SHORT COMMUNICATION

Exclusion of calcitonin/ α -CGRP gene defect in a family with autosomal dominant supravalvular aortic stenosis

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Supravalvular aortic stenosis (SVAS) is a structural heart defect characterised by a congenital narrowing of the ascending aorta, either localised or diffuse, originating at the superior margin of the sinuses of Valsalva. SVAS may occur as a sporadic defect or segregate as an autosomal dominant trait. It is also found in some subjects with Williams syndrome (WS).

It has been hypothesised that mutations in the calcitonin/α-CGRP gene may be relevant in causing either SVAS or WS.²³ However, Bennett *et al*³ reported exclusion of the calcitonin gene as a candidate gene in a small family with autosomal dominant SVAS using a gene specific probe which failed to show concordant segregation. Similarly, studies performed on patients with WS using Southern blot analyses (to show large deletions or gene rearrangements) and RNase protection assays (to identify point mutations) have not indicated any abnormality of the calcitonin-CGRP gene.⁴⁵

The purpose of our study was to determine if a mutation within the calcitonin-CGRP gene could be implicated in other families segregating for SVAS, thus exploring the possibility of

genetic heterogeneity for autosomal dominant SVAS.

Materials and methods

Clinical investigations of our three generation family with 17 affected subjects (fig 1) have been reported.⁶ High molecular weight DNA was isolated from peripheral blood leucocytes using an Applied Biosystems 340A Nucleic Acid Extractor, digested with the appropriate restriction enzyme, fractionated on agarose gels, and blotted onto nylon membrane (MSI). The DNA probe was labelled with ³²P-CTP by random priming (Amersham) and used for hybridisation as previously described.⁷

The probe pGBCT2 used for linkage analysis recognises a *PvuII* RFLP 13 kb downstream of the 3' end of the calcitonin/ α -CGRP gene on chromosome 11p15.8 Alleles of 9.5 kb and 8.5 kb are observed with frequencies of 0.436 and 0.564, respectively.8

Two point calculations were performed using the computer program LINKAGE version 4.7,9 assuming a penetrance of 86% and a gene frequency of 0.0001. To be certain that the degree of penetrance did not influence our

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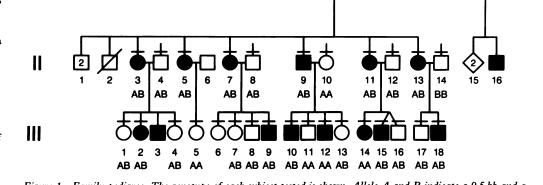


Figure 1 Family pedigree. The genotype of each subject tested is shown. Allele A and B indicate a 9.5 kb and a 8.5 kb fragment, respectively, identified by pGBCT2. Filled symbol = affected.

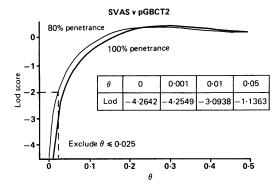


Figure 2 The lod scores versus recombination values (θ) for loci pair: SVAS-pGBCT2.

conclusions, other calculations were performed using values of 80% and 100%.

Results

The genotype of family members tested is given beneath each subject's symbol (fig 1). Linkage analysis results showed negative log odd scores throughout the θ values tested (fig 2), with lod scores of -4 to -2 for values of θ less than 0.03.

Discussion

Investigators have speculated that a disturbance of calcium regulation may be involved in the formation of SVAS and WS.23 As calcitonin is involved in calcium regulation, mutations within the calcitonin/α-CGRP gene could plausibly lead to development of SVAS. On the basis of a single recombinant event between an affected and unaffected sib pair in a small family with SVAS, the calcitonin gene was excluded as the site of mutation.3 However, it remains possible that SVAS is a heterogeneous disorder and that in some families the causative mutation may arise within the calcitonin gene. Our study of a larger family using genetic linkage analysis provides strong evidence for exclusion of the calcitonin/a-CGRP gene as the primary defect responsible for autosomal dominant SVAS in a second family. Thus, if a disturbance of calcitonin/ α -CGRP secretion/regulation is involved in the aetiology of SVAS, such events may be secondary to defects at a locus extrinsic to the primary gene.

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