Published in final edited form as:

Neurosci Lett. 2008 January 24; 431(1): 31–35. doi:10.1016/j.neulet.2007.11.014.

Meta-analysis of whole genome linkage scans for intracranial aneurysm

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Abstract

Background and Purpose: Genetic predisposition likely plays an important role in the development of intracranial aneurysms. We carried out a genome search meta-analysis to identify loci associated with intracranial aneurysm.

Methods: We identified previous whole genome linkage analyses by searching PUBMED. Five studies reported by separate investigators where detailed data could be obtained were included in our analysis. We synthesized the available genome-wide scan data by using a heterogeneity-based genome search meta-analysis.

Results: We identified two linkage sites on chromosome 3 and 17 which had p values <0.01 for association with intracranial aneurysm.

Conclusion: Our findings confirm the association of a locus on chromosome 17 and identify a new linkage site on chromosome 3 for intracranial aneurysm. The new locus contains a number of potential gene candidates including kininogen-1 precursor, fibroblast growth factor-12 and endothelin converting enzyme 2.

Keywords

Intracranial aneurysm; genome linkage scan; meta-analyses

Introduction

Approximately 10% of patients with a subarachnoid haemorrhage have a family history [1]. Prospective studies suggest that subarachnoid haemorrhage occurs in approximately 2% of first degree relative of patients who have presented with this problem, an incidence that is four to ten times that of the general population [1,2]. A number of whole-genome linkage studies have now been performed in families in which there are multiple members with a history of intracranial aneurysm [3-10]. The aim of this study was to carry out a genome search meta-analysis in order to identify genetic loci that may play a role in the development of intracranial aneurysm.

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Methods

Selection of genome scans and data collection

Suitable studies for inclusion in the meta-analysis were identified by searching PUBMED database with the terms “intracranial aneurysm” and “genome” or “linkage”. Hand searching of reference lists of identified articles was also carried out. We identified eight whole-genome scans between the years 2001 and 2007 [3-10]. Studies included in our analysis all assessed the linkage of autosomal genome sites with familial intracranial aneurysm. Study inclusion required presentation of detailed genome maps from autosomal chromosomes for further analysis. We did not consider the second stage of scans where candidate regions from the first stage were fine mapped. One study was excluded because data from affected individuals had already been included in a previous report [3]. Where studies failed to present sufficient data for analysis the information was requested from the corresponding authors. Two studies were not included due to a lack of linkage scores [4,5]. Finally, five intracranial aneurysm whole-genome scans met the inclusion criteria and were included in the genome scan meta-analyses [6-10]. The following information was recorded from the selected studies: Number of families assessed, definition of affected and unaffected cases, ethnicity, number of affected cases, and method of analysis and linkage sites identified. Linkage data (LOD scores) were extracted from each study for the rank-ordering procedure.

Data analysis

We synthesized the available genome-wide scan data by using a heterogeneity-based genome search meta-analyses (HEGESMA) proposed by Zintzaras and Ioanidis [11] to identify genetic regions with linkage to intracranial aneurysm. In brief, the genome (autosomes) was divided into 120 approximately 30-cM regions (referred to as bins) defined by the Marshfield genetic linkage maps

(<http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp>). For each study, every bin was assigned a within-study rank based on the maximum linkage score within the bin. Bins were ranked in descending order from 120 to 1 (rank 120 designates the bin with the most significant result). The summed rank (R_{sum}) across studies was computed for each bin. The P-value for summed ranks (P_{sumrnk}) and the P-value for the heterogeneity (Q) statistics were determined by 10,000 permutations of the ranked data sets that were multiplied by a weighting factor proposed by Levinson et al. [12] to allow results to reflect the relative contribution of each study and allow for multiple testing. The P_{sumrnk} measures whether the R_{sum} in a specific bin is significantly higher than expected; a significant P-values for the Q statistic indicates heterogeneity of linkage evidence between studies, non-significant values suggest consistent linkage evidence across studies. All computations were performed using the HEGESMA_v2.0 software available online at <http://biomath.med.uth.gr>. Two large Japanese studies [8,10] were included in a sensitivity analysis, excluding the three Caucasian studies [6,7,9] with the highest maximum log of the odds ratio (LOD) reported, which were also the three smallest studies.

