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SHORT COMMUNICATION

Identification of an autosomal dominant locus for intracranial aneurysm through a model-based family collection in a geographically limited area

Chul-Jin Kim[1], Seung Soo Park[1], Hyun-Seo Lee[2], Hea-Jong Chung[2], Woori Choi[2], Jin-Ho Chung[2], Jin-Man Kim[3] and Seong-Tshool Hong[2]

Intracranial aneurysm (IA) is characterized by an abnormal bulging of one of the arteries in the brain and is heavily affected by genetic factors. Although IA is a very serious disease because of its severity and prevalence in the general public, the gene causing IA has not yet been identified due mainly to the lack of definitive genetic loci for the disease. Following a model-based family collection that recruited families from a geographically limited area that inherited IA as an autosomal dominant trait, we conducted a genome-wide linkage analysis. Significant evidence of linkage to IA was found on chromosome 8p22.2 with a maximum two-point logarithm of the odds ratio score of 3.61 under an autosomal dominant model of inheritance. The methods described in this study could be applied to localize disease-causing genes of other complex diseases through either a genome-wide linkage analysis or a genome-wide association study.
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INTRODUCTION

Intracranial aneurysm (IA) is characterized by an abnormal bulging of an artery in the brain, which eventually ruptures and leads to bleeding into the brain or the subarachnoid space.[1] It is estimated that up to 1 in 15 people will develop an IA during their lifetime.[2] Epidemiological evidence suggests that genetic factors have a more important role than environmental factors in the etiology of IA. The risk of IA in first-degree relatives of patients with IA is four times higher than in the general public, whereas the relative risk in siblings is six times higher than in the general public.[3]

Because IA is a common disease with severe consequences and is strongly influenced by genetic factors, there have been worldwide efforts to clone the disease-causing gene of IA.[4] However, despite the recent identification of several IA-susceptibility genes through genome-wide association studies (GWAS),[5–8] the disease-causing gene of IA has not been cloned, and the pathogenic mechanism of IA remains unclear. The genetic factors causing IA seem to be quite heterogeneous, autosomal dominant and autosomal recessive, and undetermined modes of inheritance for familial IA have been reported.[9] Although a total of seven genetic loci have been suggested for IA-causing genes to date, definitive loci for IA (that is, those with a two-point logarithm of the odds ratio (LOD) score >3) are not known.[10]

Because IA is potentially caused by a variety of different genes,[11] a typical collection of families with IA for linkage analysis could be genetically heterogeneous even if all of the IA patients in the collection were phenotypically the same. Thus, genetic heterogeneity in the cause of IA in each family could offset a positive LOD score at each locus during linkage analysis. For our study, we recruited only IA-afflicted families from a geographically limited area who also shared a common mode of inheritance, which presumably means that their IA is caused by a homogenous genetic factor. The genome-wide linkage analysis of this family collection resulted in the successful localization of an IA-causing gene to chromosome 8p22.2.

MATERIALS AND METHODS

Clinical diagnosis and pedigrees

A genome-wide linkage analysis was conducted to find the locus of the IA-causing gene. A total of 600 patients who were treated for IA at either Chonbuk National University Hospital (Chonju, South Korea) or Hana Hospital (Ilsan, South Korea) were identified. After consent was obtained, the patients or their relatives were interviewed to determine whether there were other family members who had IA. When additional affected persons were identified within a family, their medical records were thoroughly examined to confirm the diagnosis of IA. Because the genetic cause of IA seems to be heterogeneous,[11] we focused only on the families that met the following two

[1]Department of Neurosurgery and Research Institute of Clinical Medicine, Chonbuk National University Medical School and Hospital, Chonju, Chonbuk, South Korea; [2]Laboratory of Genetics and Department of Microbiology, Chonbuk National University Medical School, Chonju, Chonbuk, South Korea and [3]Department of Neurosurgery, Hana Hospital, Ilsan, Kyungkido, South Korea
Correspondence: Professor ST Hong, Laboratory of Genetics and Department of Microbiology, Chonbuk National University Medical School, Chonju, Chonbuk 561-712, South Korea.
E-mail: seonghong@jbnu.ac.kr
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criteria: first, a dominant inheritance pattern and, second, paternal and maternal lines both originating in Chonbuk province. Families with known genetic diseases associated with IAs, such as Ehlers–Danlos syndrome type IV, Marfan syndrome, neurofibromatosis type I, autosomal dominant polycystic kidney disease or achondroplasia,[12] were excluded from this study.

