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ARTICLE

Elastin: mutational spectrum in supravalvular aortic stenosis

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Supravalvular aortic stenosis (SVAS) is a congenital narrowing of the ascending aorta which can occur sporadically, as an autosomal dominant condition, or as one component of Williams syndrome. SVAS is caused by translocations, gross deletions and point mutations that disrupt the elastin gene (*ELN*) on 7q11.23. Functional hemizygosity for elastin is known to be the cause of SVAS in patients with gross chromosomal abnormalities involving *ELN*. However, the pathogenic mechanisms of point mutations are less clear. One hundred patients with diagnosed SVAS and normal karyotypes were screened for mutations in the elastin gene to further elucidate the molecular pathology of the disorder. Mutations associated with the vascular disease were detected in 35 patients, and included nonsense, frameshift, translation initiation and splice site mutations. The four missense mutations identified are the first of this type to be associated with SVAS. Here we describe the spectrum of mutations occurring in familial and sporadic SVAS and attempt to define the mutational mechanisms involved in SVAS. SVAS shows variable penetrance within families but the progressive nature of the disorder in some cases, makes identification of the molecular lesions important for future preventative treatments. *European Journal of Human Genetics* (2000) **8**, 955–963.

Keywords: elastin; supravalvular aortic stenosis; mutations

Introduction

Elasticity of the skin, lungs and large blood vessels depends on the presence of elastic fibres in the extracellular matrix. Abnormalities of elastic fibres, which are composed mainly of amorphous elastic (95%) and microfibrils, cause several cardiovascular, connective tissue and skin disorders, including Marfan syndrome, supravalvular aortic stenosis (SVAS) and Cutis Laxa.[1–4]

SVAS is an obstructive vascular lesion with an incidence of 1/20 000 births, described in 1842 by Chevers,[5] and was the first disorder to be associated with the elastin gene (*ELN*). The aortic narrowing can occur as a discrete hourglass deformity or as diffuse aortic hypoplasia and may be associated with other vascular lesions, the association with pulmonary arterial stenoses being well recognised. SVAS can occur sporadically or as an autosomal dominant condition with reduced penetrance.[6] The severity of SVAS is variable but it may be progressive, leading to cardiac failure and early death

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without surgical intervention. A chromosomal translocation t(6;7)(p21.1;q11.23) that segregated with SVAS in a family first suggested that mutations in the *ELN* gene located at 7q11.23 might be a cause of SVAS.[7] Subsequently, gross deletions of *ELN* were identified in two cases of SVAS.[8,9] We, and others, have since described point mutations in the *ELN* gene in both familial and sporadic cases of SVAS.[10,11]

SVAS also occurs as one component of Williams-Beuren syndrome (WBS),[12] a complex developmental disorder caused by a deletion of about 1.4 Mb of chromosome 7q11.23, which takes out a number of contiguous genes, including *ELN*.[13] Comparing WBS patients and patients with isolated SVAS, it appears that hemizygosity for elastin causes SVAS and, sometimes, peripheral artery stenosis, and hernias, but not the other features of WBS.[14]

In an attempt to define the molecular pathology of SVAS, we have screened 100 patients, ascertained from a range of sources in the UK and abroad, with either familial or sporadic supravalvular stenosis and/or other vascular abnormalities, for mutations in all 34 exons of the *ELN* gene. The relationship between the type of mutation found and the penetrance of SVAS within families as well as other possible genotype/phenotype correlations were investigated. In this way we can attempt to identify the pathogenic mechanisms involved in this cardiovascular disorder.

Materials and methods

Clinical ascertainment of patients

The samples were referred in from various centres. Patients were ascertained based on cardiological diagnosis of SVAS on echocardiogram, (+/-) angiography. SVAS patients were classified as severe (s) if they had surgical procedures, moderate (m) if they were still under follow-up by clinicians and mild, if stated as mild and often under no further follow-up.

Elastin FISH and microsatellite analysis

Elastin FISH was carried out using the Oncor WSCR probe according to the manufacturer's instructions (Appligene Oncor Lifescreen, Hertfordshire, UK). Haplotype analysis of patients was carried out by PCR amplification of genomic DNA using the following markers:

D7S2476 F: GGGCAACATAGCACGATT

D7S2476 R: CAGGAGTCAGTTAGATAAGGTCAC

ELN Helg18/19F: 5' ATG AGA CGT GGT CAA GGG TAT

ELN Helg18/19R: 5' GGG ATC CCA GGT GCT GCG GTT

LIMK1 GT F: TGGGGCAGGAGAATGATGTG

LIMK1 GT R: AGTCTTCTTTGCGGGCTATGTTA

D7S613 F: CAGCCTGGGTAACAAAAGC

D7S613 R: CCTCCCTCCCTAATCCATG

All amplifications were carried out using 100 ng of genomic DNA and 10 pmol of each primer in 20 μl reaction volumes. Cycle conditions were: 95°C for 2 min, then 27 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, with a final extension step of 5 min at 72°C. PCR products were electrophoresed on 8% polyacrylamide gels (acrylamide:N,N' bisacrylamide 19:1) for 3 h at 300 V then visualised by silver staining.

