Society for Cardiovascular Pathology
The Cardiovascular Pathology of Genetic Disorders: When a Good Gene Goes Bad
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The Genetics of Atherosclerosis
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Introduction
Atherosclerosis of the coronary arteries is a complex multifactorial process involving multiple pathways that are influenced by both genetic and environmental factors. Our knowledge of the biology of atherosclerotic vascular disease has significantly increased over the last few decades. After an initial focus on lumen narrowing, atherosclerosis is now viewed as a response to injury that at all stages includes a significant inflammatory component. The development, progression, and complications of atherosclerosis involve multiple cell types and mediators, as well as pathways of inflammation, cell proliferation, vascular remodeling, extracellular matrix changes, calcification, thrombosis, and cell death. Furthermore, atherosclerosis is a non-linear process and the risk of acute coronary syndromes is dynamic and fluctuates over time. Although atherosclerosis generally progresses with age, there are periods of quiescence punctuated by episodic increases in plaque inflammation and growth. Mechanisms mediating ‘instability’ of atherosclerotic plaque have been postulated and the term ‘vulnerable plaque’ coined.

Coronary heart disease (CHD) is heritable common ‘complex’ disease from the genetic standpoint. Several rare Mendelian forms have been documented and have provided valuable insights into the common form of the disease as highlighted below.

Mendelian disorders associated with CHD.
Mendelian disorders associated with CHD such as familial hypercholesterolemia, comprise single-gene traits that are transmitted in an autosomal dominant, recessive or X-linked manner. For example, mutations in genes encoding LDL receptor (LDLR), ligand-binding domain of apolipoprotein B (APOB) - 100, and proprotein convertase subtilisin/kexin type 9 (PCSK9), result in familial hypercholesterolemia transmitted in an autosomal dominant manner. The examination of disease pathophysiology and gene function in such Mendelian disorders has increased our understanding of the etiology of the common ‘complex’ trait of CHD. Additionally, common variation in genes implicated in Mendelian disorders may determine disease susceptibility in the general population. Several Mendelian disorders of lipid metabolism associated with increased CHD risk have yielded novel insights into the mechanisms of CHD (Table 1). Mendelian causes of atherosclerosis not primarily related to the lipoprotein pathway are summarized in Table 2.

In the prototypical Mendelian lipoprotein disorder, Familial Hypercholesterolemia, elevated LDL particles are deposited initially in the subendothelial space as fatty streaks. Subsequent recruitment of inflammatory cells such as macrophages leads to formation of foam cells. Death of foam cells leads to migration of smooth muscle cells from the media and subsequent proliferation to form a fibrous cap. Extracellular accumulation of lipids and deposition of calcium follow. Enlargement and progression of atheroma leads to rupture of the lesion or progressive stenosis of the artery. Xanthomas, localized collections of lipid rich foam cells that histologically resemble atheroma, can occur in the skin or in tendons. In Tangiers disease accumulation of lipids is both intra and extracellular, mainly in the intima but also in deeper layers. These lipid containing cells are mostly smooth muscle cells with lipid droplets. In Fabry’s disease, glycosphingolipids accumulate in vascular endothelium, the intima and in medial smooth muscle cells.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of inheritance</th>
<th>Gene (chromosome)</th>
<th>Lipid abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial hypercholesterolemia</td>
<td>AD</td>
<td>LDLR (19p13.2)</td>
<td>Elevated plasma levels of total and LDL cholesterol</td>
</tr>
<tr>
<td>Familial ligand-defective apolipoprotein B</td>
<td>AD</td>
<td>APOB (2p24)</td>
<td>Elevated plasma levels of VLDL and LDL cholesterol</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>AD</td>
<td>PCSK9 (1p32)</td>
<td>Elevated plasma levels of total and LDL cholesterol</td>
</tr>
<tr>
<td>Type III hyperlipoproteinemia</td>
<td>AR</td>
<td>APOE (19q13.2)</td>
<td>Elevated plasma levels of [beta]-VLDL</td>
</tr>
<tr>
<td>Hyperlipoproteinemia (a)</td>
<td>AD</td>
<td>LPA (6q27)</td>
<td>Elevated plasma levels of lipoprotein(a)</td>
</tr>
<tr>
<td>Familial combined hyperlipidemia</td>
<td>AR or MF</td>
<td>SOD2, CETP, LCAT APOA1, APOC2 APOA4, USF1</td>
<td>Elevated plasma levels of LDL and VLDL cholesterol</td>
</tr>
<tr>
<td>Atherosclerosis susceptibility</td>
<td>AD or MF</td>
<td>19p13.3~p13.2 (gene locus)</td>
<td>Preponderance of small, dense LDL particles, increased levels of triglyceride-rich lipoproteins, and low levels of HDL cholesterol</td>
</tr>
<tr>
<td>Fish eye disease</td>
<td>Unknown</td>
<td>LCAT (16q22.1)</td>
<td>Elevated plasma levels of serum triglycerides, VLDL, and low HDL cholesterol</td>
</tr>
<tr>
<td>Tangier disease</td>
<td>ACD</td>
<td>ABCA1 (9q22-q31)</td>
<td>Absence of HDL cholesterol and very low plasma levels of apolipoprotein A1</td>
</tr>
<tr>
<td>Cerebrotendinous xanthomatosis (CTX)</td>
<td>AR</td>
<td>CYP27A1 (2q33-qter)</td>
<td>Plasma cholesterol levels are not elevated, but deposits of cholesterol and cholesterol occur in virtually every tissue, particularly in the Achilles tendons, brain, and lungs</td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td>XL</td>
<td>GLA (Xq22)</td>
<td>Accumulation of glycosphingolipids in the vascular endothelium and visceral tissues throughout the body</td>
</tr>
<tr>
<td>Sitosterolemia</td>
<td>AR</td>
<td>ABCG8 (2p21)</td>
<td>High plasma levels of plant sterols</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABCG5 (2p21)</td>
<td></td>
</tr>
</tbody>
</table>

Additional details and references are provided in reference 9⁹
Table 2. Mendelian disorders associated with CHD that are not related to the lipoprotein metabolism.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of inheritance</th>
<th>Gene (chromosome)</th>
<th>Pathology Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocystinuria</td>
<td>AR</td>
<td>CBS (chr21q22.3)</td>
<td>Abnormally high concentrations of homocysteine and its precursor, methionine (due to defective CBS); associated with oxidative stress, endothelial injury and platelet-mediated intimal proliferation of smooth muscle cells.</td>
</tr>
<tr>
<td>Pseudoxanthoma elasticum</td>
<td>AR</td>
<td>ABCC6 (chr16p13.11)</td>
<td>Fibroelastic thickening and disorganization of the intima, fragmentation and calcification of elastic fibers in the arterial wall.</td>
</tr>
<tr>
<td>Progeroid syndromes- classic Hutchinson-Gilford progeria syndrome and Werner syndrome</td>
<td>HGPS may occur due to de novo mutations and Werner’s is AR</td>
<td>1) LMNA (chr1q21.2). 2) WRN (chr8p12)</td>
<td>Loss of medial smooth-muscle cells, secondary maladaptive vascular remodeling, intimal thickening, disrupted elastin fibers, and deposition of extracellular matrix; sclerotic plaques and stenosis in aorta and coronaries.</td>
</tr>
</tbody>
</table>
Non Mendelian forms of atherosclerosis are by far the most common. There is a significant heritable component as exemplified by twin and family studies.\textsuperscript{9,10} The familial clustering of CHD is partly due to heritability of known CHD risk factors, but there is evidence to suggest that family history contributes to an increased risk of CHD independently of the known risk factors.\textsuperscript{11} A history of early CHD in a first-degree relative approximately doubles the risk of CHD, although the reported relative risk ranges from 1.3–11.3.\textsuperscript{9} Challenges in discovering genetic susceptibility variants for CHD include phenotypic heterogeneity, genetic heterogeneity, modest gene effects, gene–gene and gene–environment interactions, and rare variants causing complex disease. Evidence from genetic studies implicating cellular proliferation and inflammation in atherosclerosis is summarized below.

**Cellular proliferation.** The importance of cellular proliferation was highlighted by the discovery that SNPs in a 58-kilobase (kb) interval on chromosome 9p21 were associated with CHD. Nearly one-quarter of persons of European ancestry are homozygous for the risk allele. The risk interval is a relative ‘gene desert’ and the mechanism linking the region to CHD remained unknown until a report in *Nature* in 2010 by Visel and colleagues.\textsuperscript{12} Deletion of the orthologous region in mice affected cardiac expression of nearby genes, as well as vascular smooth muscle cell proliferation through a cis-acting mechanism. Primary cultures of aortic smooth muscle cells from these mice exhibited excessive proliferation and diminished senescence, a cellular phenotype consistent with atherogenesis.\textsuperscript{4,12}

**Inflammation.** Evidence implicating candidate genes in inflammation pathways has come mostly from candidate gene association studies (Table 3) and not yet from GWAS.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene name</th>
<th>SNPs</th>
<th>SNP class</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX5AP</td>
<td>Arachidonate 5-lipoxgenase-activating protein</td>
<td>Haplotype A</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4986978</td>
<td>Intron</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>Chemokine (C-X3-C motif) receptor 1</td>
<td>rs3732378</td>
<td>NonSyn (T280M)</td>
</tr>
<tr>
<td>MMP3</td>
<td>Matrix metallopeptidase 3</td>
<td>rs3025058</td>
<td>Regulatory</td>
</tr>
<tr>
<td>SERPINE1</td>
<td>Serpin peptidase inhibitor, clade E, member 1</td>
<td>rs1799889</td>
<td>Regulatory</td>
</tr>
<tr>
<td>PSMA6</td>
<td>Proteasome subunit, α type, 6</td>
<td>rs1048990</td>
<td>5’ UTR</td>
</tr>
<tr>
<td>TNFSF4</td>
<td>Tumor necrosis factor (ligand) superfamily, member 4</td>
<td>rs3850641</td>
<td>Intron</td>
</tr>
<tr>
<td>LTA4H</td>
<td>Leukotriene A4 hydrolase</td>
<td>Haplotype K</td>
<td>—</td>
</tr>
</tbody>
</table>