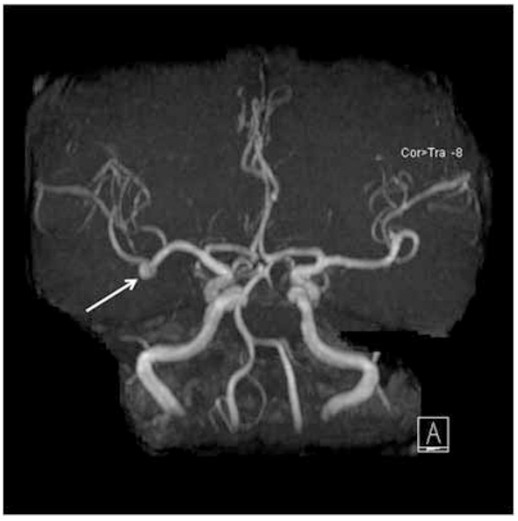

Figure 1 Magnetic resonance angiography of a typical IA patient. The 21-year-old male patient has a right middle cerebral artery bifurcation aneurysm (arrow).

A total of five families with dominant inheritance of IAs agreed to join this study. Magnetic resonance angiography was performed on all of the members of the five families aged 20 years or older, regardless of clinical signs and symptoms of IA. The magnetic resonance angiographies of each family member were examined by both a radiologist and a neurosurgeon.

Genotyping and linkage analysis

Genomic DNA was extracted from blood samples from the IA families for linkage analysis. The entire genomes of the IA families were mapped at 10 cM intervals using the ABI Linkage Mapping Set (ABI, Carlsbad, CA, USA). Two-point and multipoint LOD score calculations were carried out using the MLINK and LINKMAP programs from the LINKAGE package (v5.1).[13] A large-scale population study indicates that the prevalence of IA slightly but steadily increases with age,[14] so we conducted our linkage analysis with different age-dependent penetrance rates. The prevalence of IA was estimated from previous IA linkage studies by other groups.[15] The penetrance of IA was set to be 70% by age 20–40 years, 80% by age 41–60 years and 90% for those over 60 years of age. The frequency of IA in the general population was set at 1%. Heterogeneity tests were carried out using the HOMOG program.

RESULTS

As an attempt to recruit only IA families that shared a common mode of inheritance, family histories and patterns of IA inheritance were thoroughly studied. Although we found that IA segregated as an autosomal dominant trait in 17 families among the 35 IA families