Mutation detection and sequencing

ELN exons were amplified using primers and amplification conditions described previously.[10]

In addition exon 18 was amplified using the following primers to avoid the microsatellite repeat:

ELN: X18F: ATA CTC TAC TAA CCA CCC TTC TA

ELN: X8R: ACA CAC ACA CAC AGC CCA GCT C

Mutations were detected by a comparison of SSCP and heteroduplex analysis, products run on 1 mm non-denaturing 8% polyacrylamide gels and visualised by silver staining as previously described.[10] Mutations were initially characterised by fluorescent cycle sequencing (Thermo Sequenase II, Amersham, Bucks, UK) of column purified PCR products in both orientations with a matched control on an ABI 373 sequencer.

Mutation screening of pedigrees

Screening for the exon 17 mutation in family no. 91 *Mlu*I digested PCR products were separated on a 2% agarose gel and visualised by ethidium bromide staining.

Screening for the exon 6 mutation in family no. 106 *Bst*NI digested PCR products were separated on a 2% agarose gel and visualised by ethidium bromide staining.

Cloning exon 26 alleles in patient no. 11

Exon 26 PCR amplified products from patient no. 11 were cloned using the TOPO TA cloning kit (In Vitrogen, The Netherlands) according to the manufacturer's instructions. Plasmid minipreps were made from 10 clones, using the Qiagen miniprep kits, and sequenced in both orientations using the M13 reverse and forward vector primers (fluorescent cycle sequencing, Amersham). The mutations were confirmed in at least four different clones to insure that false base changes due to Taq polymerase proofreading errors were accounted for.

ARMS PCR of S442G polymorphism

ARMS PCR was carried out using primers designed to amplify the mutant allele only:

