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Elastin Mutation and Cardiac Disease

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Abstract. Characterization of the molecular basis of structural cardiac disease includes elucidating the pathogenesis of certain vascular disease by demonstrating mutations of the Elastin gene as the cause of familial supravalvular aortic stenosis (SVAS) and Williams' syndrome (WS). Defining the etiology of SVAS has clinical implications in terms of prenatal and presymptomatic diagnosis and possible earlier intervention with medical therapy. This review considers the evidence relating Elastin mutations to SVAS and WS and outlines the possible mechanisms by which these mutations give rise to cardiac disease. Finally, the implications which Elastin mutation identification has on current clinical practice and future research directions are considered.

Key words: Supravalvular aortic stenosis — William's syndrome — Elastin

Supravalvular aortic stenosis (SVAS) is an uncommon but well characterized form of left ventricular outflow obstruction. First recognized by an Italian pathologist, Mencarelli, in 1930, it has been identified with increasing frequency during the past 30 years. The lesion involves the ascending aorta and may often be observed with associated pulmonary arterial stenoses or stenoses of other arteries, especially at major branch points [13]. The onset and severity of disease varies but, if untreated, may result in heart failure, myocardial infarction, and death. Surgical intervention is the only therapeutic option in symptomatic cases. SVAS is classically associated with Williams' syndrome (WS), which comprises accompanying features of mental retardation, characteristic personality, specific cognitive profiles, occasional infantile hypercalcemia, dysmorphic facial features, and connective tissue disease abnormalities (Fig. 1). However, SVAS has also been well documented in the absence of WS, either as a sporadic disease or inherited as a distinct autosomal-dominant condition. The histopathological features of the ascending aortic changes and the natural history do not differentiate between these three clinical situations.

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The Emergence of Distinct Phenotypes with SVAS

In 1961 Williams et al. [28] recognized the association of SVAS with mental retardation and a characteristic facies. Their observations were extended by Black and Bonham-Carter in 1963 [1]. Most of these cases were sporadic, although familial cases were eventually reported [2, 27]. In parallel with the literature developing around WS, cases of SVAS in the absence of WS were reported. Eisenberge et al. [5] reported two families with isolated SVAS. These observations were followed by a few reports of single pedigrees in which SVAS was identified in members of successive generations in a pattern consistent with autosomal-dominant inheritance [13, 14, 16, 20, 26]. Schmidt et al. [22] presented compelling evidence for autosomal-dominant inheritance of isolated SVAS by describing a large three-generation family in whom 5 of 9 siblings were affected with SVAS of mild to moderate degree. Twenty additional relatives comprising parents, additional siblings, and children of the original 5 patients were evaluated by physical and echocardiographic examinations, and 17 of 27 members of this family were found to be affected. None of the patients in this family had any features to suggest WS.

Chiarella et al. [3] considered a large family of 80 members and documented transmission of the SVAS trait with incomplete penetrance (86%) and variable expressivity, resulting in several affected individuals having subclinical disease.

A Role for Elastin in the Pathogenesis of SVAS?

Initial evidence supporting a role for Elastin in the pathogenesis of SVAS was derived from pathological and physiological studies. Pathological studies of patients

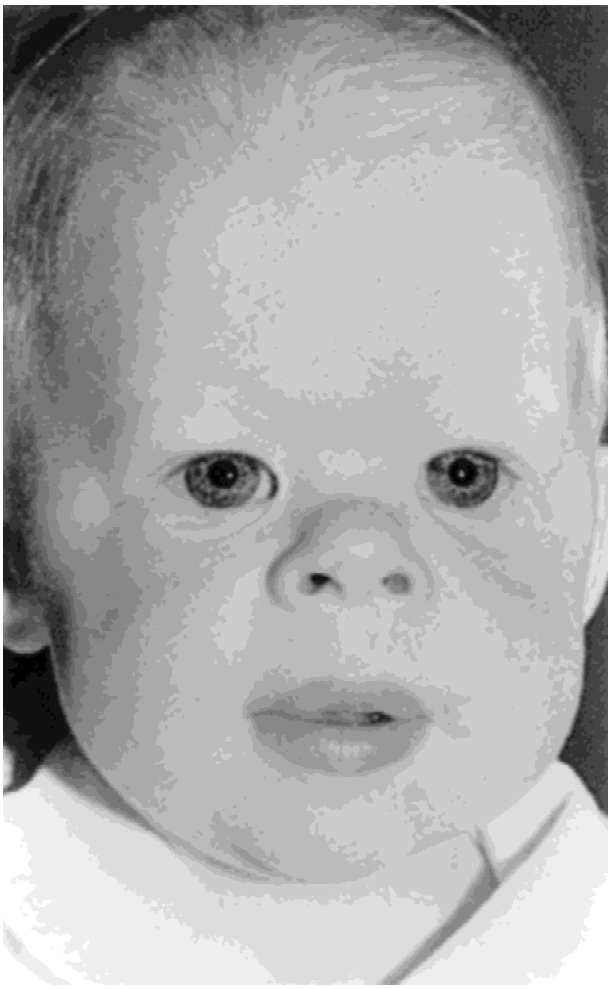

Fig. 1. Typical features of Williams' syndrome.

