Intracranial aneurysms (IAs) have a substantial genetic component. In a genome-wide linkage search, Onda et al found 3 candidate regions, with the strongest linkage near the elastin gene (ELN, OMIM 130160). Ruigrok et al found association between IA and ELN intron 5 and exon 22. These findings were not replicated in 2 European studies or a Japanese population. In Farnham et al, we examined 13 IA pedigrees, and confirmed linkage of IA to a 7cM region comprising the ELN gene. Here, we examine ELN as a candidate gene responsible for the Utah IA pedigrees.

Materials and Methods

Our ELN mutation screening set consisted of 16 individuals: 1 affected individual from each of the 13 families was chosen to represent the haplotype on chromosome 7 cosegregating with IA. In 1 family, 2 different haplotypes were present, so 2 individuals were chosen, 1 to represent each haplotype. Two normal population controls with no history of IA were analyzed for comparison. Affected individuals had a confirmed medical diagnosis of IA as described in Farnham et al.

The 16 genomic DNA samples were amplified by the polymerase chain reaction using primers corresponding to each of 34 ELN exons and their neighboring intronic regions, and to the promoter and 3’UTR regions described in Onda et al. The polymerase chain reaction products were screened for mutations by DHPLC. Potential DNA variants were polymerase chain reaction-amplified and sequenced. Variants were considered possible candidates if the mutation was new to, or was significant in, the Japanese study. One affected and the oldest unaffected sibling was screened for a rapid and informal determination of segregation of the variant with IA.

Results

Variants were identified in the promoter region, exons 4 and 6, and the 3’UTR (Table). No further variation was found in the remaining 32 exons and exon/intron boundaries. For comparison, the frequencies of the Japanese variants are shown with the Utah results. A rare change at position −38 in the promoter region, present in the Japanese population, was not found in the Utah families. Variants in intron 6 and at position +549 in the 3’UTR were unique to the Utah cohort. None of these changes was present in the 2 controls.

Because Onda et al found no significant difference between controls and affected individuals for the variants in promoter (−972 or −1042), intron 4, and 3’UTR (+659), and we observed similar frequencies, no further segregation study was performed on these sites. The 3’UTR (+502) variant, which showed borderline significant difference (P=0.085) in the Japanese study, and the 2 novel variants identified in this study (3’UTR +549 and intron 6 −94) were
examined by testing one affected and unaffected sibling in each pedigree for segregation with IA.

Neither affected nor unaffected siblings in the single pedigree with the 3′ UTR G>A (+549) insertion carried informative haplotypes, so no inference could be made between this mutation and IA. The intron 6 variant was observed in 2 pedigrees (Table); however, the G>A (=94) mutation was found only in the unaffected sib of 1 pedigree, and was absent in the 2 individuals from the second pedigree. The 3′ UTR (+502 ins A) variant was observed in 3 pedigrees. In the first pedigree, both the affected and the unaffected sibling also carried the variant. In the second pedigree, the 2 affected individuals shared both chromosome 7 haplotypes and the DNA change; the unaffected individual did not have the DNA variation. In the third pedigree, the A insertion was not observed in the affected sibling, and informative unaffected siblings were not available.

**Discussion**

We did not find compelling evidence of segregating mutations or polymorphisms in ELN explaining the IA aggregation observed in the Utah pedigrees. The absence of the mutations for both intron 6 and the 3′ UTR variant in affected siblings rules out these mutations as the cause for IA. Two explanations might account for these results. The analysis in Farnham et al7 suggested a 7-cM region; it is possible that an unidentified gene in this region is responsible for the linkage evidence. Second, in this study, only exonic, promoter, and 3′ UTRs of interest in Onda et al2 were screened. Other regions of the ELN gene might be causal for IA.

Onda et al2 and Ruigrock et al3 have found significant associations between IA and intron 20/intron 23, and intron 5/intron 22, respectively. Such results, in addition to Farnham et al,7 indicate that regulatory pathways for ELN synthesis and maintenance are likely to be involved in IA, rather than ELN structure. Additional sequencing of regulatory regions should be pursued.

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**References**


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