Genome-wide Linkage in Three Dutch Families Maps a Locus for Abdominal Aortic Aneurysms to Chromosome 19q13.3

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Objectives. Elucidation of the genetic background of familial abdominal aortic aneurysm (AAA) suggests a genetic etiology.

Methods and results. We carried out a genome-wide scan in three Dutch families with four or five affected siblings. Suggestive loci were further studied by subsequent fine mapping of the locus performed in 101 affected sib-pairs. The genome-wide scan was performed with 400 DNA markers and results were given as non-parametric, multipoint linkage scores (NPL). We observed a suggestive linkage for AAA (NPL score 3.25 at D19S902, 72.72 cM) on chromosome 19q in the three families. After fine mapping on chromosome 19, the NPL score became nominal in the 101 affected sib-pairs. A separate analysis of the three families with fine mapping revealed a peak with significant evidence for linkage (NPL score 3.95 at D19S904, 78.08 cM) on chromosome 19q. This peak was situated to the right compared to the region found in a previously published article for familial AAA on chromosome 19q.

Conclusions. Our results identified a candidate locus in three Dutch families with AAA at chromosome 19q13.3. Separate analysis of these three families provides evidence for genetic heterogeneity.

Keywords: Genome-wide linkage; Familial abdominal aortic aneurysm.

Introduction

Abdominal aortic aneurysm (AAA) is a late onset disease. AAA rarely occurs before the age of 50, but becomes increasingly common thereafter, affecting 4–9% of men above the age of 65.1–4 Rupture, the main complication of AAA, is associated with a mortality of 65–85%.5–7 Many factors are accepted as important in AAA development, including age, gender, smoking and hypertension.1,6,9 AAA is frequently familial, with up to 18% of brothers of the proband having AAA and familial factors may influence the age of onset of AAA.10–14 Recently, 233 multiplex families with AAA were collected from nine different countries, in which almost one fifth of the families had four or more affected family members, suggesting a genetic component.15

In genetic studies, the unravelling of a disease like AAA is complicated by the late onset of the disease because at the time of diagnosis (affected) parents have often deceased; initially unaffected family members still may develop an AAA in the future (incomplete penetrance); no DNA is available from affected siblings who might or might not have died from AAA, and finally children of the siblings are yet too young to have developed an AAA. Consequently, available DNA samples are usually restricted to a single generation. Additionally, many families are small in size. The majority of the affected sib-pairs consist of families with only two affected siblings. Large families will be more informative and their unaffected family members may contribute to information on the transmittance of alleles.16

The search for susceptibility genes for AAA has proven difficult. Several candidate gene studies were unable to provide consistent evidence for an association between putative susceptibility genes and AAA.17 However, in a recent collaborative study significant linkage was found on chromosome 19q13, near marker D19S416 at 58.69 cM, with a LOD score of 4.75 (P = 0.00014).18
Because in this collaborative study, the number of affected family members was an important covariate and genetic heterogeneity was evident, we performed a separate analysis of three large families, that were part of the above mentioned collaborative study, but came from a single population (Dutch).

**Methods**

**Clinical diagnosis and definitions**

An AAA was defined as an aortic diameter greater than 3 cm in the infrarenal portion of the aorta, or a 50% increase in diameter of the suprarenal aorta, confirmed by CT-scan or ultrasound investigation.19

Large families were defined as at least four or more first-degree relatives, who were still alive and diagnosed with an AAA, as confirmed by a physician. In these families unaffected brothers and sisters were also invited to participate, as they are informative in the hereditary transmittance of alleles.

Affected sib-pairs were defined as one or more siblings, who were still alive and diagnosed with an AAA, as confirmed by a physician.

No samples or data were available from other generations, as the age of onset of AAA is above the age of fifty, at the time point of diagnosis (affected) parents have often deceased and the children are often too young to already have developed an AAA.

**Family collection**

The procedure of family collection and participating hospitals has been described in detail previously, but afterwards one additional hospital agreed to participate in our study.20 We selected three large families with four or more affected family members for participation into a medium density genome-wide linkage study (Fig. 1).

High-density mapping was performed in these three families. Additionally, another 55 families, comprising of 79 affected sib-pairs, were recruited, yielding a total collection of 101 affected sib-pairs from 58 families. These 101 affected sib-pairs belonged to 46 families with two, seven families with three, four families with four, and one family with five affected siblings. None of the families showed any clinical symptoms of Ehlers Danlos type IV or Marfan syndrome.

The medical records of the index patients and their relatives were reviewed and the diagnoses verified. Subsequently, the index patients and their relatives were invited for recording of their medical and family history and collection of blood samples for DNA analysis. The presence of major risk factors for AAA, i.e. smoking, hypertension (WHO criteria), diabetes mellitus, hypercholesterolaemia and atherosclerotic manifestations presenting as peripheral, coronary and cerebral arterial occlusive disease, was not significantly different in comparison with AAA patients without a familial predisposition (unpublished data, for definitions see).21

This study was approved by the local ethics committee and all patients and their family members gave informed consent.