ELN X20R: 5'CCCATCCCTTCTCAACCCATGTC

ELN X20F ARMS M:
5'CCCTGGAGTCGCAGGTGTCCCAG

ELN X20F ARMS N:
5'CCCTGGAGTCGCAGGTGTCCCGA

Table 1 Spectrum of elastin gene mutations in SVAS

Patient	*F/S*	*Nucleotide position of mutation*	*Mutation*	*Effect of mutation*	*Clinical details*
112	F	Exon 1	Δ bases 1 to 28	Del ATG start codon	SVAS(m), family history of SVAS
661	F	5'UTR	Δ bases 4 to 8	Del ATG start codon	SVAS(m), VPS, PPAS in proband, SVAS(s), PPAS in father
070	S	5'UTR	–38 C>T	New start codon?	SVAS(s), PPAS, brachiocephalic vessel narrowing, coronary ostial stenosis
		Exon 9			
		450 C>G	Y150X	Nonsense	
322	F	Exon 9			SVAS in proband. AVS(s), PVS(s), PAS(s), AH in one daughter and SVAS(m), PVS(m), PPAS in other daughter
		450 C>G	Y150X	Nonsense	
332	F	Exon 9			SVAS(m) in proband. Brother SVAS(m). Son SVAS(s), SVPS(s) and PPAS(s)
		450 C>A	Y150X	Nonsense	
108	S	Exon 9			SVAS(m), PAS. Parents have normal ECHO
		450 C>G	Y150X	Nonsense	
120	F	Exon 10			SVAS(s), PPAS(m) in proband; mother has SVAS(m)
		526A>T	K176X	Nonsense	
134	F	Exon 10			PPAS(m) in proband
		526A>T	K176X	Nonsense	
67	S	Exon 21			SVAS
		1324 C>T	Q442X	Nonsense	
136		Exon 21			SVAS, PPAS
		1324 C>T	Q442X	Nonsense	
290	F	Exon 21			SVAS(s), AH, PPAS(s), PAS in proband. Half brother SVAS(s), SVPS(m)
		1324 C>T	Q442X	Nonsense	
101	S	Exon 25			SVAS(s), PPAS, bilateral inguinal hernias
		1708 C>T	R570X	Nonsense	
64	F	Exon 2	Base 105^106 ins T	Frameshift/stop at codon 64 (exon 4)	SVAS, Takayashus in proband. SVAS in daughter
106	F	Exon 6	Base 279 del T	Frameshift/stop at codon 121 (exon 7)	SVAS(s) proband; cousin SVAS(s) whose son has SVAS(s), PPS(m) sudden death; variable penetrance (see pedigree in Figure 3)
		Exon 24			
		1653T>A	G551G	Silent mutation	
3	S	Exon 8	Base 406 del G	Frameshift/stop at codon 136 (exon 8)	SVAS(s), PAS(s)
128	F	Exon 12	Base 639 del G	Frameshift/stop at codon 322 (exon 18)	SVAS(s), PPS(s) in proband; SVAS(s) in mother, variable penetrance in other family members
340	F	Exon 16	Base 842^843 ins TIGG	Frameshift/stop at codon 312 (exon 17)	SVAS(s), PPAS, AH in proband. Maternal uncles SVAS(s), AH. Cousin reportedly PPAS
80	F	Exon 18	Base 983 del C	Frameshift/stop at codon 333 (exon 18)	SVAS, SPS(s); father surgery for valvular PS, PPS, daughters PPS
93	F	Exon 18	Base 1048 del G	Frameshift/stop at codon 463 (exon 22)	SVAS in proband, father had hole in the heart
130	F	Exon 20	Base 1192 del G	Frameshift/stop at codon 463 (exon 22)	SVAS and PPAS in proband, sisters, father and uncle
118	F	Exon 20	Base 1260 del C	Frameshift/stop at codon 463 (exon 22)	SVAS in proband. SVAS(s) in mother
411	F	Exon 21	Base 1357 del G	Frameshift/stop at codon 463 (exon 22)	PVS(m), SVAS(m) in proband. Multiple PPAS, ASD in father
12	F	Exon 26	Base 1788^1789 ins T	Frameshift/stop at codon 615 (exon 26)	SVAS(s), PPAS in proband; variable familial penetrance (14)
590	S	Exon 26	Base 1727 del G	Frameshift/stop at codon 674 (exon 28)	SVAS(s), PPAS, superior mesenteric artery stenosis(s), coarctation and stenosis of brachiocephalic vessels
16	F	Exon 28	Base 1989^1990 ins GG	Frameshift/stop at codon 674 (exon 28)	SVAS(s), PPAS
69	F	Exon 3	Donor splice Base 163+1 G→T	Splicing defect	SVAS in siblings and father
68	?F	Exon 8	Acceptor splice Base 377–2 A→C	Splicing defect	SVAS(s) in proband. Sibling died of 'cardiac problem' at 18 months
66	S	Exon 15	Acceptor splice Base 746–1 G→C	Splicing defect	SVAS, RAS, PAS(m)
91	F	Exon 17	Acceptor splice Base 890–1 G→C	Splicing defect	SVAS (see pedigree in Figure 2)
116	F	Intron 23	Base 1501+11^12 ins C	Possible splicing defect	Familial vascular stenoses

Table continues on next page

Table 1 – *continued from previous page*

Patient	*F/S*	*Nucleotide position of mutation*	*Mutation*	*Effect of mutation*	*Clinical details*
630	F	Intron 23	Base 1501+11^12 ins C	Possible splicing defect	Vascular stenoses
102	S	Exon 3 163 G>A	A55T	Missense	Isolated PPAS
500	S	Exon 30 2120 C>A	A707D	Missense	SVAS(m)
11	S	Exon 26 1828G>C 1829G>A	G610Q	Missense	SVAS(s); mutations on the same allele
15	F	Exon 13 659 C>T	P220L	Missense	SVAS(s) in proband and mother
Boeckel *et al*[24]	F	Exon 18	Base 1040 del C	Frameshift/stop in exon 22	Familial SVAS and PAS with variable penetrance, ranging from mild to severe
Li *et al*[11] K1179	F	Exon 26	Base 1821 del C	Frameshift/stop in exon 28	Familial SVAS
Li *et al*[11] K2017	S	Exon 3	Donor splice Base 163+1 G→A	Splicing defect	SVAS
Li *et al*[11] K2044 K2260/2205	F	Exon 16	Acceptor splice Base 800–3 C→G Base 800–2 A→G	 Splicing defect Splicing defect	Familial SVAS
Urban *et al*[20] SVAS 1+7	F	Exon 16	Acceptor splice Base 800–3 C→G	Splicing defect	Familial SVAS with variable penetrance, ranging from mild to severe

Clinical details: F: familial; S: sporadic; (m): mild/moderate; (s): severe; SVAS: supravalvular aortic stenosis; SVPS: supravalvular pulmonary stenosis; VAS: valvular aortic stenosis; VPS: valvular pulmonary stenosis; PAS: pulmonary artery stenosis; PPAS: peripheral pulmonary artery stenosis; AH: aortic hypoplasia; ASD: atrial septal defect: RAS: renal artery stenosis; Del: deletion; ins: insertion.