Additional details and references are provided in reference 9\textsuperscript{9}
Genome-wide Association Studies (GWAS) have provided significant insights into the pathogenesis of non-Mendelian forms of atherosclerosis\textsuperscript{13} by discovery of nearly 130 loci influencing various atherosclerotic vascular diseases and related intermediate traits.\textsuperscript{13} The susceptibility variants that have been uncovered increase the risk modestly, typically by 10-40\% per risk allele. However, because many of the alleles are frequent in the population, the population attributable risk is significant.\textsuperscript{14} Many more such loci of weak effects will likely be identified by GWAS of large sample sizes and also as a result of meta-analyses that combine results of several studies. Table 4 summarizes the SNPs identified by GWAS as being associated with CHD.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Gene(s) in that location</th>
<th>Function of gene product(s)</th>
<th>RAF</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10757278</td>
<td>9p21.3</td>
<td>Cyclin-dependent kinase inhibitor 2A/2B (CDKN2A,2B)</td>
<td>Regulation of cell cycle</td>
<td>0.45</td>
<td>1.28</td>
</tr>
<tr>
<td>rs599839</td>
<td>1p13.3</td>
<td>Cadherin, EGF LAG seven-pass G-type receptor 2 (CELSR2); Proline-serine-rich coiled-coil 1 (PSRC1); sortilin 1 (SORT1)</td>
<td>Involved in contact-mediated communication; May participate in p53-mediated growth suppression; An essential player in adipocyte and muscle glucose metabolism</td>
<td>0.78</td>
<td>1.20</td>
</tr>
<tr>
<td>rs3008621</td>
<td>1q41</td>
<td>Melanoma inhibitory activity family, member 3 (MIA3)</td>
<td>Regulates cell adhesion and migration</td>
<td>0.26</td>
<td>1.10</td>
</tr>
<tr>
<td>rs501120</td>
<td>10q11.2</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0.16</td>
<td>1.33</td>
</tr>
<tr>
<td>rs9818870</td>
<td>3q22.3</td>
<td>Muscle RAS oncogene homolog (MRAS)</td>
<td>Responsible for a variety of normal cellular functions</td>
<td>0.15</td>
<td>1.15</td>
</tr>
<tr>
<td>rs3184504</td>
<td>12q24</td>
<td>SH2B adaptor protein 3 (SH2B3)</td>
<td>Mediates the interaction between the extracellular receptors and intracellular signaling pathway</td>
<td>0.39</td>
<td>1.13</td>
</tr>
<tr>
<td>rs9982601</td>
<td>21q22</td>
<td>Solute carrier family 5, member 3 (SLC5A3); Mitochondrial ribosomal protein S6 (MRPS6); Potassium voltage-gated channel, Isk-related family, member 2 (KCNE2)</td>
<td>Permits sodium ion transport; Involved in protein synthesis within the mitochondrion; Regulates neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume</td>
<td>0.13</td>
<td>1.20</td>
</tr>
<tr>
<td>rs12526453</td>
<td>6p24</td>
<td>Phosphatase and actin regulator 1 (PHACTR1)</td>
<td>Inhibits phosphoprotein phosphatase inhibitor</td>
<td>0.65</td>
<td>1.12</td>
</tr>
<tr>
<td>rs6725887</td>
<td>2q33</td>
<td>WD repeat domain 12 (WDR12)</td>
<td>Affects maturation of the large ribosomal subunit</td>
<td>0.14</td>
<td>1.17</td>
</tr>
<tr>
<td>rs1122608</td>
<td>19p13</td>
<td>Low-density lipoprotein receptor (LDLR)</td>
<td>Involved in receptor-mediated endocytosis of specific ligands</td>
<td>0.75</td>
<td>1.15</td>
</tr>
<tr>
<td>rs11206510</td>
<td>1p32</td>
<td>Proprotein convertase subtilisin/kexin type 9 (PCSK9)</td>
<td>Involved in cholesterol homeostasis</td>
<td>0.81</td>
<td>1.15</td>
</tr>
<tr>
<td>rs1746048</td>
<td>10q11</td>
<td>Chemokine (C-X-C motif) ligand 12 (CXCL12)</td>
<td>Activates leukocytes</td>
<td>0.84</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Additional details and references are provided in reference 15.\textsuperscript{15} RAF= risk allele frequency; OR= odds ratio
Gene Abbreviations. ABCA1, ATP-binding cassette, subfamily A, member 1; ABCG5, ATP-binding cassette, subfamily G, member 5; ABCG8, ATP-binding cassette, subfamily G, member 8; ACD, autosomal codominant; AD, autosomal dominant; APOA1, apolipoprotein A1; APOC2, apolipoprotein C2; APOA4, apolipoprotein A4; APOB, apolipoprotein B; APOE, apolipoprotein E; AR, autosomal recessive; CETP, cholesteryl ester transfer protein (plasma); CYP27A1, cytochrome P450, family 27, subfamily A, polypeptide 1; GLA, α-galactosidase; HDL, high-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPA, lipoprotein Lp(a); MF, multifactorial; SOD2, superoxide dismutase 2 (mitochondrial); USF1, upstream transcription factor 1; VLDL, very low-density lipoprotein; XL, X-linked. CBS: cystathionine-beta-synthase; ABCC6, multidrug resistance-associated protein 6; LMNA, lamin A;

References
Cardiomyopathies arise as primary and enigmatic disorders of the myocardium or secondary to established cardiovascular disease, such as chronic ischemia, hypertension, diabetes, valve disease or congenital malformation. Historical clinical studies recognized that in addition to lacking a recognized etiology, familial clustering was a prominent feature of primary cardiomyopathies, an observation that fostered application of human genetic strategies to investigate the role of genetics. Several decades of productive human genetics research has led to the discovery of thousands of distinct gene mutations that cause primary cardiomyopathies. In addition to defining molecular etiologies, definition of cardiomyopathy gene mutations have fostered novel insights into mechanism, improved clinical classifications, and uncovered relationships between inherited and presumed acquired forms of cardiac remodeling. These concepts will be exemplified through a review of genetic causes of hypertrophic remodeling and will be considered in the context of dilated cardiomyopathies.

Hypertrophic Remodeling:
Unexplained hypertrophy of the left ventricle (LVH) is a prevalent myocardial disorder affecting 1 in 500 individuals (1). LVH that asymmetrically involves the interventricular septum prompts the clinical diagnosis of hypertrophic cardiomyopathy (HCM), the most common cause for death on the athletic field. HCM is caused by mutations in cardiac genes that encode protein constituents of the sarcomere, the contractile unit within myocytes. More than half of all HCM is due to mutations in \textit{MYH7} or \textit{MYBPC3}, which encode β myosin heavy chain and myosin binding protein C, respectively (2, 3). Analyses of the biophysical consequences of human HCM mutations in MYH7 revealed the unexpected finding that these increase rather than decrease myosin properties (4), therein refuting the notion of LVH as a compensatory response to sarcomere dysfunction.
Characterization of LVH that occurs in association with electrophysiologic manifestations and extra-cardiac phenotypes resulted in discovery of additional genetic pathways. Mutations in the gamma-2 regulatory subunit of AMP-activated protein kinase (PRKAG2) that result in glycogen accumulation in the heart (5, 6) and in the lysosome-associated membrane protein-2 (7), which disrupts cardiac autophagy provide evidenced that multiple distinct pathophysiologic mechanisms produce LVH. With The widespread availability of clinical genetic testing for LVH provides molecular criteria for discriminating between HCM and other forms of hypertrophic remodeling (8, 9), information that is relevant to the clinical outcomes of patients.

Discovery of genetic causes of hypertrophic remodeling has also allowed analyses of the relationship between LVH that is inherited and LVH found in the general population. Genetic analyses of participants in the Framingham Heart Study with LVH attributed to prevalent cardiovascular conditions (10) and in pediatric cohorts with unexplained LVH (11) have revealed mutations in the same genes that cause familial disease. As such, the role of genetics in LVH appears to be quite broad.

Human gene mutations also cause unexplained dilated cardiomyopathy (DCM) (12). Unlike LVH genes, the pathways in which DCM genes participate are more diverse. DCM mutations occur in sarcomere protein genes (13), but at distinct residues from mutations that cause HCM. Moreover these DCM mutations cause reciprocal consequences on biophysical functions than HCM mutations (14). DCM mutations also occur in proteins involved in force transmission (e.g., desmin (15), titin (16), and Z-disc proteins (17)), in phospholamban (18), the molecular regulator of calcium reuptake into the sarcomplasmic reticulum, and in proteins with unclear functions in myocyte biology, such as the nuclear envelope protein lamin A (19, 20). While specific phenotypes (such as progressive conduction system disease) can implicate a particular DCM gene, clinical manifestations are often overlapping. As such, definition
of the pathogenic mutation in a given patient can require analyses of the entire spectrum of DCM genes (21).

Until recently, the considerable genetic diversity and large size of many DCM genes hindered the success of gene-based diagnosis in DCM. High throughput next generation DNA sequencing overcomes these barriers. With expanded use of these technologies, the opportunity to classify DCM according to molecular etiology will rapidly increase, an opportunity that will advance early recognition of patients at risk for disease development and potentially provide new intervention strategies.

