

TRANSITIONS IN THE EXPRESSED mRNA OF THE GENE FOR FERRITIN LIGHT-CHAIN DETECTED IN FIBROBLASTS CULTURED FROM A SURGICAL SPECIMEN OF AN ABDOMINAL AORTIC ANEURYSM

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Introduction

Highly correlated genetic markers indicating susceptibility for abdominal aortic aneurysm (AAA) continue to be elusive. The problems of variable penetrance, asymptomatic onset and the frequent attribution of sudden death to "myocardial infraction" have been impediments to the usual candidate-gene or 'reverse' genetic approaches.

A recent report finds that the most significant region relating to familial susceptibility for intracranial aneurysm is located in the vicinity of chromosome 19q12-13. [1] A recent report from our research group describes clustering of abdominal, thoracic and cerebral vessel aneurysms all within the same kindred. [2] Intracranial and abdominal aortic aneurysms share similar genetic risk factors. [3] Accordingly, a mutation in this gene might have consequences relevant to both disease processes.

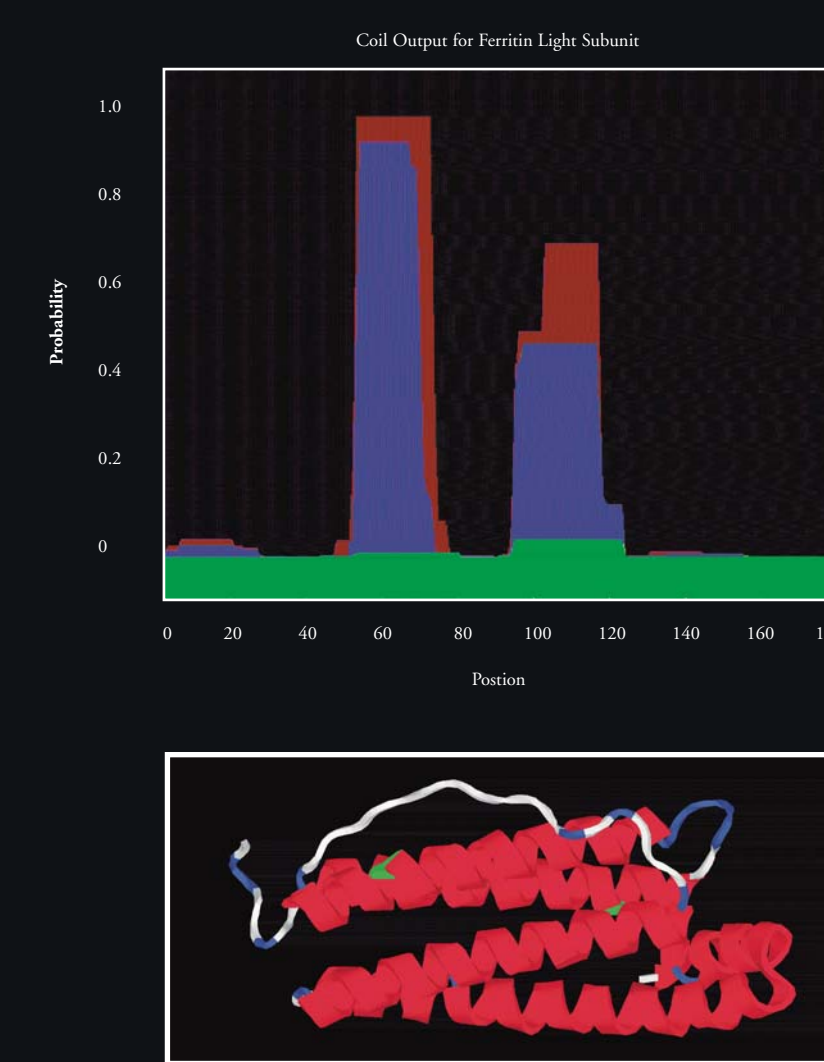
We have reviewed our library of cDNA sequences cloned from abdominal aortic aneurysm (AAA) tissue fibroblasts cultured from the adventitia of AAA and selected a ferritin-like gene for further study. [4] This gene was chosen because it maps in the human genome to a hot spot (chromosome 19q13) for susceptibility to cerebral artery aneurysm (CAA).

Methods

cDNA expression libraries were screened with antibodies to vitronectin and fibrinogen. Fibroblast mRNAs were amplified with primers designed to detect matrix cell-adhesion-like molecules. The sequence of the ferritin-like cDNA was translated into its predicted protein sequence. Three nucleotide transitions resulted in two amino acid substitutions: D(18)N and L(52)F. BlastP (NCBI) was used to compare alignments with known ferritin genes. Searches in multiple reading frames and multi-dimensional computational tools (BLAST, TMpred, TopPred, SAPS and SSpro) were used to predict conformational consequences of the detected transitions.