Results

Individual genome scans

The characteristics of five individual genome scans [6-10] included in our meta-analyses are given in Table 1. These five studies assessed a total of 263 individuals from 118 families, with two studies providing over 90% of the cases [8,10]. The number of autosomal markers used for genome scans ranged from 390 to 10,000. All studies demonstrated sites with LOD scores ≥ 3 , however the loci identified varied between investigations. Loci included 1p34.3-36.13 (LOD 4.2), 5q (LOD 3.2), 7q11 (LOD 3.2), 11q24-25 (LOD 4.3) and 17cen (LOD 3.0) (Table 1).

Meta-analyses of intracranial aneurysm genome scans

Figure 1 shows the summed ranks (R_{sum}) for each bin (vertical axis) plotted against the bin location within the chromosomes (horizontal axis). Our primary analysis identified 5 loci which were associated with intracranial aneurysm at a p value ≤ 0.05 : 17p12-q21.33 ($P_{\text{sumrnk}} = 0.0011$), 3q27.3-3qter ($P_{\text{sumrnk}} = 0.0024$), 11q24.1-qter ($P_{\text{sumrnk}} = 0.0253$), 1p35.3-p32.2 ($P_{\text{sumrnk}} = 0.0284$) and 14q24.1-q32.12 ($P_{\text{sumrnk}} = 0.0463$). As a sensitivity analysis we also assessed findings by pooling the two large Japanese studies [8,10]. Significant loci associated with intracranial aneurysm were similar (Table 2). Association with chromosomal sites at 17p12-q21.33 ($P_{\text{sumrnk}} = 0.0044$), 3q27.3-3qter ($P_{\text{sumrnk}} = 0.0097$), 14q24.1-q32.12 ($P_{\text{sumrnk}} = 0.0113$) and 11q24.1-qter ($P_{\text{sumrnk}} = 0.0115$) were confirmed. We detected no significant heterogeneity of linkage evidence between studies. Table 2 summarizes the ten chromosomal bins with the lowest p values for association with intracranial aneurysm ordered by summed ranks (R_{sum}) and the sensitivity analysis.

Discussion

Most intra-cranial aneurysms likely result from a combination of environmental and genetic factors. Histology studies suggest the importance of vascular smooth muscle cell loss and degeneration of the internal elastic lamina for intracranial aneurysm formation [1]. Endothelial injury and inflammation have also been suggested to play a role in aneurysm formation based on studies in animal models [13,14]. The familial predisposition to subarachnoid haemorrhage and the relatively young age of presentation support the importance of genotype in the development of intracranial aneurysms [1,2]. In this meta-analysis we identified two regions on chromosome 3 and 17 to be associated with familial intracranial aneurysm, with p values < 0.01 (Table 2, Figure 1). Our findings with respect to chromosome 17 confirm that from Yamada and colleagues' study, which contributed 35% of the affected cases for our analysis [8]. Candidate genes in this area of the genome include those important in inflammation, immunity and endothelial function (Table 3). In a more recent analysis by the Kyoto group the investigators selected out 9 candidate genes in this linkage region to assess in more detail [15]. The authors reported mutations at a number of locations within *TNFRSF13B* in patients with familial intracranial aneurysm and identified a protective haplotype based on allelic variation within this gene in a case-control study [15]. The product of *TNFRSF13B* is believed to play an important role in B cell function and mutations in this gene have also been linked to immunodeficiency [15]. A clear functional mechanism for *TNFRSF13B* in intracranial aneurysm is yet to be defined.

We also identified a linkage site on the long arm of chromosome 3 which has not previously been associated with intracranial aneurysm (Table 1). Candidate genes in this genome region include *KNG1* (kininogen-1 precursor), *FGF12* (fibroblast growth factor 12) and endothelin converting enzyme 2 (Table 3). A defect in kininogen has been associated with internal elastic lamina breaks and aortic aneurysm development within a rat model [16]. The fibroblast growth factor family of peptides play an important role in the control of vascular smooth muscle function and thus defects in this pathway may be relevant to aneurysm formation [17].