Exon 2 of the elastin gene was used as an internal control for amplification. Cycling conditions were: 95°C for 2 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final extension step of 5 min at 72°C. The products were separated on a 2% agarose gel and scored for the presence of the polymorphism.

Results

Mutations in the *ELN* gene

One hundred unrelated patients with SVAS and vascular abnormalities were screened for mutations in the elastin gene, using a combination of SSCP and heteroduplex analysis. All these patients gave a positive FISH signal with elastin probes, showing that they did not have major deletions of the *ELN* gene (data not shown). Mutations were identified in 35 of the patients. These are described in Table 1. Additionally five common polymorphisms were identified. Other previously published SVAS mutations are also listed in Table 1 to provide a comprehensive list of mutations published to date.

Premature termination mutations

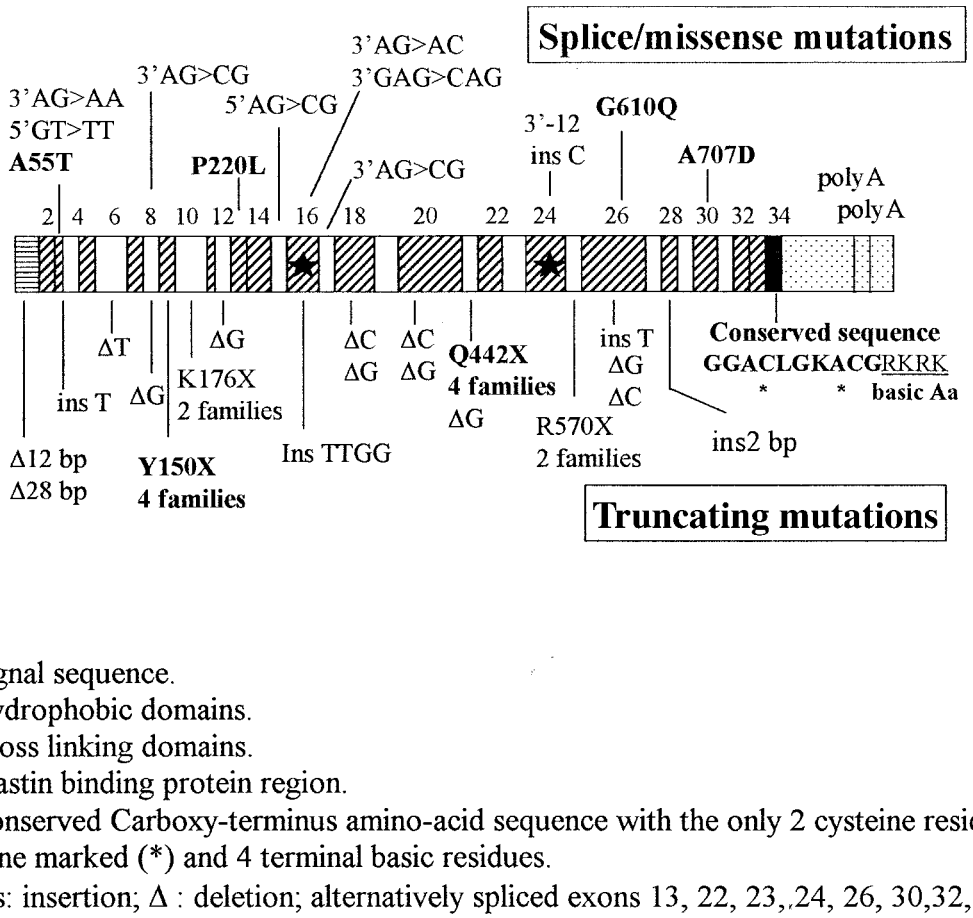
Twenty-three nonsense or frameshift mutations were detected and, if translated, would result in truncated proteins lacking the C-terminal region of elastin. The C-terminus encodes important functional domains that includes for example: exon 30 (the site of a 10 bp element thought to control mRNA decay), and exon 34 (encodes residues required for interaction with the MAGP protein during elastogenesis (see Figure 1).