References:


18. Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, Kranias EG, MacLennan DH, Seidman JG, Seidman CE. Dilated cardiomyopathy and heart


Single Gene Disorders in Congenital Heart Disease

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Co-Director, Cardiovascular Genetics
Associate Director, Heart Institute Research Core
Associate Professor, Pediatric Cardiology
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Cincinnati Children’s Hospital
Associate Professor, Adult Cardiovascular Diseases
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The Speaker has no disclosures
Single Gene Disorders in Congenital Heart Disease

- Significant effort devoted to identifying the genetic contributions to cardiovascular disease
  - Cardiomyopathy
  - Arrhythmias
  - Vasculopathy
  - Cardiovascular malformations
Single Gene Disorders in Congenital Heart Disease

- Congenital heart disease (CHD) remains the most common birth defect worldwide.
- Accounts for 25-30% of all birth defects.
- Birth prevalence 6-8/1000 live births.
  - ~40,000 children born with CHD in the US each year.
  - Additional 40,000 are born with subclinical disease.
- Numbers increasing as improvements in diagnostic techniques allow for detection of milder forms of disease.
Single Gene Disorders in Congenital Heart Disease

- With improvements in medical and surgical management, the majority of these patients survive into adulthood
  - Currently >1 million adults with CHD

- Significant impact on health care system
  - Reach reproductive age

- Understanding of genetics critical to these patients and their families
Genetic Basis of Congenital Heart Disease

- Chromosomal
  - Submicroscopic chromosomal aberrations
- Mendelian
  - Autosomal dominant (AD)
  - Autosomal recessive (AR)
  - X-linked
- Epigenetic-imprinting
- Multifactorial or Complex
Single Gene Disorders in Congenital Heart Disease

- Single gene disorders have been increasingly described in association with CHD

- Syndromic
  - Holt-Oram syndrome (TBX5)

- Nonsyndromic
  - NKX2.5
## Embryological Mechanisms

### Normal
- establishment of cardiogenic field
- formation of the heart tube
- chamber specification
- rightward looping
- chamber formation and valve development
- neural crest contribution to outflow tract

### Pathological
- laterality and looping - heterotaxy
- mesenchymal cell (neural crest) migration - TOF, TGA
- extracellular matrix - AV canal
- targeted growth - TAPVR
- apoptosis - muscular VSD
- hemodynamic (flow) defects - LVOT, RVOT, PDA
Holt-Oram Syndrome

- ASD, VSD - 66%
- 17% with complex lesion e.g. HLHS
- Thumb anomaly, (absence in 19/44, hypoplasia in 17/44, triphalangeal thumbs in 8/44) absence of radius (10/44).
- AD 12q21.3-q22, mutations in the \textbf{TBX5} gene (601620) - transcription factor
Expression of Murine Tbx5 in the embryonic heart and limbs
TBX5

- Transcriptional activator of chamber-specific genes
  - cardiac specification
  - chamber morphogenesis
  - differentiation

Human atrial septal defects
Heart Defects Associated with Tbx5 mutations

- ASD (141)
- PDA (10)
- PLSVC (9)
- TAPVR (5)
- AA (4), PAA (8), TRA (2)
- MVD (10)
- ECD (7), DORV (3), TOF (8)
- HLH (4), TRAB (2)
- VSD (67)
Noonan, Cardio-Facio-Cutaneous, and Costello Syndromes

- Neuro-cardio-facio-cutaneous (NCFC) syndromes
  - Noonan syndrome
  - Costello syndrome
  - Cardio-facio-cutaneous (CFC) syndrome
  - LEOPARD syndrome
NCFC syndromes result from DNA mutations that result in alteration of complex protein signaling pathways
- RAS/RAF/MEK
- Controls cell growth

There is a significant amount of clinical overlap between these disorders
However, each is characterized by mutations in specific genes
Noonan, Cardio-Facio-Cutaneous, and Costello Syndromes

- Most Noonan syndrome patients have mutations in PTPN11 (~50%)
  - Mutations in SOS1, K-RAS, and RAF1 account for ~25%

- Most Costello syndrome patients have mutations in H-RAS

- Most patients with CFC syndrome have mutations in B-RAF
  - Also may involve MEK1 and MEK2
The RAS/RAF/MEK signaling pathway plays important roles in different cellular mechanisms:
- Metabolism, differentiation, cell death

The malfunction of this pathway during embryologic development may result in multiple clinical abnormalities:
- Developmental delay
- Mental retardation
- Musculoskeletal disease
- Cardiomyopathies (Heart muscle disease)
Noonan Syndrome

- Possible parent to child inheritance
  - But many cases are new mutations with no prior family history
- Occurs in every 1:1000 to 1:2500 live births
- Findings may include wide set eyes, low-set ears, breast bone abnormalities, neck webbing, bleeding abnormalities, short stature
- Mild intellectual deficits may also occur
Noonan Syndrome

- Cardiac disease is well described and occurs in ~50% to 80% of people
- Pulmonic stenosis is the most common finding (20% to 50% cases)
- Hypertrophic cardiomyopathy (HCM) may occur in 20% to 30%
- Vascular involvement may also occur
  - Pulmonary arteries
  - Aorta

Noonan Syndrome
Pulmonic Stenosis
Noonan Syndrome
Aortic Dilation
Noonan Syndrome
Hypertrophic Cardiomyopathy
Cardio-Facio-Cutaneous Syndrome

- CFC is characterized by mental retardation, characteristic facies, ectodermal abnormalities, and cardiac disease.
- Recent review of 38 patients with proven mutations known to cause CFC.
- 71% found to have cardiac disease.

# Cardio-Facio-Cutaneous Syndrome

## Table 1

<table>
<thead>
<tr>
<th>Feature</th>
<th>BRAF (32)</th>
<th>MEK1 4)</th>
<th>MEK2 (2)</th>
<th>Respondents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary valve stenosis</td>
<td>11/27</td>
<td>3/4</td>
<td>0/2</td>
<td>42</td>
</tr>
<tr>
<td>ASD</td>
<td>9/27</td>
<td>0/3</td>
<td>0/2</td>
<td>28</td>
</tr>
<tr>
<td>VSD</td>
<td>7/27</td>
<td>0/3</td>
<td>0/2</td>
<td>22</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>11/28</td>
<td>1/3</td>
<td>1/2</td>
<td>39</td>
</tr>
<tr>
<td><strong>Hair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curly hair</td>
<td>29/32</td>
<td>3/4</td>
<td>2/2</td>
<td>92</td>
</tr>
<tr>
<td>Absent or sparse eyebrows</td>
<td>24/29</td>
<td>4/4</td>
<td>2/2</td>
<td>86</td>
</tr>
<tr>
<td>Sparse hair</td>
<td>27/32</td>
<td>3/4</td>
<td>2/2</td>
<td>84</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nevi</td>
<td>23/31</td>
<td>3/4</td>
<td>2/2</td>
<td>76</td>
</tr>
<tr>
<td>Keratosis pilaris</td>
<td>16/22</td>
<td>1/2</td>
<td>2/2</td>
<td>73</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>13/23</td>
<td>2/3</td>
<td>2/2</td>
<td>61</td>
</tr>
<tr>
<td>Haemangiomas</td>
<td>11/27</td>
<td>3/3</td>
<td>1/2</td>
<td>47</td>
</tr>
<tr>
<td>Red itchy skin</td>
<td>11/28</td>
<td>3/4</td>
<td>1/2</td>
<td>44</td>
</tr>
<tr>
<td>Ichthyosis</td>
<td>7/23</td>
<td>1/2</td>
<td>0/2</td>
<td>30</td>
</tr>
<tr>
<td>Café-au-lait macules</td>
<td>7/27</td>
<td>0/4</td>
<td>2/2</td>
<td>27</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>5/21</td>
<td>2/3</td>
<td>0/2</td>
<td>27</td>
</tr>
<tr>
<td>Cutaneous lymphangioma</td>
<td>2/29</td>
<td>0/3</td>
<td>0/2</td>
<td>6</td>
</tr>
</tbody>
</table>

Costello Syndrome

- Complex developmental disorder
  - Characteristic craniofacial features
  - Neurocognitive delay
  - Failure to thrive
  - Endocrine and skeletal disease
  - Predisposition to neoplasias
  - Cardiac disease
Costello Syndrome

- Many different types of heart disease seen
  - Malformations
  - Tachyarrhythmias
  - Cardiac hypertrophy
    - May be isolated to the left ventricle (LV), both ventricles, or may result in a dilated cardiomyopathy
### TABLE II. Cardiovascular Malformations in 28 Patients With Costello Syndrome

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>New patients</th>
<th>Literature</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>27</td>
<td>67</td>
<td>94</td>
</tr>
<tr>
<td>Cardiovascular malformation, total*</td>
<td>4 (14%)</td>
<td>24 (36%)</td>
<td>28 (30% all patients) (50% any abn)</td>
</tr>
<tr>
<td>Right-sided obstruction</td>
<td>3</td>
<td>10</td>
<td>13 (46%)</td>
</tr>
<tr>
<td>Pulmonic stenosis, valvar or NOS</td>
<td>1</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Pulmonic stenosis, ASD</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary artery stenoses</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Right and left sided obstruction</td>
<td>0</td>
<td>1</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Pulmonic stenosis, BAV, AS, MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left-sided obstruction</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Septal defect</td>
<td>0</td>
<td>9</td>
<td>9 (32%)</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Not specified</td>
<td>1</td>
<td>4</td>
<td>5 (18%)</td>
</tr>
<tr>
<td>Conotruncal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atrioventricular canal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Single ventricle</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heterotaxy</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AS, aortic stenosis; BAV, bicuspid aortic valve; CVM, cardiovascular malformation; MS, mitral stenosis; NOS, not otherwise specified.