with SVAS identified abnormalities in Elastin structure and function. Perou [18] showed that the diseased media in the aorta of a single patient with SVAS contained excessive smooth muscle and reduced elastic tissue in the form of broken and disorganized elastic fibers. O'Connor et al. [16] examined tissue from six individuals with SVAS, inherited as autosomal dominant, sporadic, or as part of WS, and demonstrated that there was a common histological abnormality which comprised a haphazard arrangement of elastic fibers, excessive collagen and hypertrophied smooth muscle, and scant ground substance in the medial layer of the aorta in all patients. This contrasted with the highly organized arrangement in normal medial tissue. A quantitative deficiency of elastic fibers was also noted, resulting in an abundance of smooth muscle clumps and bundles in the diseased media compared to the normal aorta in which elastic tissue is most abundant.

The physiology of the vascular system, particularly the aorta, suggests that abnormalities in Elastin could cause SVAS. Reduced vascular elasticity would have a deleterious effect on vascular resistance and since hemodynamic stress is greatest in the ascending aorta, this structure would be the most severely affected [7]. Conversely, pulmonary artery stenosis often improves with time in SVAS patients; the lower pulmonary vascular resistance may possibly allow more normal growth of the arteries despite abnormalities of the media [15].

Such initial evidence implicating Elastin in the pathogenesis of SVAS gained increased momentum as the findings of genetic studies unfolded.

Genetic Studies

The mapping of the autosomal-dominant SVAS phenotype to chromosome 7q near the Elastin locus further implicated Elastin as a candidate for the disease gene. Ewart et al. [6] conducted linkage analysis using a large family with 13 affected individuals and showed that the disease gene was located to 7q in the Elastin region. Further support for a mutation of Elastin at 7q11.23 came with evidence that SVAS cosegregates in an autosomal-dominant fashion with a familial 6,7 translocation [15], subsequently shown to disrupt the Elastin gene [4]. Ewart et al. [6] demonstrated hemizygosity at the Elastin locus in affected members of familial as well as sporadic WS, which were confirmed by fluorescent *in situ* hybridization (FISH) and Southern blot analyses, and they concluded that WS can be caused by submicroscopic deletions within chromosomal subunit 7q11.23 involving the whole of the Elastin gene, with deletions exceeding 250 kb. FISH, using cosmid probes flanking either end of the Elastin locus to determine hemizygosity at this locus, has since been found to give a positive result in 96% of patients with classical WS [12] (Fig. 2). Therefore, it could be postulated that mutations involving part of the Elastin gene cause familial SVAS, whereas large deletions involving the entire Elastin gene and adjacent loci cause WS [7]. While a causal association between mutations of Elastin and SVAS remained unproven, there was strong evidence that this was likely.

Large DNA rearrangements which disrupt Elastin were associated with the disease in three SVAS families [4, 7, 17]. Ewart et al. [7] describe a family with autosomal-dominant transmission of SVAS and in whom a 100-kb deletion at the 3′ end of the Elastin gene cosegregated with the disease. DNA sequence analysis localized the breakpoint between Elastin exons 27 and 28—the same region disrupted by the SVAS-associated translocation [4]. The data presented thus indicated that mutations in the Elastin gene could cause SVAS and also suggested that Elastin exons 28–36 may encode critical domains for vascular development. Olson et al. [17] reported SVAS without features of WS in a family with a 30-kb deletion involving exons 2–27 with breakpoints in