Splice site mutations

Base substitutions at the GT/AG consensus splice sites were seen in four patients: no. 66 (intron 14 acceptor, AG > AC), no. 68 (intron 7 acceptor, AG > CG), no. 69 (intron 3 donor, GT > TT) and no. 91 (intron 16 acceptor, AG > CG). Two families (nos 116, 630) had the same insertion of a single C base, 11 bases downstream from the start of intron 23. This variant was not seen in 200 controls and further elastin mRNA analysis would be required to see if it affects splicing. In all cases the exons flanking the altered splice site are frame-neutral, so that aberrant splicing would not be expected to disrupt the reading frame.

Changes affecting initiation of translation

Two families (nos 112 and 661) had small deletions in exon 1 encompassing the ATG initiation codon. These deletions should not prevent transcription, but lack of translation would lead to a null allele and haploinsufficiency for elastin. In patient no. 070 a novel upstream ATG codon is created at position –35 nucleotides (ACG > ATG). However, the lack of a Kozak consensus sequence[15] surrounding this site suggests



Signal sequence.
Hydrophobic domains.
Cross linking domains.
★ Elastin binding protein region.
Conserved Carboxy-terminus amino-acid sequence with the only 2 cysteine residues in the gene marked (*) and 4 terminal basic residues.
Ins: insertion; Δ : deletion; alternatively spliced exons 13, 22, 23,,24, 26, 30,32,33.

Figure 1 *ELN* cDNA showing the functional domains and the mutations detected.

it is unlikely to be functional. This patient had an additional clearly pathogenic nonsense mutation, Y150X. Parental samples were not available to check whether the two changes were in *cis* or *trans*.

Missense mutations

Four patients had missense mutations not present in 200 controls. In family no. 15 the exon 13 mutation P220L was present in the proband and his affected mother. A sporadic case (patient no. 500) had A707D in exon 30. Another sporadic case (patient no. 11) had a dinucleotide substitution in exon 26, GG > CA, resulting in the aminoacid substitution G610Q. Sequencing of cloned PCR products showed that the two changes were in *cis*. One of the two substitutions, 1828G > C, encoding G610R, is a common polymorphism (discussed below) and was present in the patient's father, but the second change occurred *de novo*. Finally, patient no. 102 has an apparent missense mutation, A55T, which replaces the last nucleotide of exon 3 (G) with (A). Such substitutions are known to affect the adjacent splice site because of their proximity to the consensus splice junction (GT).

Recurrent mutations

The Y150X (exon 9) and Q442X (exon 21) mutations were each seen in four apparently unrelated patients. K176X and the arguably pathogenic intron change, 1501 + 11∧12insC were each seen in two patients. Haplotype analysis of the patients using flanking and intragenic markers (D7S2476, ELN Helg18/19, LIMK1, D7S613) showed no evidence of founder effects for these mutations (data not shown).

Intragenic polymorphisms

Four novel intragenic polymorphisms were found:

Intron 4 G (.96) or A (.04) at position 196 + 71;

Intron 23 T (.64) or C (.36) at position 1501 + 24;

Intron 26 G (.93) or C (.07) at nucleotide 1828, giving the expressed polymorphism G610R;

Intron 32 C (.73) or T (.27) at position 2273–34;

A previously reported polymorphism S422G[16] was scored in 100 controls. We found 41 GG homozygotes, 41 SG heterozygotes and 18 SS homozygotes (allele frequencies: S .385, G .615).

Intrafamilial variability

Clinical details collected on two large families (no. 91 and no. 106) with multiple affected members and defined mutations illustrate the phenotypic variability within families. Faster mutation-specific digestion tests were set up for screening other family members. Family no. 91 had 13 carriers of a splice site mutation. Five were asymptomatic, whilst other mutation carriers had symptoms ranging from mild to severe SVAS requiring surgery (Figure 2). In family no. 106, mutation testing could only be performed on the proband III-4 and her sister III-7. In addition there are three sudden infant deaths, one an infant with proven SVAS and two with cardiovascular problems; two surviving children with SVAS or pulmonary stenosis, and three other possibly affected adults (Figure 3).

No *ELN* mutations were found in 64 patients with SVAS included in the study. These were clinically typical SVAS patients but the majority were sporadic. Eight cases were reported as familial but linkage support for the role of elastin was not carried out due to the small number of family members available for analysis. The SSCP/HD technique employed in this study has a 90% detection rate, so we cannot exclude the presence of point mutations in these patients. The presence of whole exon deletions within the *ELN* gene (not seen by FISH analysis), is also possible, and skin fibroblasts are being obtained from these patients for further screening. Alternatively, mutations in another gene could also cause SVAS.