Endothelin is a potent endothelium-derived vasoconstrictor [18,19]. Endothelin has been implicated in multiple cardiovascular disorders including hypertension and endothelial dysfunction [20]. The endothelin converting enzyme 2 (*ECE2*, 3q28-q29), residing within our linkage region on distal chromosome 3, participates in endothelin synthesis. A number of studies have suggested a role for endothelin in intracranial aneurysm [21,22]. Elevated concentrations of circulating endothelin have been demonstrated in patients following intracranial aneurysm rupture and endothelin has been implicated in vasospasm in these subjects [21]. Recent studies within a rat model however suggest that endothelin may play a more direct role in intracranial aneurysm development [22]. Sadamasa and colleagues

demonstrated upregulation of endothelin within aneurysms and reduced development of aneurysms in rats receiving an endothelial B receptor antagonists [22].

Interpretation of whole genome meta-analyses needs to take into account a number of limitations. Firstly the technique requires detailed presentation of linkage data, which we were unable to obtain in two identified studies [4,5]. Secondly, the summation that is performed involves the mixing of genetically distinct populations. In our analysis the main population was from Japan, with smaller contributions from the USA and the Netherlands. In order to address this issue however we carried out a sensitivity analysis in which we limited assessment to the two larger Japan studies. Our findings from the later assessment were similar to the main analysis (Table 2). Given the size of the 3 smaller studies however the relevance of our findings to non-Japanese populations is unclear. Replication of findings is an important requirement in any genetic association study and larger investigations in other populations are still required.

In conclusion this genome search meta-analysis suggests the linkage of 3q27.3-3qter and 17p12-q21.33 with intracranial aneurysm. A number of candidate genes in these regions warrant further study in larger populations including endothelin converting enzyme 2.

Acknowledgements

Research undertaken by JG is funded by the National Institute of Health, USA (RO1 HL080010-01), NHMRC, Australia (project grant 379600, fellowship 431503) and NHF, Australia.

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Figure 1.

Summed rank scores from five whole genome linkage studies for intracranial aneurysm. Individual autosomes were sub-divided into ~30 cM bins represented by a diamond shaped dots. For example, chromosome 1 comprises of ten bins (1.1-1.10), chromosome 3 contains eight bins (3.1-3.8), and chromosome 17 has four bins (17.1-17.4), and so forth, according to the chromosomal length. Bins are ordered ascendant from 1 corresponding to the bin 1.1 to 120 (bin 22.2). Chr= Chromosome; 1% and 5% boundary at $p < 0.01$ and 0.05 respectively.

Table 1

Summary of studies included in meta-analysis.

Reference	Location	Ethnicity	Criteria for cases	Criteria for unaffected	Number of families included	Number of cases	Weighting factor	Markers	Analysis	Linkage sites
Ozturk et al. (2006)	Yale, L.A, USA	Caucasian	≥2 family members affected by IA	Negative MRAs or CTAs screening	2	4	0.2763	2 stage: i) Affimetrix GeneChip Mapping 10K Xba Array, ii) microsatellite (n=13)	Multi-point analysis of linkage assuming AD with 70-99% penetrance	11q24-25 (LOD 4.3), 14q23-31 (LOD 3.00)
Nahed et al. (2005)	New Haven, USA	Caucasian	≥2 family members affected by IA or SAH	age≥30 yr and no SAH (n=3) or imaging ICA (n=8)	1	6	0.3384	2 stage: i) Affimetrix GeneChip Mapping 10K Xba Array, ii) microsatellite (n=23)	Multi-point analysis of linkage assuming AD with 70-90% penetrance	1p34.3-36.13 (LOD 4.2)
Yamada et al. (2004)	Japan	Japanese	≥3 family members affected	age≥60 yr and negative imaging	29	93	1.3322	2 stage: i) ABI PRISM Linkage Mapping Set v2 (400 microsatellite markers), ii) fine mapping at 1 to 2 cM densities	Multi-point nonparametric (model-free) method	17cen (LOD 3.00) 19q13 (LOD 2.33) Xp22 (LOD 1.80)
Roos et al. (2004)	Amsterdam, the Netherlands	Caucasian	Large Dutch consanguineous family with IA in one generation	age≥18 yr and negative imaging	1	6	0.3089	2 stage: i) Screening set 6; Marshfield clinic, Marshfield Wis (n=390 microsatellite markers), ii) fine mapping	Parametric testing was performed using a recessive mode of inheritance and assuming penetrance of 70% to 90%	2p13 (LOD 2.94), 5q (LOD 3.23)
Onda et al. (2001)	Tokyo, Japan	Japanese	≥2 family members affected and IA >5 mm	No history of SAH and negative imaging	85	154	1.7143	Linkage Mapping Set version 2 (Applied Biosystems), (404 microsatellite markers)	Multi-point nonparametric linkage analyses	5q22-31 (LOD 2.24), 7q11 (LOD 3.22), 14q22 (LOD 2.31)