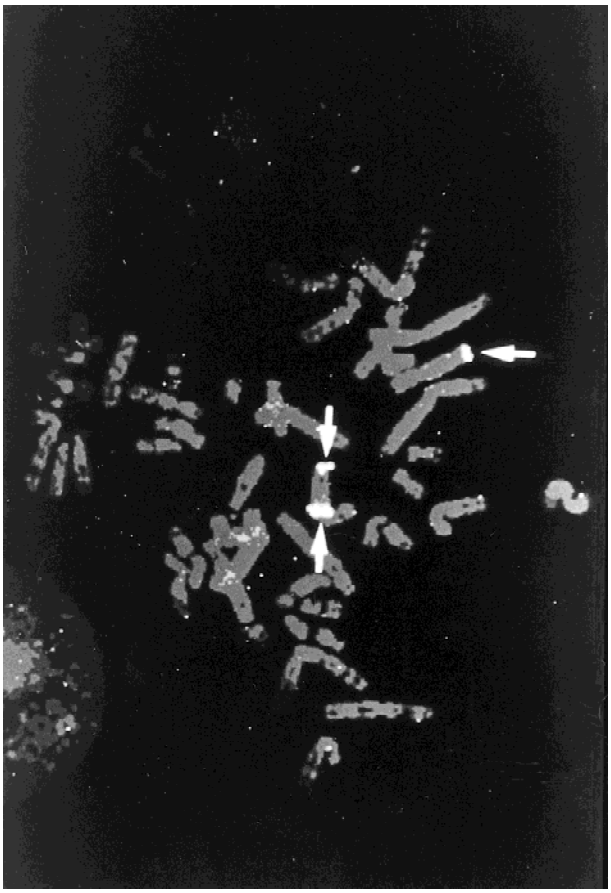

Fig. 2. FISH study to demonstrate the deletion of Elastin in Williams' syndrome. The chromosome 7's are identified by a telomeric chromosome 7-specific probe. Elastin hemizygosity is clearly demonstrated.

introns 1 and 27 within the Elastin gene. This deletion not only disrupts the same region of the gene at the 3′ end as reported by Ewart et al. [7] but also extends within the gene almost to the 5′ end.

To date, Elastin gene deletion associated with SVAS has been observed almost exclusively in WS. The single published exception to this has been the case of Fryssira et al. [9]. This single patient, who presented a *de novo* deletion of Elastin as demonstrated by FISH studies using cosmids from the 5′ and 3′ ends of the Elastin gene, had none of the facial features of WS but required surgical treatment of SVAS. Two further examples of this rare phenomenon have recently been communicated [23].

Olson et al. [17] postulated that more subtle Elastin gene defects such as point mutations might account for some or most cases of SVAS based on the observation that a deletion was not detected in a large SVAS family despite there being linkage to the Elastin gene region. Data demonstrating that point mutations do indeed cause both familial and sporadic SVAS have been presented. The complete genomic structure of the human Elastin gene has been determined, thus allowing the development of oligonucleotide primers for mutational analysis of this locus. Tassabehji et al. [24] described two SVAS families in whom the probands manifested severe SVAS, had normal FISH of Elastin thus showing no evidence of a large deletion, and demonstrated point mutations in exons 21 and 26. Li et al. [11] identified Elastin point mutations associated with the disease in four SVAS families and three sporadic cases, including nonsense, frameshift, and splice site mutations. In one case Elastin mutation was demonstrated to have arisen *de novo*.

In terms of the relationship between SVAS and WS, although it is now clear that the molecular basis of both involves Elastin, the precise basis of the phenotypic differences between these two clinically distinct situations is not ascertained. However, speculation that WS may represent a more extensive deletion of the Elastin locus incorporating adjacent genes and thereby accounting for the additional phenotypic characteristics is supported by evidence that LIM-kinase is also deleted in 20 WS cases tested [24].

Finally, characterization of the Elastin gene indicates that it comprises 2361 base pairs (bps) split into 34 small exons ranging in size from 30 to 225 base pairs and extending over ~47 kb of genomic sequence. The introns range from 82 bp to ~10 kb and, as previously reported, the 3′ region of the gene is rich in Alu sequences. Two microsatellite repeats are also described. An (AG)n repeat is located ~230 bp upstream of the 5′ acceptor splice site of exon 18 and a (GT) 17–20 repeat at 10 bp downstream from the 3′ donor splice site of exon 18.

Function of Elastin

Elastin is the major component of elastic fibers which form a network contributing to the elasticity and resilience of tissues, such as skin, lung, and arterial blood vessels. Mature elastic fibers are composed of two morphologically distinct components: amorphous Elastin and 10- to 12-nm microfibrils. The Elastin protein is a well-characterized, highly hydrophobic, nonglycosylated polypeptide of approximately 830 amino acids and is thought to form a random coil. The Elastin polypeptides are encoded by an mRNA of ~3.5 kb which serves as a template for the translation of Elastin polypeptides in cells such as aortic smooth muscle and skin fibroblasts. Following intracellular post-transitional modification, the Elastin polypeptides are secreted into the extracellular millieu where they assemble into functional fibers. The fibrillogenesis of elastic fibers is initiated by deposition of microfibrils which provide a scaffold onto which the individual Elastin molecules align. After secretion, individual Elastin molecules are cross-linked to one another via lysine residues by the copper-dependent

enzyme lysine oxidase to form a complex interacting network of elastic fibers. The intermolecular cross-links, known as desmosines, contribute to the insolubility of Elastin. Cysteine residues near the carboxyl terminus of the polypeptide are thought to be important for the interaction with the cysteine-rich protein fibrillin in the arrays of microfibrills [25].