**Genotyping**

We extracted DNA from peripheral blood samples, using DNAzol reagent (Invitrogen, Molecular Research Center, Inc., Cincinnati, OH). For the genome-wide linkage analysis, the ABI Prism Linkage Mapping Set v 2.5-MD10 (Applied Biosystems, Torrence, CA, USA) was used. This set consists of 400 DNA markers distributed throughout the genome, with an average distance of 10 cM. These 400 markers were multiplexed in 96 polymerase chain reactions (PCRs). PCR was set up with a Tecan Miniprep 75 pipetting robot (Tecan, Maennedorf, Switzerland) in 384 well plates, that were cycled on a Geneamp 9700 Viper (Applied Biosystems, Torrence CA, USA). The PCR products were pooled using a separate Tecan Miniprep 75 pipetting robot and were supplemented with an LIZ500 internal size standard. Fragment analysis was performed on an ABI 3100 sequencer (Applied Biosystems, Torrence, CA, USA).

For the 101 sib-pairs, additional markers, derived from the ABI Prism Linkage Mapping Set v 2.5-HD5, with an average distance of 5 cM (panel 78 and 79), were used for fine mapping of chromosome 19. For the three large families, also markers were used with a distance of 1 or 2 cM22 in the area of the suggestive linkage observed in the genome-wide scan. The fragment analysis data were analysed with the Genemapper v 2.5 software (Applied Biosystems, Torrence, CA, USA).

**Data management and statistical analysis**

For data conversion and analysis, the Allegro version 1.1 computer program for multipoint genetic linkage analysis was used.23 Results were presented as non-parametric multipoint LOD scores (NPLs), since, we dealt with family members of one generation and no convincing evidence was present from other
generations. Marker orders and genetic distances were obtained from the documentation of Applied Biosystems. Allele frequencies and sizes of the markers were obtained from the website of CEPH (www.cephb.fr) to construct the input files for the linkage programs. Criteria for significance were based on guidelines published by Lander and Kruglyak:4 a LOD score > 3.6 (P-value < 0.00002) indicates genome-wide significance, a LOD score between 2.2 and 3.6 (P < 0.0007) indicates genome-wide suggestive linkage and a LOD score between 0.6 (P < 0.05) and 2.2 (P < 0.01) is considered nominal.

The critical maximum NPL-score thresholds for significance at the 5% level were estimated by simulation with the Allegro computer program.23 We created 1000 artificial data sets for which we used the original pedigree structure, phenotype information and allele frequencies noted in the experimental dataset of the three families. This simulation was generated under the null hypothesis of no linkage.

By using the sample-size of 101 affected sib-pairs, we had at least a 90% power to detect ‘significant linkage’ (NPL score of 3.6)24–27 for a locus with a locus-specific relative risk of four in the absence of locus heterogeneity.

Since, the exact mode of inheritance is not known15 and the data available were not sufficient to establish an exact age-dependent penetrance, linkage was also assessed using a dominant mode of inheritance with a reduced penetrance of 80% and a recessive mode of inheritance. For both parametric analyses, the programs MEGA2 (data conversion) and SIMWALK2 (calculations) were used (data not shown).28–30

Fig. 1. The Figure shows the family trees of three families with abdominal aortic aneurysms (AAA). Filled squares and circles indicate those in whom AAA was diagnosed. I–II = different generations and year of birth and death. Pat II:2 in family I did not participate in the study.
Results

Genome-wide scan

Results of the genome-wide scan are shown in Fig. 2. According to the criteria of Lander and Kruglyak, 20 chromosomal regions showed nominal NPL score peaks. Three chromosomal regions showed suggestive evidence for linkage. The highest NPL score observed for the three families was 3.25 ($P = 0.04$) at D19S902 (72.72 cM, 19q13), followed by 2.30 ($P = 0.02$) at D1S2842 (273.46 cM, 1q42–43) and 2.24 ($P = 0.03$) at D8S258 (41.55 cM, 8p21).

Fine mapping on chromosome 19

The fine mapping of chromosome 19, for which 22 markers were used, was performed in all of the 101 affected sib-pairs, including the affected sib-pairs of the three large families. The results are shown in Fig. 3. The highest NPL score peak observed in the 101 affected sib-pairs was 1.57 ($P = 0.08$) at D19S894 (15.55 cM, 19p13).

In this group, the NPL score was negative or around zero in the region of suggestive evidence for linkage observed in the genome-wide scan performed in the three families.

The three large families, for which 41 markers were
used, were analysed separately (Fig. 4) and showed significant evidence for linkage, with a NPL score of 3.95 ($P < 0.001$) at D19S904 (78.08 cM, 19q13.3).