Discussion

We have identified 35 patients with point mutations in the elastin gene many of whom have other vascular abnormalities. There appear to be mutation hotspots in exons 9 (Y150X) and 21 (Q442X – also reported in a sporadic case by Li *et al*[15]) of the *ELN* gene. Mutation K176X was detected in two of our families, and mutation R570X was also described in a sporadic SVAS case by Li *et al*.[11] None of these families are known to be related and we found no evidence for a founder effect in our cases. Marked intra and interfamilial variation in phenotype is seen and there does not appear to be any good genotype–phenotype correlation, ie cases with missense mutation or splicing were just as likely to have severe SVAS as cases with truncating mutations. Disease severity within our SVAS families has varied from asymptomatic carriers of the mutation, to individuals dying in infancy from severe cardiac disease. Long term follow-up of aortic and pulmonary vascular lesions in WBS patients with SVAS has shown that individuals with moderate pressure gradients (exceeding 20 mm Hg) can present with pressure increases later in life, often requiring surgical intervention for the life-threatening vascular narrowing.[17,18] Isolated SVAS may also be progressive and such patients require follow-up but the situation with regard to monitoring of mutation carriers with no apparent cardiovascular abnormalities is not resolved.

Two mechanisms for the pathology of SVAS can be proposed: the defects in elastin could be quantitative (haploinsufficiency for elastin) or qualitative (aberrant tropoelastin resulting in abnormal elastic fibres).

The hypothesis of a quantitative defect appears the most attractive because hemizygosity for elastin is already established as the mechanism for SVAS in patients with partial deletions involving *ELN* and in WBS.[13] All classic WBS patients described are hemizygous for the elastin gene, but there is considerable variability in the severity of vascular lesions seen. Variable penetrance is typical of diseases associated with haploinsufficiency, where genetic background is expected to have a major modifying effect on development of the phenotype.

Functional hemizygosity also appears to be the mechanism by which the majority of the SVAS point mutations exert their effect. Mutations affecting the *ELN* translation start site are essentially null alleles (6%), and the truncating mutations (66%) probably result in haploinsufficiency for elastin through nonsense mediated mRNA decay.[19]

It is not, however, clear if splicing and missense mutations would result in a simple loss of function. These mutations could result in exon skipping, or frameshifts through activation of cryptic splice sites. Indeed, Urban *et al*[20] have described a SVAS patient with an acceptor splice site mutation that causes both exon skipping and activation of a cryptic splice, leading to a frameshift. Because the elastin gene has many small in-frame exons, exon skipping should be well tolerated, especially since elastin undergoes naturally occurring alternative splicing *in vivo*. It is worth noting that all the splicing mutations we detected at the consensus splice junctions are predicted to induce skipping of exons not normally spliced out. This suggests that they are important for the structural integrity of the elastic matrix and their exclusion is likely to be pathogenic. If abnormal tropoelastin proteins are synthesised they could interfere with elastic fibre assembly in a dominant negative manner, resulting in aberrant fibre formation during vasculogenesis and SVAS.

The role of the missense mutations in the pathology of SVAS is more intriguing. Three occur in exons reported to be spliced out *in vivo* (exons 13, 26 and 30).[20,21] Alternative splicing-out of a proportion of these mutated exons should result in some degree of phenotypic rescue; however, all three patients had severe SVAS. One explanation could be that the missense mutations activate cryptic splice sites that induce additional splicing out of that exon. This could upset the overall balance of tropoelastin isoforms present at certain developmental stages, which may be important for tissue-specific elastic fibre assembly. In fact, a specific element within exon 30 of tropoelastin mRNA has been shown to be involved in posttranscriptional regulation of elastin production in mature tissue,[22] and increased splicing out of this exon may have pathogenic consequences on tropoelastin expression during development.