*Additional mitral valve abnormalities: prolapse, myxomatous or redundant [new patients 3 and 23; Martin and Jones, 1991], thick mitral and/or aortic valve tips [new patient 4; Suri and Carroll, 1998, patient 1; Izumikawa et al., 1993, patient 1]; regurgitation without mitral valve abnormality [new patient 22], unspecified murmur [Torrelo et al., 1995].
Costello Syndrome

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>New patients</th>
<th>Literature</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>27</td>
<td>67</td>
<td>94</td>
</tr>
<tr>
<td>Cardiac hypertrophy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (22%)</td>
<td>26 (39%)</td>
<td>32 (34% all pts) (54% any abn)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>5</td>
<td>11</td>
<td>16 (50%)</td>
</tr>
<tr>
<td>Definite HCM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Possible HCM</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Probably not HCM, concentric LVH +/- subAS</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>LVH NOS</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Biventricular hypertrophy, LV &gt; RV</td>
<td>1</td>
<td>3</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Definite HCM</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Possible HCM</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Definitely not HCM</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Not specified, unclear</td>
<td>0</td>
<td>12</td>
<td>12 (38%)</td>
</tr>
</tbody>
</table>

# Costello Syndrome

## TABLE IV. Rhythm Disturbances in 31 Patients With Costello Syndrome

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>New patients</th>
<th>Literature</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patients</strong></td>
<td>27</td>
<td>67</td>
<td>94</td>
</tr>
<tr>
<td><strong>Rhythm disturbance</strong></td>
<td>8 (30%)</td>
<td>23 (34%)</td>
<td>31 (33% all pts)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(56% any abn)</td>
</tr>
<tr>
<td>Atrial, primary tachycardia</td>
<td>5 (62%)</td>
<td>18 (78%)</td>
<td>23 (74%)</td>
</tr>
<tr>
<td>SVT, +/- PACs</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>MAT, +/- PACs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>“Multifocal SVT”, PACs, PVCs</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chaotic atrial rhythm</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Atrial, NOS</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tachycardia NOS, probably atrial</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fibrillation, flutter</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fibrillation, “conduction defects”</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Premature atrial contractions</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Premature nodal contractions</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ventricular, total</td>
<td>2</td>
<td>2</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Premature ventricular contractions</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Neonatal VT and multifocal PVCs, persistent atrial fibrillation</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Complete heart block, “VT”</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Not otherwise specified</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

MAT, multifocal tachycardia; NOS, not otherwise specified; PAC, premature atrial contraction; PVC, premature ventricular contraction; SVT, supraventricular tachycardia; VT, ventricular fibrillation.
# Genetics of Syndromic Associated Cardiovascular Disease

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Cardiac anomalies</th>
<th>Causative gene(s)</th>
<th>Gene MIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Disease genes for syndromic cardiovascular malformations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alagille syndrome</td>
<td>PS, TOF, ASD, peripheral pulmonary stenosis</td>
<td>JAG1, NOTCH2</td>
<td>601920, 600275</td>
</tr>
<tr>
<td>Char syndrome</td>
<td>PDA</td>
<td>TFAP2B</td>
<td>601601</td>
</tr>
<tr>
<td>CHARGE syndrome</td>
<td>ASD, VSD, valve defects</td>
<td>CHD7, SEMA3E</td>
<td>608892, 608116</td>
</tr>
<tr>
<td>Costello syndrome</td>
<td>PS, HCM, cardiac conduction abnormalities</td>
<td>HRAS</td>
<td>190020</td>
</tr>
<tr>
<td>Ellis van Creveld</td>
<td>ASD</td>
<td>EVC, EVC2</td>
<td>604831, 607261</td>
</tr>
<tr>
<td>Heterotaxy syndrome</td>
<td>DILV, DORV, d-TGA, AVSD</td>
<td>ZIC3, CFC1</td>
<td>300265, 605194</td>
</tr>
<tr>
<td>Holt-Oram syndrome</td>
<td>ASD, VSD, AVSD, progressive AV conduction system disease</td>
<td>TBX5</td>
<td>601620</td>
</tr>
<tr>
<td>LEOPARD syndrome</td>
<td>PS and cardiac conduction abnormalities</td>
<td>PTPN11, RAF1</td>
<td>176876, 164760</td>
</tr>
<tr>
<td>Noonan syndrome</td>
<td>PS with dysplastic pulmonary valve, AVSD, HCM, CoA</td>
<td>PTPN11, KRAS, RAF1, SOS1</td>
<td>176876, 190070, 164760, 182530</td>
</tr>
<tr>
<td>Rubinstein Taybi</td>
<td>ASD, VSD</td>
<td>CREBBP, EP300</td>
<td>600140, 602700</td>
</tr>
<tr>
<td>Smith Lemli Opitz</td>
<td>VSD, ASD, AVSD</td>
<td>DHCR7</td>
<td>602858</td>
</tr>
</tbody>
</table>
Velocardiofacial Syndrome (VCFS)

- Learning disability (66%),
- Cleft palate or pharyngeal hypotonia (49%)
- Cardiac anomalies (74%) - TOF, Interrupted Aortic Arch Type B, etc
- Genitourinary abnormalities
- 22q11 deletion, 5-10% inherited
- DiGeorge Syndrome - hypocalcemia, thymic aplasia

Single Gene Disorders in Nonsyndromic Cardiovascular Disease

- The etiology of most nonsyndromic disease is unknown
- Last decade has seen an increase in identified genes
  - NKX2.5
  - GATA4
  - T box genes
  - NOTCH1
Morphologic Development of the Heart
Cardiac Lineages

Second lineage

- Second lineage progenitors lie medial and caudal to the first lineage progenitors of the crescent

- PAM – pharyngeal arch mesoderm

- DPM – dorsal pericardial mesoderm

First Lineage
Cardiac crescent
Contribution of Neural Crest
Contributions of the Cardiac Neural Crest
NKX2.5

- NK-homeobox transcription factor
- Plays a key role in cardiac chamber development
- Also important for conduction system morphogenesis
- Importance to myocardial function as well
- Associated with multiple structural lesions
NKX2.5

GATA4

- GATA4 is an essential transcription factor for cardiac morphogenesis
- Required for normal myocardial growth and right ventricular development
- Important for normal endocardial cushion derived tissue development (atrioventricular valves)
### GATA4

<table>
<thead>
<tr>
<th>Cardiac lesion</th>
<th>Patients, # (%)</th>
<th>GATA4 alteration, # (%)</th>
<th>Probands with family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardial cushion defects</td>
<td>42 (39)</td>
<td>2 (4.8)</td>
<td>0</td>
</tr>
<tr>
<td>Double inlet LV</td>
<td>9 (8)</td>
<td>1 (11.1)</td>
<td>0</td>
</tr>
<tr>
<td>ASD/VSD</td>
<td>8 (7)</td>
<td>1 (12.5)</td>
<td>1</td>
</tr>
<tr>
<td>Cardiomyopathy b</td>
<td>48 (45)</td>
<td>0 (0)</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>4 (3.7)</td>
<td></td>
</tr>
</tbody>
</table>

a  GATA4 alteration is defined as a non-synonymous sequence alteration not found in control individuals.

b  24 of these patients were via personal communications from M. Sarkar, C. Seidman, and J. Seidman.

---

T Box Transcription Factors

- DNA consensus sequence TCACACCT
- T-box is a 180 amino acid DNA-binding domain, generally comprising about a third of the entire protein (17-26 kDa)
- Similarity to the DNA binding domain of Mus musculus (Mouse) Brachyury (T)
- Conserved from Drosophila *Dorsocross* complex
- 7 T-box transcription factors expressed in cardiac development – Tbx1,2,3,4,5,18,20
## Genotype-Phenotype Correlations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal Location</th>
<th>Cardiac defect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK2</td>
<td>2q23-q24</td>
<td>Primum type ASD, MVP</td>
<td>Joziasse [52]</td>
</tr>
<tr>
<td>BMPR2</td>
<td>2q33</td>
<td>AVSD, ASD, PDA, PAPVR + PAH</td>
<td>Roberts [90]</td>
</tr>
<tr>
<td>CFC1/Cryptic</td>
<td>2q21.1</td>
<td>Heterotaxia, TGA, DORV, common AV canal, AA hypoplasia, pulmonary artresia, DIRV</td>
<td>Bamford [7, 41]</td>
</tr>
<tr>
<td>Cited2</td>
<td>6q23.3</td>
<td>TOF, VSD, ASD, anomalous pulmonary venous return, RVOT obstruction</td>
<td>Sperling [102]</td>
</tr>
<tr>
<td>CRELD1</td>
<td>2p13</td>
<td>AVSD, cleft mitral valve, ASD type I, heterotaxy</td>
<td>Sheffield [98]</td>
</tr>
<tr>
<td>Elastin</td>
<td>7q11.2</td>
<td>Supravalvular AoS</td>
<td>Robinsonson [91]</td>
</tr>
<tr>
<td>FOG2</td>
<td>8q23</td>
<td>TOF</td>
<td>Metcalfe [66]</td>
</tr>
<tr>
<td>GATA 4</td>
<td>8p23.1-p22</td>
<td>ASD, AVSD, pulmonary valve thickening, insufficiency of cardiac valves</td>
<td>Pizzuti [82]</td>
</tr>
<tr>
<td>JAG1</td>
<td>20p12</td>
<td>TOF, VSD with aortic dextroposition, PPS</td>
<td>Okubo [78]</td>
</tr>
<tr>
<td>KRAS</td>
<td>12p12.1</td>
<td>ASD, VSD, valvular PS, HCM, HOCM, MVP, IVP, LVH</td>
<td>Garg [35]</td>
</tr>
<tr>
<td>MYH6</td>
<td>14q12</td>
<td>Secundum ASD</td>
<td>Eldadah [29]</td>
</tr>
<tr>
<td>NKx2.5</td>
<td>5q34</td>
<td>ASD, VSD, TOF, AoS, VH Pulmonary atresia, Mitral valve anomalies, conduction disturbances</td>
<td>Schubbert [96]</td>
</tr>
<tr>
<td>NKx2.6</td>
<td>8p21</td>
<td>IA</td>
<td>Ching [21]</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>9q34.3</td>
<td>Bicuspid aortic valve, mitral valve stenosis, TOF, VSD</td>
<td>Schott [95]</td>
</tr>
<tr>
<td>PROSIT240</td>
<td>12q24</td>
<td>TGA</td>
<td>König [57]</td>
</tr>
<tr>
<td>IBX1</td>
<td>22q11.2</td>
<td>Interrupted aortic arch, IA, other aortic arch anomalies</td>
<td>Heathcote [43]</td>
</tr>
<tr>
<td>TRX5</td>
<td>17q24.1</td>
<td>ASD, AVSD</td>
<td>Garg [36]</td>
</tr>
<tr>
<td>Zic3</td>
<td>Xq26.2</td>
<td>TGA, DORV, ASD, AVSD</td>
<td>Mohamed [70]</td>
</tr>
</tbody>
</table>