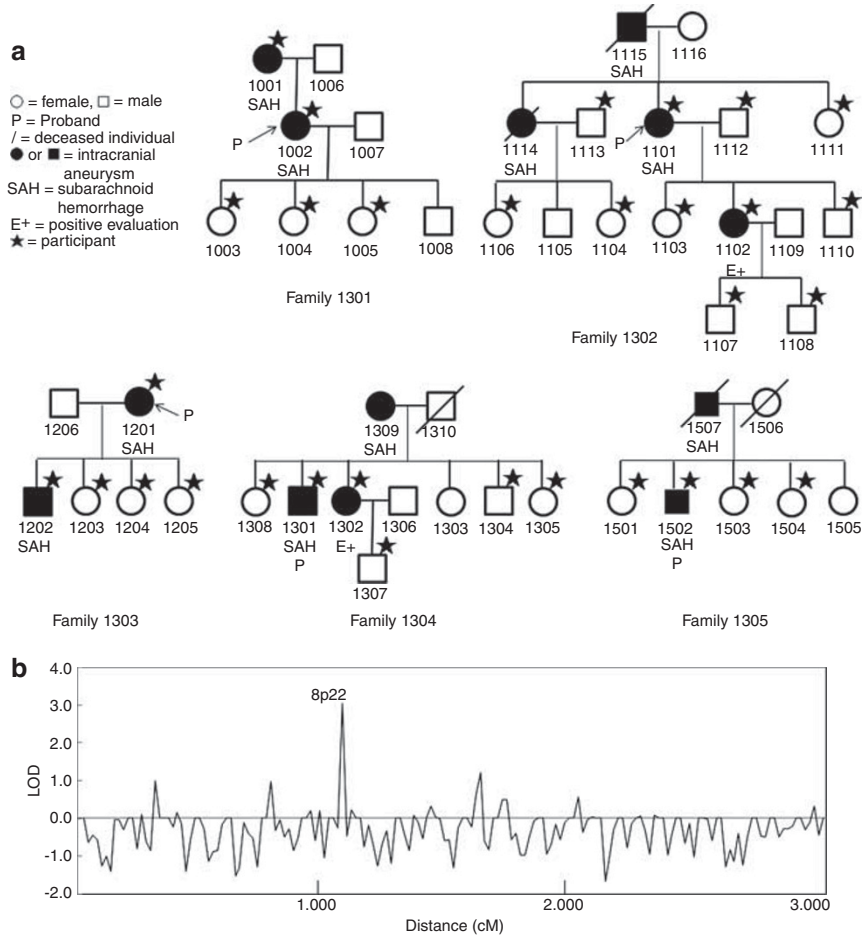

Figure 2 Familial intracranial aneurysm pedigrees (**a**) and the genome-wide screening results of the intracranial aneurysm families (**b**). Affected and unaffected individuals are represented by filled and unfilled markers, respectively. Markers with diagonal lines indicate deceased individuals.

Table 1 Two-point LOD score results between the disease locus and microsatellite markers on chromosome 8

		Recombination fraction (θ)				
Markers	*Physical distance (Mb)*	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*
D8S550	9.81	−0.99	0.13	1.04	1.49	0.98
D8S552	11.28	3.61	3.50	3.07	2.69	1.92
D8S1827	13.36	3.14	3.05	2.83	2.42	1.79
D8S549	14.19	2.87	2.81	2.62	2.31	1.71
D8S258	18.91	−0.51	−0.19	0.32	0.76	0.61

Abbreviation: LOD, logarithm of the odds ratio.

examined, only 5 families had maternal and paternal lineages that both originated from the Chonbuk province of Korea. From these families, all living first-degree relatives of IA patients aged 20 years or older were invited for magnetic resonance angiography screening. Each individual with an IA larger than 5 mm was diagnosed as an IA patient. Figure 1 shows an anteroposterior magnetic resonance angiography image of a typical IA patient with a right middle cerebral artery bifurcation aneurysm. The five families studied had a total of 13 affected individuals and 34 unaffected individuals (Figure 2). DNA samples were available from 31 individuals, including 9 affected and 22 unaffected individuals. These families were not consanguineously related to each other.

All families in this study were genotyped using a total of 400 microsatellite markers, spanning the genome at an average resolution of 8.4 cM. In parametric genome-wide screening, an LOD score of >3.0 was found only around marker D8S549 on chromosome 8p22 (Figure 2). No other chromosomal regions had an LOD score suggestive of linkage (>1.5). Fine mapping with additional markers was performed for the 8p22.2 region (Table 1). The highest two-point LOD score was 3.61 with marker D8S552 ($\theta=0$).

DISCUSSION

Despite recent research progress, neither the cause nor the pathogenic mechanisms leading to IA has been established, which is mainly because of the failure to identify the causative genes.[16] The genetic heterogeneity of IA makes it very difficult to identify IA-causing loci through linkage analysis.[11] The genetic heterogeneity contributes negative values to the LOD score calculation at the site of a disease-causing gene. To overcome these negative-offset effects, linkage analysis of complex diseases such as IA has been conducted using large collections of families with the disease. However, mere large collection analysis is not only tedious and time consuming but also does not eliminate the negative-offset effects. In this study, we successfully found an IA locus by performing a genome-wide linkage analysis after a model-based family collection. This collection recruited families from a geographically limited area that inherited IA as an autosomal dominant trait. This approach could be applied to localize the causative genes of other complex diseases through genome-wide linkage analysis. We suggest that our approach could identify disease-associated or disease-causing genes much more easily and efficiently than current methods if used for GWAS.

Recently, a large scale GWAS on IA using a high-density single-nucleotide polymorphism array was performed on a total of 20 072 individuals.[7] This study identified five loci showing evidence for association with IA on 8q, 9p, 10q, 13q and 18q. However, the highest odds ratio reported was only 1.31, suggesting that the genetic etiology of IA is very heterogeneous. Therefore, familial linkage studies of IA must examine whether the identified loci encode disease-causing genes or simply disease-susceptibility genes. The 8q locus is especially interesting because it was repeatedly identified by different large-scale GWAS studies.[8] There is a strong possibility that the 8p22.2 locus, identified in this work, is essentially the same locus as the 8p locus identified by the large-scale GWAS studies.

We have mapped a new locus for autosomal dominant IA to 8p22.2 through a model-based family collection in a geographically limited area. We suggest that our approach is an excellent method for determining a disease locus for other complex diseases. The 8p22.2 region comprises of 23 genes and contains several interesting candidate genes for IA such as vacuolar protein sorting 37 homolog A, myotubularin related protein 7, fibrogen-like 1 and fibroblast growth factor 20. Because these genes are involved in growth or maintenance of blood vessels, they are excellent candidates for an IA-causing gene.

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