Fig. 2 shows the genome-wide scan on chromosome 19 showing a plateau between 72.72 (D19S902) and 92.56 cM (D19S418). This plateau contained several hundreds of genes. After fine mapping, the region became more specific, ranging between 78.08 (D19S904) and 84.08 cM (D19S571) and containing around 100 genes.

**Simulation experiments**

The significance of the observed NPL statistics was estimated by simulation. The threshold for the NPL score occurring with probability of 5% by chance only, was 3.20. In the genome-wide scan, the multipoint NPL score was 3.25 on chromosome 19q13 at D19S902 and after fine mapping the NPL score at D19S904 was 3.95. In a genome wide scan as defined by Lander and Kruglyak, these values are above the thresholds of genome-wide ‘suggestive’ and ‘significant’ linkage. Despite that the sample size of 101 affected sib-pairs had a 90% power to declare a difference, the multipoint NPL score turned out to be non-significant (NPL = 1.57) at chromosome 19q13.

**Discussion**

We identified a candidate locus for familial AAA on chromosome 19q in three Dutch families with four or more affected siblings. In order to investigate whether the suggestive linkage that was initially found on chromosome 19 in the three large families, suggested a common locus in familial AAA, linkage analysis was also performed in the 101 affected sib-pairs. However, no evidence of linkage was detected in these 101 affected sib-pairs. The high NPL score in the three large families, compared to the low values in the sib-pair analysis, indicates that many of the affected sib-pairs originate from families in which aneurysms are of a non-genetic origin or of a different genetic origin. As isolated pairs of sibs are often coincidental and not the result of a common genetic defect, the average age of onset of AAA in these sibs is expected to be higher than observed in the multiplex families. Beside the index patients, the majority of the first degree-relatives are diagnosed by screening and the number of participants in our study is too small to draw conclusions on age differences.

Fine mapping in the three large families resulted in significant linkage with a NPL score of 3.95. Although, the location at chromosome 19q is in agreement with the previous published article, closer comparison showed that the peaks have no overlap. This may be an indication of heterogeneity as these results suggest that two different genes may be involved in AAA.

Despite gender differences in age at onset there were no indications for sex-linked inheritance. Apparently, the much younger age of males is not a result of X-linked inheritance. Apart from a genetic basis, also a gene-environment interaction may contribute to the familial clustering of AAA.

As AAA is a disorder of later life the formation of an aneurysm can be due to very gradual change of the strength of the aortic wall during life. Reduced strength of the aortic wall can be caused by changes in the synthesis and break down of the extracellular matrix. Consequently, candidate genes can be found in several functional groups; the structural proteins of the connective tissue, the proteinases, the enzymes involved in the posttranslational modification and
transcription factors involved in the regulation of these genes.

Fine mapping on chromosome 19 of the families with four or more affected siblings showed that the candidate locus became smaller. Candidate genes will be selected with a potential interaction with genes involved in the formation of the extracellular matrix and in genes involved in development and growth, e.g. collagen and elastin. 32–38

Internet search (www.ncbi.nlm.gov) for candidate genes on chromosome 19q13.3 revealed two putative candidate genes for the pathogenesis of AAA.

Fibroblast growth factor 21 (FGF21), the protein encoded by this gene, is a member of the FGF family. FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes including embryonic development, cell growth, morphogenesis, tissue repair and invasion. The function of this growth factor has not yet been elucidated. 39

Hyaluronan synthase 1 (HAS1) is a member of the newly identified vertebrate gene family associated with encoding of putative hyaluron synthases. Increased HAS1 expression leads to increased hyaluronan (HA) production. HA is actively produced during wound healing and tissue repair thereby providing a framework for the ingrowth of blood vessels and fibroblasts. 40

These candidate genes have influence on cell growth, the growth direction and tissue repair. Therefore, a mutation in one of these genes may influence the stability of the aortic wall, which eventually may result in dilatation. Sequencing of FGF21 in one of the three families did not reveal mutations in this gene (unpublished data).

In this region on chromosome 19 also many hypothetical genes are located, with unknown function. Sequencing of all these genes is a major effort; so additional fine mapping of chromosome 19 in other families may be necessary to zoom in on this region. Additionally, micro-array studies may give more insight in the expression of genes in the aneurysm wall, especially for genes located on chromosome 19.

In conclusion, the results of our genome-wide linkage analysis and subsequent fine mapping performed in three large families with familial AAA identified a locus for familial AAA at chromosome 19q13.3. Comparing the results with the previous collaborative study 18 leads to the conclusion that genetic heterogeneity between different countries or even families is very likely. Genetic studies may define subgroups of patients at risk for familial AAA who would benefit most from ultrasound screenings programs.

References


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