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NATIVE FERRITIN LIGHT CHAIN
High scoring uncharged segments:
score= 1.00 frequency= 0.709
(LAGSVTIPNFQYHMCW )
score= 0.00 frequency= 0.000 (BZX)
score= -8.00 frequency= 0.291
(KEDR)
M_0.01= 25.62 (cv= 16.03, lambda=
0.32210, k= 0.22047, x= 9.59;
90% confidence interval NATIVE
LIGHT CHAIN
for segment length: 32 +- 18)
M_0.05= 20.56 (x= 4.53)
1) From 15 to 38: length= 24,
score= 2.40
15 AAVNSLVNLY LQASVTVLSL GFYF
L: 5(20.8%); A: 3(12.5%); S:
3(12.5%); Y: 4(16.7%);
# of segments (>=20 residues) exceeding
M_0.05: 1
* Refers to 5% Significance Level
NATIVE LIGHT CHAIN
Location (Quartile) Spacing Rank
P-value Interpretation
14: 39 (1) *( 25)* 1 of 52
0.0043 large maximal spacing
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ANEURYSM
High scoring uncharged segments:
score= 1.00 frequency= 0.703 (
LAGSVTIPNFQYHMCW )
score= 0.00 frequency= 0.000 (BZX)
score= -8.00 frequency= 0.297 (
KEDR )
Expected score/letter: -1.674;
Average information/letter: 0.388
Minimal length of displayed segments
set to: 20
M_0.01= 25.02 (cv= 15.58, lambda=
0.33141, k= 0.22986, x= 9.43;
90% confidence interval for segment
length: 31 +- 17)
M_0.05= 20.10 (x= 4.51)
# of segments (>=20 residues) exceeding
M_0.05: none
ANEURYSM
There are no unusual spacings.
Sequence based protein characterization
(SAPS) of the two proteins.
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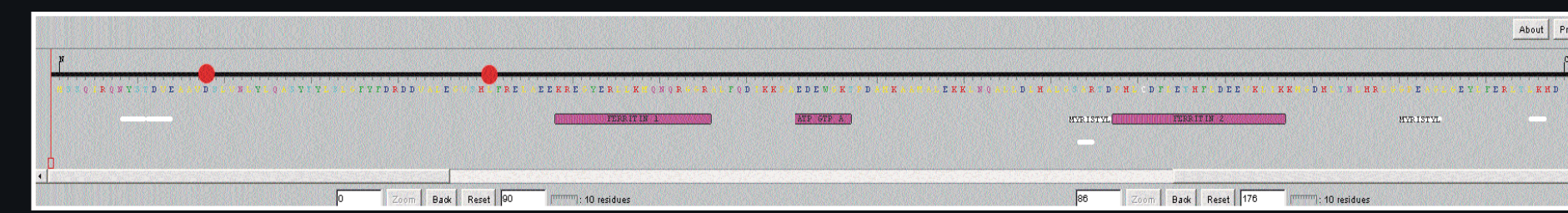
Aneurysmal ferritin light chain: green segments indicate areas of amino acid substitution which knock out native large neutral spacing and charge pattern.

The D->N substitution at position 18 reduced the tendency to cluster and also knocked out a statistically significant length of uncharged sequence indicating a decrease in tendency to cluster.