Transcription Factor Gene Families

- Homeobox (NKX2.5, HOXA13)
- Paired-Box (PAX2, PAX6)
- Forkhead (FOXC2)
- T-Box (TBX1,3,5, 20)
- Zinc-finger (GLI3, ZIC2, ZIC3)
- GATA (GATA4)
**Tbx1 gene**

b. wild-type E10.5

c. Absence of the left fourth PAA in a Tbx1+/− embryo at E10.5

d. PAA morphology in a Tbx1−/− embryo

e. Compound heterozygous Df(16)1/Tbx1tm1Bld embryo

PAA, pharyngeal arch artery; AS, aortic sac; DA, dorsal aorta; IC, internal carotid artery.
Asymmetric Disposition of Visceral Organs in Humans

- Situs Solitus: normal disposition of organs
- Right Isomerism (Asplenia Syndrome)
- Left Isomerism (Polysplenia Syndrome)
- Situs Inversus: complete mirror-image reversal of organ asymmetry
- Heterotaxy: one or more of the individual organ systems with reversed L/R polarity
Looping

- Nodal
- PitX2

- ZIC3
- ACVR2B
- LEFTYA
- CRYPTIC
- NKX2.5
- CRELD1
Heterotaxy Summary

- ZIC3 mutations in X-linked heterotaxy; also seen in 1% isolated CHD
- Sporadic males and an affected female observed
- ZIC3 mutation analysis is available
- Pedigrees show high rate of birth defects – isolated CHD, NTD, clubfoot, GI anomalies
- Other single gene defects have been identified, but collectively likely < 10% of cases, thus many genetic causes remain unidentified
- Association of heterotaxy cases with gestation diabetes, twin pregnancies, cocaine use
Conclusions

- Genetics increasingly recognized as having significant influence on cardiovascular disease
- Improving technologies provide opportunity for novel gene discovery
- Opportunity for better genotype-phenotype correlations
- Increasing utility of genetic testing in clinical practice to deliver more complete care to patients and their families
SUDDEN CARDIAC DEATH IN STRUCTURALLY NORMAL HEARTS

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Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Introduction

Sudden cardiac death occurs in individuals with structurally normal hearts but this is a rare occurrence. Investigation of such an event requires careful, thorough examination of the heart to determine that it is truly normal in terms of gross and microscopic anatomy, and a complete autopsy to rule out non-cardiac causes of death. A sudden, unexpected death in the absence of demonstrable pathological changes at autopsy is strong presumptive evidence that the cause of death was a spontaneous, lethal ventricular tachyarrhythmia. While other causes of death with no apparent anatomic abnormalities are possible (eg, status epilepticus), these are usually associated with specific clinical histories.

The great majority of sudden deaths with structurally normal hearts occur in young individuals (<35 years of age). Many (perhaps most) of these patients have “channelopathies,” genetic diseases in which mutations in genes encoding Na+, K+, and Ca2+ channel proteins are responsible for sudden death syndromes. Although these syndromes are rare, they have provided valuable insights into molecular mechanisms of lethal arrhythmias.

Arrhythmia mechanisms in structurally normal hearts

In the common settings of sudden death associated with ischemic heart disease or cardiomyopathies, structural remodeling of the heart provides anatomic substrates that alter the temporal and/or spatial patterns of impulse propagation (conduction) in the heart greatly increasing the likelihood of an reentrant arrhythmia. Such structural abnormalities are absent in the ion channelopathies. However, temporal and spatial heterogeneity in depolarizing and repolarizing wavefronts certainly occur in the channelopathies. They arise, in general, because of the normal spatial heterogeneity in the expression of several gene products involved in forming ion channels. Several K+ channels are normally distributed in spatially complex patterns that can vary in epicardial – endocardial distribution, apical – basal distribution, and/or in one chamber compared to another. Mutations affecting the function of an individual ion channel may, therefore, create an abnormal pattern of heterogeneity which can initiate a tachyarrhythmia in response to a transient acute triggering event such as sympathetic activation or an electrolyte imbalance. Lethal arrhythmias in structurally normal hearts are generally related to triggered activity, which refers to the initiation of an impulse that arises consequent to a preceding impulse or series of impulses. Triggered activity is manifest as early or late afterdepolarizations, inappropriate impulses that arise before or after full repolarization of a cell or group of cells in which abnormal ionic conditions prevail. Early afterdepolarizations arise in the long QT and Brugada syndromes. Conditions favoring the formation of early afterdepolarizations include hypoxia, acidosis, decreased intracellular K+ and increased intracellular Ca2+ concentrations. Late or delayed afterdepolarizations occur in catecholaminergic polymorphic ventricular tachycardia and are related to abnormal intracellular Ca2+ homeostasis.

Abnormal automaticity and triggered activity both have the potential to activate the heart at an ectopic site, before the next sinus beat activates the myocardium in the normal, coordinated temporal and spatial sequence. Depending on multiple factors such as the site in which the ectopic beat originates and the refractoriness of adjacent tissue, the abnormal beat may give rise to a sustained arrhythmia either by reentry or a non-reentrant mechanism.
**Action potential in non-pacemaker cardiac myocytes.** A: The action potential consists of 4 phases which arise as ensembles of specific ion flows mediated by various ion channels and pumps, and which correspond to various waveforms on the surface ECG. B. Early afterdepolarizations occur during phase 2/3 of the action potential before full repolarization. C. Late (delayed) afterdepolarizations occur during phase 4 when the membrane potential has reached its diastolic or resting level.

Below is a brief consideration of the major ion channelopathies associated with sudden death in individuals with structurally normal hearts.

**Long QT Syndrome:** This condition is defined by prolongation of the QT interval and T wave abnormalities on the surface electrocardiogram [ECG] along with clinical features of syncope, ventricular arrhythmias or sudden, unexpected death. More than 10 different types of congenital long QT syndrome have been defined (see Table). Most are caused by loss-of-function mutations in genes encoding proteins that form various K⁺ channels. The loss of function prolongs repolarization of the cardiac action potential (thereby increasing the QT interval on the surface ECG) and promotes arrhythmias by increasing the likelihood of early afterdepolarizations. The long QT syndrome can also be caused by gain-of-function mutations in SCN5A, the gene encoding the cardiac Na⁺ channel protein. These mutations prolong the QT interval by allowing leakage of depolarizing current during the repolarization phase. Mutations in proteins responsible for ion channel trafficking or scaffolding, such as ankyrin B and caveolin-3, have also been implicated in long QT syndrome.

**Brugada Syndrome:** This is an autosomal dominant disease in a structurally normal heart with characteristic ST segment elevation in the right precordial leads, right bundle branch block and susceptibility to life-threatening arrhythmias. Loss-of-function mutations in SCN5A are identified in approximately 25% of cases. More than 80 different mutations have been identified and linked to various loss-of-function mechanisms including failure of sodium channel protein expression and changes in the voltage- and time-dependence of sodium channel current activation, inactivation or reactivation. Mutations in several other genes have been
identified (see Table). However, nearly 50% of patients with BrS have no identifiable mutation in any of the candidate genes associated with this syndrome. It remains controversial whether patients with BrS have truly normal hearts. Mouse models expressing mutant forms of SCN5A show cardiac fibrosis. Autopsy and biopsy studies have reported structural changes in the hearts of BrS patients raising questions about the relationship between the cardiac Na⁺ channel protein and myocardial structure. There may also be considerable overlap in clinical profiles such that some patients with Brugada syndrome also have atrial fibrillation, conduction defects with sinus abnormalities, or prolonged QT interval. Thus, genotype:phenotype relationships in Brugada syndrome are highly complex and serve to underscore our incomplete knowledge of the pathogenesis of this type of inherited arrhythmogenic disease.