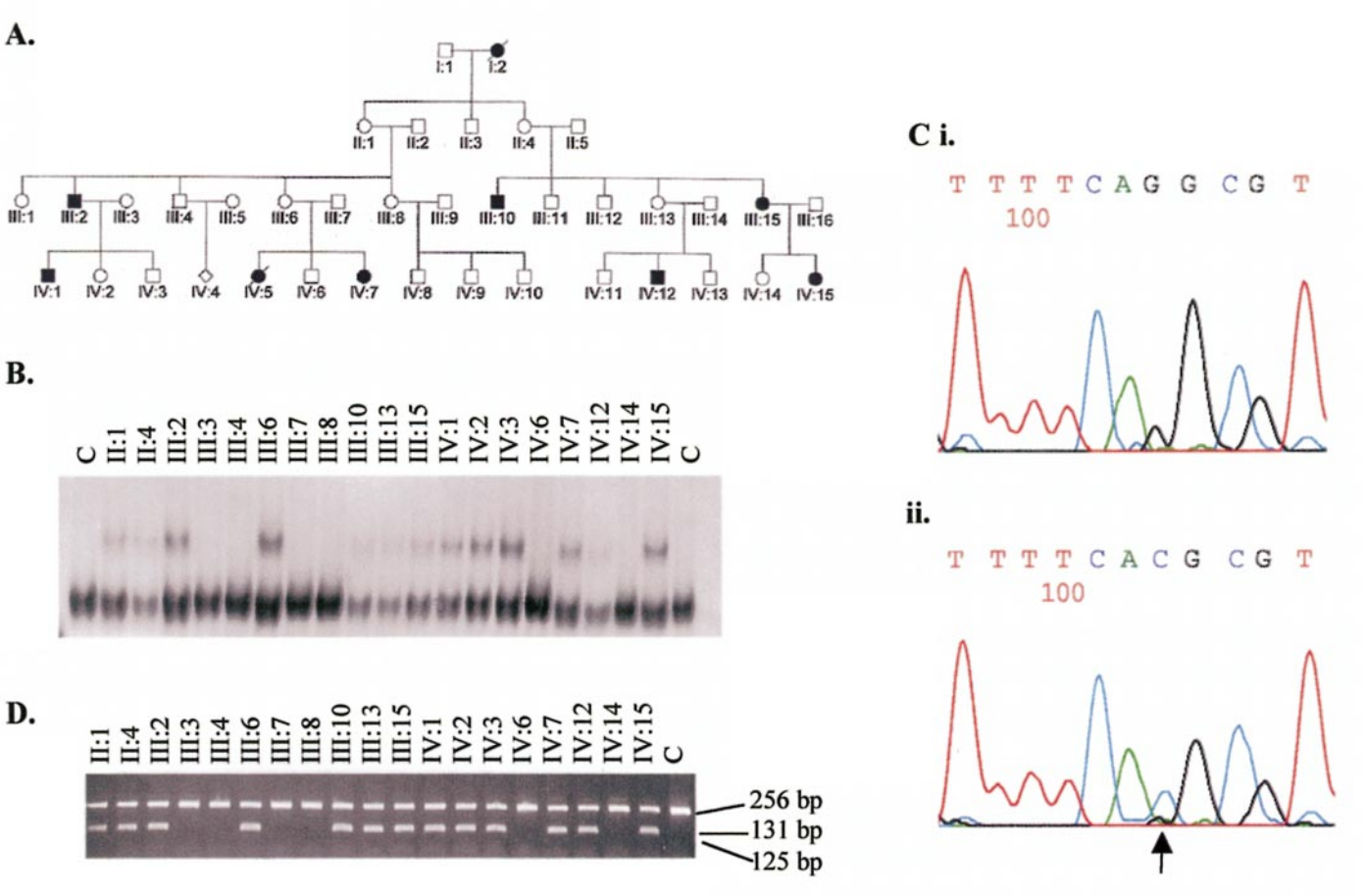


Figure 2 Pedigree and mutation analysis of family no. 91 **A** Family pedigree III-2, III-10, III-15, IV-7: severe SVAS requiring surgery; II-4, IV-12: moderate SVAS; IV-1, IV-15: mild SVAS; III-13, IV-2, IV-3, IV-14: no detected SVAS; II-1 III-3; III-4; III-6; III-7; III-8; IV-6: no reported vascular abnormalities **B** Heteroduplex analysis of exon 17 PCR product, showing a shift in the affected family members and some not reported with vascular problems; an indication of variable penetrance **C** Double strand sequence analysis of exon 17 shows: **i** normal mother III:2, and **ii** proband IV:1 with an acceptor splice site mutation at base 890–1 G → C base substitution **D** Confirmation of the mutation by *Mlu*I digest analysis. An *Mlu*I restriction site is created in the presence of the mutation resulting in two bands (131 bp + 125 bp = mutant allele; 256 bp = normal allele). Normal members of the family are homozygous for the normal allele (256 bp band); affected members are heterozygous for the normal and mutant allele (256 bp, 131 bp and 125 bp bands seen).

