (van der Voet et al. 2004). But the evidence of linkage at chromosome 7q11 in our Japanese sib-pair analysis could not be replicated by Yamada et al. who studied samples of 71 blood relatives from 14 multigenerational familial IA cases of Japanese origin (Yamada et al. 2003). In subsequent genome-wide linkage analysis the latter group examined familial aneurysm samples in 29 families with three or more affected individuals in which they found three regions on Chromosome 17 cen, 19q13, and Xp22 (Yamada et al. 2004).

In this study and our previously published genome-wide linkage analysis (Onda et al. 2001), we mainly examined ASP, as opposed to the mentioned pedigree structures of the other previously published Japanese linkage analyses (Yamada et al. 2003, 2004).

The mode of inheritance of IA is still undetermined, most probably because of locus heterogeneity. This makes the search for disease-causing genes more difficult and only large collections of kindred or ASP enable linkage analyses to furnish meaningful results. The affected sib-pair method is the statistical approach most commonly used to identify susceptibility loci of complex genetic traits (Weeks and Lange 1988). This method differs from classic linkage analysis, in that it makes no assumptions about the mode of inheritance of a trait, and might be the reason several previously published linkage analysis IA reports point to different regions.

A different example for within-population heterogeneity is the linkage study by Roos et al. (2004) who found linkage at chromosome 2p13 in a consanguineous Dutch family but at the same time could not reproduce the result in three unrelated families . The subpopulation isolates have different population histories and may thus harbor different disease susceptibility loci.

Possible replication of the several linkages found in the different studies calls for many more families to be analyzed in the form of consortium studies, with, at the same time, distinction of subpopulations of different demographical background.

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