### Long QT Syndrome Types and Associated Genetic Mutations

<table>
<thead>
<tr>
<th>BrS type</th>
<th>Gene name</th>
<th>Chromosomal location</th>
<th>Protein name</th>
<th>Ion channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQT1</td>
<td>KCNQ1</td>
<td>11q23</td>
<td>KV1.5 (β1)</td>
<td>I_K</td>
</tr>
<tr>
<td>LQT2</td>
<td>KCNH1</td>
<td>11q23</td>
<td>HERG</td>
<td>I_K</td>
</tr>
<tr>
<td>LQT3</td>
<td>SCN5A</td>
<td>11q23</td>
<td>Na,1.5</td>
<td>I_Na</td>
</tr>
<tr>
<td>LQT4</td>
<td>AnkB</td>
<td>11q23</td>
<td>HCN1</td>
<td>I_Na</td>
</tr>
<tr>
<td>LQT5</td>
<td>KCNE1</td>
<td>11q23</td>
<td>MiRP1</td>
<td>I_K</td>
</tr>
<tr>
<td>LQT6</td>
<td>KCNE2</td>
<td>11q23</td>
<td>MiRP1β</td>
<td>I_K</td>
</tr>
<tr>
<td>LQT7</td>
<td>KCNQ2</td>
<td>11q23</td>
<td>Kir2.1</td>
<td>I_K</td>
</tr>
<tr>
<td>LQT8</td>
<td>CAHNA1c</td>
<td>11q23</td>
<td>Cav1.2α1c</td>
<td>I_Ca</td>
</tr>
<tr>
<td>LQT9</td>
<td>CAV3</td>
<td>11q23</td>
<td>Caveolin-3</td>
<td>-</td>
</tr>
<tr>
<td>LQT10</td>
<td>SCN4B</td>
<td>11q23</td>
<td>Na,1.5β</td>
<td>I_Na</td>
</tr>
</tbody>
</table>

### Brugada Syndrome Types and Associated Genetic Mutations

<table>
<thead>
<tr>
<th>BrS type</th>
<th>Gene name</th>
<th>Chromosomal location</th>
<th>Protein name</th>
<th>Ion channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrS1</td>
<td>SCN5A</td>
<td>3p21–23</td>
<td>Na,1.5α</td>
<td>α subunit I_Na</td>
</tr>
<tr>
<td>BrS2</td>
<td>GPD1L</td>
<td>3p24</td>
<td>G3PD1L</td>
<td>Interacts with α subunit I_Na</td>
</tr>
<tr>
<td>BrS3</td>
<td>CACNA1C</td>
<td>12p1.3–2.3</td>
<td>Ca,1.2</td>
<td>α subunit I_Ca</td>
</tr>
<tr>
<td>BrS4</td>
<td>CACNB2</td>
<td>10p12.33</td>
<td>Ca,β2</td>
<td>β subunit I_Ca</td>
</tr>
<tr>
<td>BrS5</td>
<td>SCN1B</td>
<td>19q13.1</td>
<td>Na,β</td>
<td>β subunit I_Na</td>
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<tr>
<td>BrS6</td>
<td>KCNE3</td>
<td>11q13–q14</td>
<td>MiRP2</td>
<td>β subunit I_K/ I_w</td>
</tr>
<tr>
<td>BrS7</td>
<td>SCN3B</td>
<td>11q23.3</td>
<td>Na,β3</td>
<td>β subunit I_Na</td>
</tr>
</tbody>
</table>

**Cathecholaminergic polymorphic ventricular tachycardia (CPVT):** In this condition, polymorphic ventricular tachyarhythmias and sudden death occur in response to catecholamine surges associated with exercise or emotional stress. Mutations in genes encoding proteins involved in regulating intracellular Ca²⁺ homeostasis and excitation-contraction coupling, such as the cardiac ryanodine receptor (RyR2) and calsequestrin, are typically seen. These mutations promote leakage of Ca²⁺ from the SR and resultant arrhythmias triggered by after-depolarizations.
**Ca\(^{2+}\) fluxes in ventricular myocytes responsible for excitation-contraction coupling.** Depolarization of the sarcolemma opens voltage sensitive Ca\(^{2+}\) channels in T-tubules which allows entry of extracellular Ca\(^{2+}\) and Ca\(^{2+}\)-induced release of intracellular (SR) Ca\(^{2+}\) stores via the ryanodine receptor (RyR). The resultant increase in Ca\(^{2+}\) (the Ca\(^{2+}\) transient) activates myofilaments leading to contraction. Resting levels are restored by reuptake of Ca\(^{2+}\) into the SR by an ATPase pump (SERCA) and by extrusion of intracellular Ca\(^{2+}\) via the Na/Ca exchanger (NCX) and sarcolemmal ATPase pumps.

**Close-up view of the SR-Ca\(^{2+}\) channel complex.** The channel is composed of 4 RyR2 subunits and is associated on the luminal side with triadin (T), junction (J) and calsequestrin (CASQ2). On the cytosolic side, the channel is located in intimate proximity to the L-type Ca\(^{2+}\) channel in the T-tubules. Each RyR2 chain can bind various regulatory molecules including FKBP12.6, calmodulin (CaM), and kinases (PKA and CaMK2) which can phosphorylated RyR2 and regulate its function.
Single Gene Disorders of the Aortic Wall

Marc Halushka MD, PhD
SCVP Companion Meeting

Within the pediatric and young adult population, there are many causes of ascending aortic disease. Most of these causes are extremely rare. However, there are four diseases that are relatively common and genetic with which pathologists should be familiar. These are Marfan Syndrome, Ehlers-Danlos Syndrome Type 4, Loeys-Dietz Syndrome and Familial Thoracic Aneurysm and Dissection. These four entities – their clinical findings, genetic findings and current areas of research - are described in the talk and in these companion notes.

**Marfan Syndrome (MFS)** – This disease was first described in 1896 and was recognized as a familial disorder in 1931. It has a prevalence of 4-6 individuals per 100,000 in the population making it one of the more common causes of genetic aortic disease. MFS is well known for the classic clinical features of pectus excavatum, arachnodactyly, tall stature and lens ectopia. MFS also causes significant cardiovascular problems. Chief among these is aortic root dilatation and dissection which, in nonsurgically repaired individuals, is the major cause of mortality in this population. Mitral valve prolapse is quite common (~40%). Also, “MFS cardiomyopathy” and arrhythmias are reported at higher rates in this population.

Mutations in fibrillin-1 cause MFS. FBN is a large 350 kDa glycoprotein that is found in the extracellular matrix. Initially, mutations in FBN1 were thought to cause structural problems in the aorta. More recently it has been found that FBN1 interacts with latent TGF-beta binding protein -1 (LTBP-1) to regulate local TGF-beta activity. Thus FBN1 is important in regulatory control of TGF-beta, resulting in the myriad of clinical features of MFS.

Current areas of active MFS research include a recent revision of the Ghent nosology (the clinical method to diagnose MFS) and insight into pathogenesis and mechanisms of therapeutic response. New treatment options include revised valve sparing surgical approaches and a large clinical trial of losartan. Recently losartan was shown in a mouse model to significantly decrease the symptomatology of MFS.

**Ehlers-Danlos Syndrome Type 4 (EDS-IV) / Vascular EDS (vEDS)** – EDS-IV is another old entity, first described in 1901. It was established as an autosomal dominantly inherited disease in 1949. This disease has a prevalence of 1 individual in 250,000 making it >10 times less common than MFS. The clinical features of EDS-IV are thin skin with visible veins, easy bruising and characteristic facial features including a thin pinched nose, thin lips, prominent ears, hollow cheeks and tightness of skin over the face. Individuals with EDS-IV have significantly shortened lifespans due to the spontaneous rupture of visceral organs and blood vessels. Medium to large arteries have the greatest propensity to rupture, while the aortic root is typically spared. There is no need for dilatation to occur prior to dissection showing the difficulty in preventing catastrophic events. Rarely, the left ventricle of the heart has spontaneously ruptured causing death.

Mutations in the type 3 pro-collagen COL3A1 gene cause EDS-IV and occur throughout the gene. They almost exclusively alter a glycine residue in the Gly-X-Y core triple-helical domain. This results in weakened collagens of connective tissues.

Active areas of research in EDS-IV include new work with a haploinsufficient Col3a1 mouse model, surgical data suggesting elective procedures in EDS-IV patients have good outcomes and a controversial study suggesting that celiprolol reduces events in EDS-IV subjects.
**Loeys-Dietz Syndrome (LDS)** – This is a recently described syndrome first reported in 2005. It is believed that many patients with LDS were previously misclassified as having MFS or EDS-IV. The incidence of LDS in the general population is unknown but thought to fall between MFS and EDS-IV. The main clinical shared features of LDS are hypertelorism and a wide/bifid uvula. LDS has been subdivided into two clinical groups. LDS type 1 is known as the “facial dysmorphogenic type” and is notable for a cleft palate, craniosynostosis and micrognathia. LDS type 2 is known as the “vascular EDS-like type” and is notable for visceral rupture, easy bruising, wide/atrophic scars, joint laxity and translucent/velvety skin. At the cardiovascular level, LDS is associated with arterial tortuosity, early aortic aneurysms and dissections, some nonaortic aneurysms and possibly with cardiomyopathy.

LDS is caused by mutations in the genes transforming growth factor receptor 1 and 2 (TGFBR1 & TGFBR2). Mutations in TGFBR2 are more frequent and mutations in either gene can cause either clinical type. These genes regulate TGF-beta signaling and are known to be involved mechanistically with the same pathways altered in MFS. Some groups are using the term “TGF-beta-opathy” to describe a number of diseases with altered TGF-beta signaling in the pathogenesis of aortic aneurysm.

Active areas of LDS research include further refinement of the phenotype. It was recently shown that LDS patients have very high rates of eosinophilic esophagitis and inflammatory bowel disease. A recently mouse model faithfully recapitulates the vascular phenotypes and is being used to study LDS mechanisms. LDS patients are being placed on losartan for treatment and clinical studies of this treatment are being planned.

**Familial thoracic aneurysm and dissection (TAAD)** – It has been known for some time that ~19% of individuals with aortic dissections have an affected first degree relative, but do not have one of the described genetic syndromes. It is thought they harbor mutations in genes that have an autosomal dominant inheritance but with decreased penetrance. Therefore this TAAD group represents a bucket of a number of different diseases waiting to get names. As a result, there are a wide range of phenotypes seen with TAAD, often in only a subset of this group.

To date, there are two genes that have been found mutated in this population. Smooth muscle myosin heavy chain 11 (MYH11) is part of the contractile apparatus of VSMCs. Mutations in MYH11 explain <2% of the TAAD population but are highly enriched in individuals with concurrent aneurysm and patent ductus arteriosus. The second known TAAD gene is smooth muscle actin, alpha 2 (ACTA2). This gene also is part of the contractile apparatus of VSMCs. Mutations in this gene account for 14% of the TAAD population, suggesting many more genes are yet to be discovered. Thus, active areas of TAAD research include refining the phenotype of the diverse TAAD population and gene discovery including exome sequencing.