SAH= Sub-arachnoid haemorrhage; ICA= Intra-cranial aneurysm; SNP= Single nucleotide polymorphism; AD= Autosomal dominant; yr= year.

Table 2
HEGESMA results showing top 10 chromosomal bins and its sensitivity test

Test	Bin number	Chromosome site	Marshfield location (cM)		Cytogenetic band	R _{sum}	P _{summk}	P-value for Q
			Start	End				
HEGESMA	102	17.2	25.14	63.62	17p12-q21.33	439	0.0011	0.1036
	28	3.8	201.14	228.14	3q27.3-3qter	432	0.0024	0.1573
	78	11.6	123.00	147.77	11q24.1-qter	404	0.0253	0.7386
	3	1.3	54.30	83.07	1p35.3-p32.2	401	0.0284	0.1066
	91	14.3	74.96	105.00	14q24.1-q32.12	390	0.0463	0.8951
	61	9.1	0.00	27.32	9pter-p22.3	386	0.0544	0.5657
	35	4.7	159.30	181.93	4q32.1-q35.1	385	0.0574	0.8310
	90	14.2	40.11	74.96	14q13.1-q24.1	379	0.0708	0.1925
	39	5.3	64.14	97.82	5q11.2-q14.3	372	0.0929	0.5967
	40	5.4	97.82	131.48	5q14.3-q23.2	370	0.0985	0.5764
Sensitivity test	102	17.2	25.14	63.62	17p12-q21.33	229	0.0044	0.1537
	28	3.8	201.14	228.14	3q27.3-3qter	225	0.0097	0.1555
	91	14.3	74.96	105.00	14q24.1-q32.12	224	0.0113	0.2538
	78	11.6	123.00	147.77	11q24.1-qter	223	0.0115	0.0257
	35	4.7	159.30	181.93	4q32.1-q35.1	217	0.0237	0.0665
	61	9.1	0.00	27.32	9pter-p22.3	209	0.0430	0.4888
	3	1.3	54.30	83.07	1p35.3-p32.2	202	0.0625	0.2858
	20	2.10	233.62	269.07	2q35-qter	196	0.0939	0.3237
	90	14.2	40.11	74.96	14q13.1-q24.1	194	0.1066	0.4786
	39	5.3	64.14	97.82	5q11.2-q14.3	191	0.1147	0.8011

Table 3

Examples of candidate genes in relation to identified linkage loci on chromosome 3 and 17.

Symbol	Gene	Locus	OMIM number	Functions
<i>FGF12</i>	fibroblast growth factor 12	3q29-qter	*601513	cell-cell signalling, signal transduction, nervous system development
<i>KNG1</i>	kininogen 1, precursor	3q27	+228960	smooth muscle contraction, inflammatory response
<i>ECE2</i>	endothelin converting enzyme 2	3q28-q29	*610145	converts big endothelin-1 to endothelin-1
<i>TNFRSF13B</i>	Tumor necrosis factor receptor superfamily member 13B	17p11.2	*604907	Inflammation and immune function
<i>NOS2A</i>	Nitric oxide synthase IIA	17cen-q11.2	*163730	Inflammation and immune function
<i>CSF3</i>	Colony stimulating factor 3	17q11.2-q12	*138970	Endothelial progenitor cells and inflammation