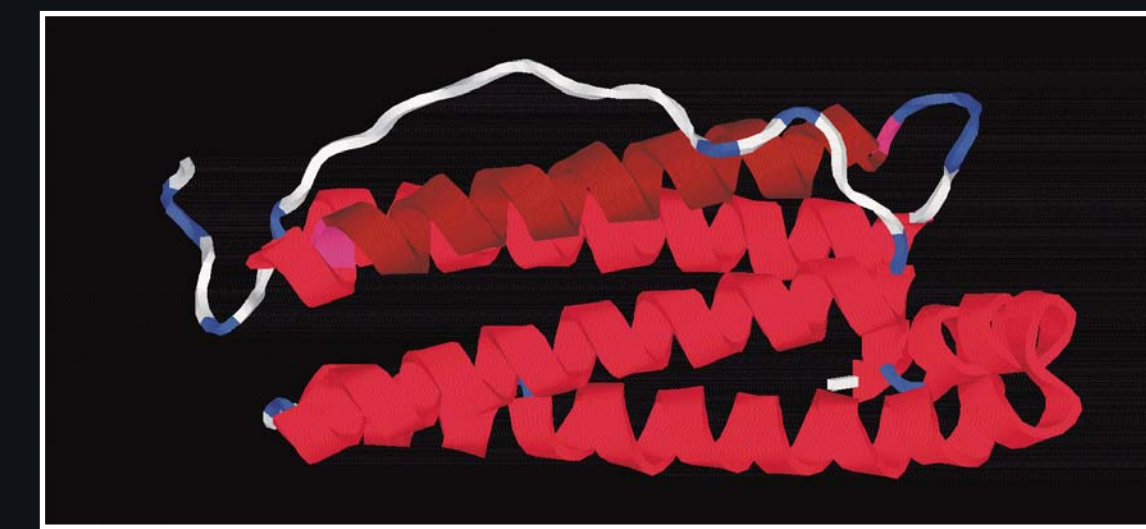
Results



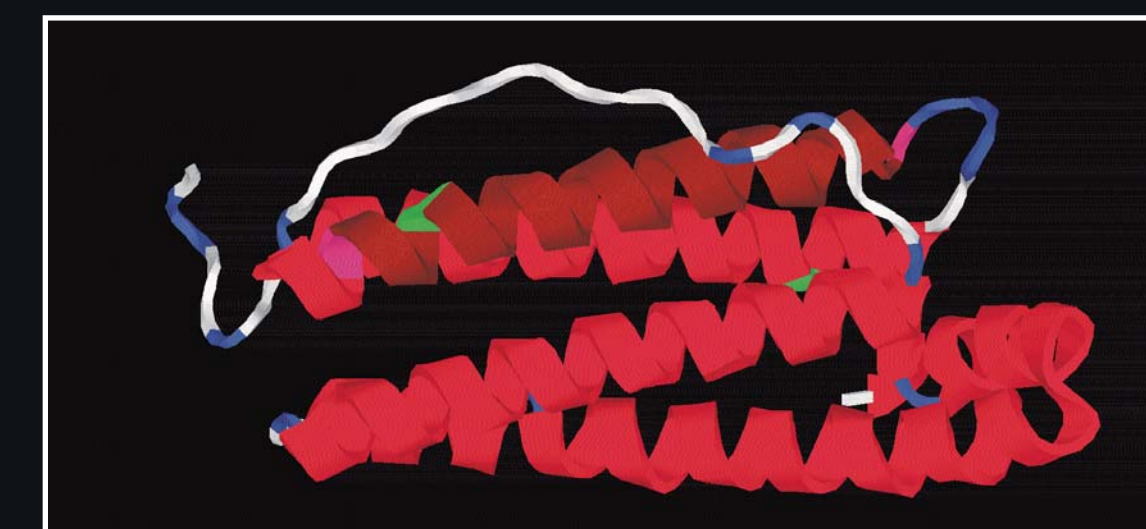
Left: Genomic origin of new mRNA with exons at 19q13.3-13.4 (P02792)



Above: Prosite Mapping of Protein Sequence. Red dots indicate sites of mutation.



Native ferritin light chain: flanking magenta segments represent distribution of a large neutral spacing. Brown segment represents a high scoring clustering segment



Aneurysmal ferritin light chain: green segments indicate areas of amino acid substitution and demonstrate their location in relation to the large neutral spacing (magenta) and high scoring clustering segment (brown) which are seen in wild-type ferritin light chain.

Discussion

A characteristic disorganization of structural elements found in aneurysms has similarities in other organ systems caused by iron toxicity. Oxidative stress is associated with the disorganization seen in liver fibrogenesis. [5] Further studies implicate lipid peroxidation with increased collagen alpha gene expression and possibilities of hepatic fibrosis. [6] Iron injury may also have a role in endothelial cell dysfunction [7] and vascular smooth muscle proliferation leading to atherosclerosis. [8]

Since the ferritin molecule is such a universal factor in the control of inflammation and containment of oxidative damage, one can only speculate as to how the specific mutations described here can be a genetic risk factor for diseases limited to the arterial tree. Such mutations should have widespread consequences in multiple organ systems. However recent findings related to the expression of artery-specific proteins with a defined regional distribution in the aorto-iliac system [9] suggest that there may be stable phenotypic changes in fibroblasts differentiated in discreet tissues. The transitions observed here might also be specific to post-translational changes or alternative splicing of the genomic ferritin light chain in the abdominal aortic or cerebral artery fibroblast.

Conclusion

The observed transition of the ferritin-like mRNA from a AAA fibroblast line suggests an alteration in the physiological functionality of the expressed protein. Since control of antioxidant damage in all forms of aneurysmal diseases is a subject of increasing relevance, the present observations and the tools of proteomics may provide a mechanism for understanding the distribution of both CAA and AAA in some families.

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