<table>
<thead>
<tr>
<th>Table of Genetic Diseases of the Ascending Aorta</th>
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<tbody>
<tr>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td>Marfan Syndrome</td>
</tr>
<tr>
<td>Vascular Ehler-Danlos Syndrome</td>
</tr>
<tr>
<td>Loeys-Dietz Syndrome</td>
</tr>
<tr>
<td>Familial TAAD</td>
</tr>
<tr>
<td>Bicuspid Aortic Valve</td>
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<td>Arterial Tortuosity Syndrome</td>
</tr>
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</table>
References:
Single Gene Disorders of the Aortic Wall

Marc Halushka MD, PhD
SCVP Companion Meeting
February 27, 2011
Causes of Aortic Aneurysm

- Loeys-Dietz Syndrome
- Turner Syndrome
- Weightlifting
- Shprintzen-Goldberg Syndrome
- Tetralogy of Fallot
- Ehlers-Danlos Syndrome IV
For surgical pathology cases of the ascending aorta it is not necessary (or possible) to make a diagnosis of a particular disease entity by histology alone.

- Should differentiate between inflammatory disease and non-inflammatory disease.
Always get an elastic stain (VVG, Movat) on an aortic pathology case.
Take Home Message #3

If you are thinking of EDS-IV – think to get EM.
Take Home Message #4

If encountering an aneurysm/dissection at autopsy – do a thorough “genetic” physical exam

Ripperger et al. For Sci Int, 2009
Main genetic syndromes

- Marfan Syndrome
- Ehlers-Danlos IV Syndrome
- Loeys-Dietz Syndrome
- Familial TAAD
Marfan Syndrome

- First described in 1896
- Recognized as a genetic syndrome with familial inheritance 1931
- Prevalence of 4-6 individuals per 100,000 in the population
- Chr. 15q identified by linkage analysis in 1990
- Fibrillin-1 (FBN1) implicated in 1991 (Dietz et al & Lee et al Nature)
MFS Clinical Features

- Pectus excavatum
- Arachnodactyly
- Tall stature
- Lens ectopia
MFS Cardiovascular Features

- Aortic Aneurysm (>65%)
- Mitral valve prolapse (~40% by 30 years of age) *
- Cardiomyopathy (One report of 25% have reduced EF in absence of significant valvular regurgitation or aortic surgery) †
- Arrhythmias (SVT common, one report of 21% of patients with ventricular arrhythmias / 4% fatal) ‡

* Rybczynski et al Am J Cardiol, 2010
† Alpendurada et al Eur J Heart Fail, 2010
‡ Yetman et al JACC 2003
Fibrillin-1 (FBN1)

- 235 kb gene with 65 exons making a 350 kDa glycoprotein
- FBN1 is part of the extracellular matrix
- Initially thought that FBN1 mutations caused structural abnormalities alone
- Interaction with LTBP-1 to regulate local TGF-β activity
- Over 600 distinct mutations have been described in FBN1

Comeglio et al Br J Ophthalmol 2002
Active areas of MFS research

- **Diagnosis**
  - Revision of Ghent nosology
  - *Pathogenesis and mechanisms of therapeutic response*

- **Treatment**
  - Losartan trial
  - Valve sparing surgical approaches
  - External aortic root repair
    (bespoke implant)

*Pepper et al J R Soc Med, 2010*
Ehlers-Danlos Syndrome Type 4

- AKA Vascular EDS
- First described in 1901 by Edvard Ehlers and in 1908 by Henri-Alexandre Danlos
- Established as autosomal dominant inheritance in 1949
- Affects 1:250,000 across all sex/ethnicities
- COL3A1 identified in 1988 (Superti-Furga et al JBC)
- Over 400 mutations in COL3A1 are known
EDS-IV Clinical Features

- Thin skin with visible veins
- Easy Bruising
- Characteristic facial features (thin pinched nose, thin lips, prominent ears, hollow cheeks, tightness of skin over the face)
- Rupture of arteries, uterus, or intestines
EDS-IV Cardiovascular Features

- **Arterial ruptures** (65% of one large cohort)
  - Common in the medium to large arteries
  - Aortic root is typically spared
  - No need for dilatation prior to dissection
  - Higher risk during pregnancy

- **Left ventricular heart wall ruptures**
Type 3 pro-collagen (COL3A1)

- 44kb gene spanning 51 exons
- 139 kDa protein that is a major collagen of connective tissues
- Core triple-helical domain (Gly-X-Y)
- Dx by gene sequencing (current)

OR

- Abnormal synthesis/ratio of COL3A1 from dermal fibroblast cultures (historical)

Pepin et al NEJM 2000
Active areas of EDS-IV research

- **Pathogenesis**
  - New work with a haploinsufficient Col3a1 mouse model

- **Treatment**
  - Celiprolol (beta blocker) therapy reduced events*
    - Caveats: Only 13/25 had COL3A1 mutation in treated group, while 20/28 had a COL3A1 mutation in control group & 17/23 events occurred in patients with mutations
  - Elective procedures in EDS-IV are reasonable and have good outcomes †

*Ong et al Lancet, 2010
† Brooke et al J Vasc Surg, 2010
Loeys-Dietz Syndrome

- First described in 2005 (Loeys et al Nat Genet)
- Patients were previously often misclassified as MFS or EDS-IV
- Mutations in TGFBR1 or TGFBR2
- Controversial, since others had found TGFBR2 mutations among MFS and TAAD cohorts
LDS Main Clinical Features

- Hypertelorism
- Wide/bifid uvula
- Arterial/aortic aneurysms
- Arterial tortuosity
LDS Type I Clinical Features

“Facial dysmorphogenic type”

- Cleft palate
- Craniosynostosis
- Micrognathia

75%
LDS Type II Clinical Features

“Vascular EDS-like”

- Visceral rupture
- Easy bruising
- Wide/atrophic scars
- Joint laxity
- Translucent/velvety skin

25%
LDS Cardiovascular Features

- Vascular tortuosity
- Early aortic aneurysm and dissection
- Nonaortic aneurysms
- Cardiomyopathy?*

*Eckman et al Circ Heart Fail, 2009
Transforming Growth Factor Receptor 1 (TGFBR1, ALK5)

- 9 exons over 31kb
- 56 kDa protein
- Forms heterodimeric complex with TGFBR2
- Internalizes TGFβ signalling activating SMAD2/3 (pSMAD2/3)

Loeys et al NEJM 2006
Transforming Growth Factor Receptor 2 (TGFBR2)

- 7 exons over 87kb
- 65 kDa protein involved in TGFβ signalling
- Mutated more frequently than TGFBR1

Loeys et al NEJM 2006
Active areas of LDS research

- **Diagnosis**
  - Further refine the phenotype
    - Eosinophilic esophagitis
    - Inflammatory bowel disease

- **Pathogenesis**
  - Mouse model

- **Treatment**
  - Losartan
Vascular TGF-β Functional Pathway

Key

- LLC – Large latent complex
- SLC – Small latent complex
- LAP – Latency associated peptide
- BMP1 – Bone morphogenic protein
- MMP2 – Matrix metalloproteinase 2
- TGFBR1, TGFBR2 – Bind TGF-β and internalize signalling
- SMAD – Signal transducer and transcriptional modulator of TGF-β signalling

Familial Thoracic Aneurysm and Dissection (TAAD)

- ~19% of individuals with dissection have an affected 1° relative
- Generally autosomal dominant but with decreased penetrance
- Multiple genes implicated
- Phenotypic heterogeneity
Smooth Muscle Myosin Heavy Chain 11 (MYH11)

- Spans 37 exons over 154 kb
- 42 kDa protein that is part of the contractile apparatus of VSMCs
- Mutations reported in 2006 (Zhu et al Nat Genet)
- Concurrent aneurysm and patent ductus arteriosus
- Explains <2 % of TAAD population
Smooth Muscle Actin, Alpha 2 (ACTA2)

- Spans 9 exons over 26 kb
- 42 kDa protein that is part of the contractile apparatus in VSMCs
- Mutations reported in 2007 (Guo et al Nat Genet)
- Explains 14% of TAAD population
- Also implicated in CAD, stroke and Moyamoya disease (Guo et al AJHG 2009)
Active areas of TAAD research

- **Diagnosis**
  - Refining the diverse TAAD population

- **Genetics**
  - Gene discovery
    - Exome sequencing
    - More targeted VSMC contractile proteins?
### Summary of Genetic Disorders of the Ascending Aorta

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Gene Location</th>
<th>Freq.</th>
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<tbody>
<tr>
<td>Marfan Syndrome</td>
<td>FBN1</td>
<td>16p13</td>
<td>Common</td>
</tr>
<tr>
<td>Vascular Ehler-Danlos Syndrome</td>
<td>COL3A1</td>
<td>2q32</td>
<td>Rare</td>
</tr>
<tr>
<td>Loeys-Dietz Syndrome</td>
<td>TGFBR1, TGFBR2</td>
<td>9q22, 3p22</td>
<td>Rare</td>
</tr>
<tr>
<td>Familial TAAD</td>
<td>MYH11, ACTA2</td>
<td>16p13, 10q23</td>
<td>Common</td>
</tr>
<tr>
<td>Bicuspid Aortic Valve</td>
<td>NOTCH1</td>
<td>9q34</td>
<td>Common*</td>
</tr>
<tr>
<td>Arterial Tortuosity Syndrome</td>
<td>SLC2A10 (GLUT10)</td>
<td>20q13</td>
<td>Very rare</td>
</tr>
</tbody>
</table>

*Note: Disease is common, NOTCH1 mutations are not associated with aneurysm*
Acknowledgements

Hal Dietz
ARRHYTHMOGENIC CARDIOMYOPATHY: from autopsy to genes and transgenic mice

Gaetano Thiene

Cardiovascular Pathology, Department of Medical-Diagnostic Sciences and Special Therapies,

University of Padua Medical School, Padua, Italy

My country may rightly claim the discovery of arrhythmogenic cardiomyopathy (ARVC/D) as a distinct heredo-familiar morbid entity. In 1736 Giovanni Maria Lancisi postmously published in Naples the book “De Motu Cordis et Aneurysmatibus” (1). Lancisi was Professor of Anatomy at the University “La Sapienza” in Rome and personal doctor of the Pope (“Archiatrii Pontifici”). In Chapter V of the book, entitled “De Hereditaria ad Cordis Aneurysmata Constitutione: De Cordis Prolapsu” (on the hereditary predisposition to cardiac aneurysms: cardiac prolapse) he reported some examples of such morbid entities and described the history of a family with disease recurrence in four generations, all featured by signs and symptoms which were in keeping with what nowadays we call AC: palpitations, dilatation and aneurysms of the right ventricle (RV), heart failure, sudden death (Fig.1). Thus, the first description of AC dates back nearly two centuries and half earlier than modern observations.