Possible Mechanisms to Explain the Molecular Pathology of SVAS

The detailed sequence of events linking Elastin mutation to SVAS is still unclear. It is postulated that point mutations may lead to the synthesis of aberrant mRNA transcripts causing functional hemizygosity, as is known to be the mechanism of SVAS in WS. Aberrant transcript synthesis might result in the production of Elastin protein lacking the carboxyl terminus. As described, the carboxyl end of Elastin contains two cysteine residues thought to be critical for the interaction with microfibrillar-associated glycoprotein during elastogenesis, and loss of this could result in aberrant elastic fiber formation during vasculogenesis [10]. The splice site mutations described by Li et al. [11] affect exons 3 and 16 and may result in removal of these exons from the mature transcript. Exon 3 encodes a hydrophobic domain that may be important for the tertiary structure of Elastin, and exon 16 encodes a relatively large hydrophobic domain that separates two alanine-rich cross-linking sites near the center of the molecule together with a hexapeptide sequence that interacts with Elastin-binding proteins on the surface of cells. Thus, deletion of exon 16 could disrupt both the structural integrity and the cellular interaction of Elastin [11].

Phenotype Variability

Understanding of the SVAS syndrome involves recognition of the extreme variability in site, extent, and severity of cardiovascular involvement from case to case. Patients with SVAS may have stenotic lesions of one, several, or all the coronary arteries, major systemic arteries, pulmonary arteries, and systemic veins [22]. Variable expressivity and reduced penetrance of SVAS is seen in both WS and familial SVAS, explaining why some cases require cardiac surgery whereas others do not. This is consistent with findings in previous families that, for example, harbor a balanced translocation [4] or have an intragenic deletion of Elastin [17]. Chiarella et al. [2] suggest that the incidence of associated pulmonary stenosis is underestimated by the absence of clinical indications for catheterization by which many of these lesions would be identified. Li et al. [11] attempted to determine if a specific Elastin mutation could be used to predict the severity of vascular disease. These authors reexamined the phenotypes in a population of SVAS cases with known mutations. They found that variability of disease within families was every bit as great as that seen between families. Within a single family, these authors reported asymptomatic individuals whose vascular abnormality was documented late in life through clinical testing as well as individuals requiring early surgical correction as a result of severe generalized vascular disease.

Implications for Treatment

Elucidating the molecular pathogenesis of SVAS may have implications for treatment in terms of presymptomatic and prenatal diagnosis and earlier intervention with drug treatment [22].

Presymptomatic diagnosis of SVAS can be made in many patients by noninvasive cross-sectional and color Doppler flow echocardiography. However, these studies are not completely sensitive, especially in detecting peripheral pulmonary stenoses [8]. Invasive cardiac catheterization and angiography are more sensitive but carry the risk of serious complications [6]. French [8] reported that even angiography was not predictive of distal pulmonary arterial stenosis in patients who developed significant late disease, suggesting scope for improvement in diagnostic methods. In addition, the signs of SVAS may change with time, because aortic involvement may worsen while pulmonary findings may improve with age. Exclusion of a known familial SVAS mutation in a given case as a result of genetic testing will identify unaffected individuals who do not require cardiac investigation. Moreover, prenatal diagnosis may be offered to families with autosomal-dominant SVAS, with the recognition that mutation identification bears no relationship to severity of phenotype. Curran et al. [4] suggested that early intervention with drug treatment to lower the heart rate and blood pressure may slow progression of the disease, whereas Rabinovitch [19] investigated the use of elastase inhibitors in preventing pulmonary hypertension and associated pulmonary arterial abnormalities.

Future Directions

The detailed genomic characterization of the Elastin gene means that detailed mutation analysis at this locus is now possible. Future studies can focus on the role of Elastin as a genetic risk factor for common vascular disease. Continued mutation analysis in SVAS and WS patients may help distinguish the vascular pathology of these conditions by determining the spectrum of disease-causing mutations. Future developments could also incorporate the investigation of other conditions for in-

volvement of Elastin mutation. Schmidt [22] suggests, on the basis of gross and histologic pathologic findings of widespread stenoses in two sisters with familial SVAS, that SVAS syndrome is a generalized arterial fibromuscular dysplasia with variable expression. That the etiology of SVAS and pulmonary arterial stenoses in familial SVAS and WS is defined by Elastin mutation raises the interesting question as to the etiology of other arterial stenoses which have a familial basis and similar histopathology.

Current research, as summarized here, will prove invaluable to the resolution of such issues which are the subject of ongoing studies.

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