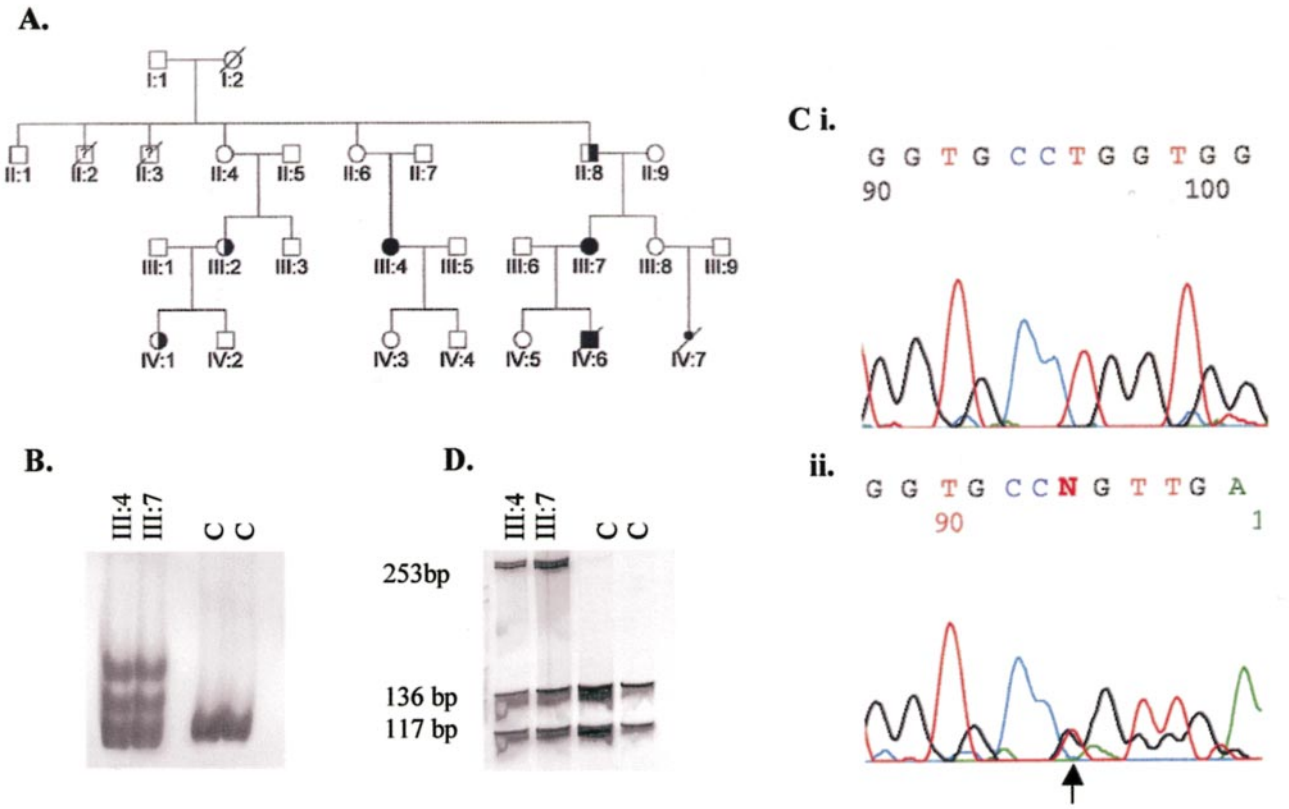


Figure 3 Pedigree and mutation analysis of family no. 106 **A** Family pedigree II-2, II-3: died suddenly in infancy; II-8: chronic lung disease, heart murmur; II-9: emphysema; III-2: cardiac murmur; III-4, III-7, IV-1: severe SVAS; IV-2: pulmonary stenosis; IV-3, IV-4: normal; IV-6: severe SVAS, mild pulmonary stenosis, peripheral pulmonary stenosis and minor biventricular hypertrophy, died in infancy **B** Heteroduplex analysis of exon 6 showing a shift in the affected family members tested: III-4, III-7; C: controls **C** Double strand sequence analysis of exon 6 shows **i** Control individual and **ii** proband III:4 with a single base deletion (T) at base 279, giving a frameshift **D** Confirmation of the mutation by *Bst*NI digestion. The T base deletion destroys a *Bst*NI site that normally cleaves the 253 bp product into fragments of 136 bp and 117 bp. The mutant 253 bp allele is present only in the affected individuals, not in the controls (C).

The physiological consequences of haploinsufficiency for elastin can be followed in studies on hemizygous mouse knockouts ($ELN^{-/+}$).[23] Both humans and mice respond to *ELN* hemizygosity during development, by increasing the number of elastic lamellar units in their aorta, thereby increasing the risk of obstructive vascular disease. Whether aberrant tropoelastin and the formation of abnormal elastic fibres would also lead to SVAS via the same physiological mechanism remains to be determined, but we expect that would be the case. Future work will concentrate on providing evidence for the existence of a dominant negative mechanism for the pathology of SVAS.

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