The first recent pathological description has been done by Laennec, as reported in his bibliography by Saintingan in 1904 (2). In Middlemarch, published in 1871 by George Eliot, the protagonist Dr. Lydate, talking to his patient, says “you are suffering from what is called fatty degeneration of the heart, a disease which was first described by Laennec... it is my duty to tell you that death from the disease is often sudden...” (3).
In 1905 Sir William Osler reported a case of a nearly 40 year old man who died suddenly while climbing a hill (4). Postmortem disclosed a biventricular myocardial atrophy, with a thinning and translucency of the ventricular free walls, that Osler immortalized with the name “parchment heart”. The heart specimen was part of Maude Abbot collection (5). Segall in 1950 reviewed the specimen and republished the case with unequivocal drawings showing paper thin walls (6) (Fig.2).

A controversial case, which was the source of subsequent misconceptions, has been reported by Uhl at the Johns Hopkins Hospital in Baltimore in 1952 (7). He published “A previously undescribed congenital malformation of the heart: almost total absence of myocardium of the right ventricle” in a 8 month old female infant who died due to congestive heart failure and no arrhythmias at the ECG. Here is the description of the heart at autopsy: “Externally the heart appears greatly enlarged... almost the entire dilated chamber (RV) was occupied by a large laminated mural thrombosis which adhered firmly to the endocardium along the anterior wall of the ventricle. Examination of the cut edge of the ventricle wall revealed it to be paper-thin with no myocardium visible... In the RV wall epicardium and endocardium lie adjacent to each other with no intervening cardiac muscle... no fibro-fatty tissue in the RV free wall was observed” (Fig.3). The early age and the peculiar pathological description points to a structural heart disease present at birth (congenital malformation), as emphasized in the title itself. Clinical presentation was neither characterized by cardiac arrhythmias nor by a family history of heart disease. Thereafter, cases in adults with paper-thin ventricular walls have been published with the eponym of Uhl’s anomaly, clearly a misnomer since the parchment heart in adult is the end-stage of a late progressive loss of the myocardium followed by fibro-fatty replacement. On the opposite, the cases reported in the literature under the age of 15 months with the eponym of Uhl’s anomaly, were all featured by heart failure and isolated RV involvement (whether segmental or diffuse) without
arrhythmias, all in keeping with the original description (8-15). The parchment heart cases reported in the adults (including the Osler heart) (6) varied from 17 to 81 years and died either of congestive heart failure or electrical cardiac arrest (16-26).

The University of Padua wrote pages that are milestones in the history of the disease (27). Sergio Dalla Volta in 1961 and 1965 first published similar cases under the name of “auricularization of the RV pressure” to emphasize the behavior of the RV chamber without an effective systolic contraction, with the blood was pushed to the pulmonary artery mainly thanks to the right atrial systole (28,29). Although the patients presented also with ventricular arrhythmias, Dalla Volta pointed more to the hemodynamic features rather than to the arrhythmogenicity of the RV. One of the original patients reported by Dalla Volta underwent cardiac transplantation 30 years later in 1995 at the age of 65, because of congestive RV failure. The left ventricle was normal, whereas the RV was hugely dilated with diffuse paper-thin free wall and complete disappearance of the myocardium (30) (Fig.4).

At the same University of Padua, the pathologist Vito Terribile in 1972 performed the autopsy of a woman with a history of palpitations and congestive heart failure, who died due to pulmonary embolism. The heart showed an extreme dilatation, mural thrombosis and “adipositas cordis” of the RV, and the left ventricular myocardium exhibited areas of “myocardiosclerosis”, all structural findings in keeping with AC (31) (Fig.5).

The interest on the arrhythmic aspects of the disease was attracted by Guy Fontaine from Paris in the ‘70s with the report of non-ischemic ventricular tachyarrhythmias, originating from the RV with left bundle branch block morphology (32). Moreover, he observed in the basal ECG delayed repolarization (“postexcitation syndrome”) at the end of the QRS complex, a feature which he named epsilon wave (33).

Frank Marcus from Tucson, fascinated by this new field of RV electrophysiology, decided to spend a sabbatical year in Paris with Fontaine at the Jean Rostand Hospital (34).
He had the time and opportunity to review the adult cases studied by Fontaine with clinical manifestation of primary RV disease. The result was a milestone paper, which was published in Circulation in 1982 (35). The disease was named “RV Dysplasia” since the histology of the myocardial specimens, resected at surgery for removal of arrhythmic foci, showed anomalous histological features of the RV myocardium consisting of fibro-fatty tissue, which were believed to be the consequence of an embryonic maldevelopment. By observing the presence of aneurysms in the inflow, apex and outflow of RV, they coined the term “triangle of dysplasia”, a pathognomonic landmark of the disease. The same group, with the help of the surgeon Guiraudon, perceived the idea of total disconnection of the RV free wall as surgical treatment of RV tachycardia, by interrupting the reentry into the left ventricle (36).

The early pioneeristic contributions of Marcus and Fontaine stimulated the interest of the electrophysiologists. Andrea Nava in Padua, thanks to the study of families with sudden death and autopsy evidence of AC from Piazzola sul Brenta (a small village close to Padua in the Veneto Region), discovered the heredo-familial nature of the disease, a monogenic disorder with a mendelian autosomal dominant transmission (37,38). Gianfranco Buja reported the occurrence of the disease both in homozygous and heterozygous twins (39).

The risk of sudden death as first manifestation of the disease was proven by the postmortem study of a series of young victims, in the setting of a project supported by the Veneto Region and carried out by Gaetano Thiene.

The first observation consisted of a young doctor, formerly cycle champion, who died suddenly in a tennis court, in a hot afternoon of May 1979 (Fig.6). Fifteen minutes after the starting of the game, he stopped, took his pulse, walked back to the border of the tennis courtyard and suddenly fainted. In his diary, written on October 4, 1978, during preparation of the Internal Medicine examination, the sentence “ventricular tachycardia of left bundle branch block morphology” was found, which retrospectively can be referred to his own ECG.
The girlfriend told that in that day he had suffered of palpitations and did an ECG. It required years to understand that the explanation of cardiac arrest and ventricular fibrillation was the fibro-fatty tissue that had been observed at autopsy in the RV free wall and at apex of the left ventricle and not conduction system abnormalities as first hypothesized (40). This experience confirmed an old concept in Medicine, namely that you see only what you look for and you recognize only what you know.

Among the 60 consecutive cases of sudden death in the young (<35 years) collected in the Veneto project, 12 of whom (20%) were found to be affected by AC. Most deaths had occurred during effort and had presented inverted T wave in the right precordial leads a the basal ECG. The novel findings were promptly submitted to the New England Journal of Medicine, which was reluctant to believe that the disease could be a so frequent cause of juvenile sudden death. Eventually, by providing all the data and illustrations, case by case, the paper was accepted for publication (41) and accompanied by a quite rewarding editorial, signed by Barry Maron, entitled “Right ventricular cardiomyopathy: another cause of sudden death in the young” (42).

A letter to the Editor was then forwarded to the New England by a group of Greek doctors (43), claiming that a very similar cardiac malignant disease was observed in Naxos in the setting of cardiocutaneous syndrome, consisting of AC, palmoplantar keratosis and woolly hair (Naxos disease) (44). They postulated that those patients might belong to families that descended from Venetians, who had landed in Naxos in 1207 (Fig.7). Soon after Domenico Corrado demonstrated that AC was a killer among the athletes, accounting for about 25% of fatalities in the Veneto Region (45), at difference from the United States where hypertrophic cardiomyopathy ranked first.

The report of AC as a major cause of sudden death in the young arose skepticism in the scientific community. Many scientists came to Padua to examine the heart specimens of this
morbid entity (Fig.8). The statement of Sir James Mackenzie is quite relevant: “There are three stages in the history of every medical discovery. When it is first announced, people say that it is not true. Then, a little later, when its truth has been borne in on them, so that it can no longer be denied, they say it is not important. After that, if its importance becomes sufficiently obvious, they say that anyhow it is not new” (46). Meanwhile postmortem observations increased with time, since our Pathology Unit became the only tertiary center for all cases of juvenile sudden death in the Veneto Region.

The interest was then focused on the in vivo recognition of the disease and risk stratification through instrumental investigations: Andrea Nava with electrocardiogram (47), Roldano Scognamiglio with echocardiography (48), Luciano Daliento with angiography (49), Thomas Wichter with 123I-meta-iodobenzylguanidine scintigraphy (50), Luca Oselladore with signal averaged ECG (51), Annalisa Angelini with endomyocardial biopsy (52), Luigi Menghetti with cardiac magnetic resonance (53), Pietro Turrini with QT dispersion (54), Franco Folino with heart rate variability (55), Hari Tandri with contrast enhanced cardiac magnetic resonance (56), Domenico Corrado with electroanatomic mapping (57).

In 1994 an international task force leaded by Bill McKenna put forward the diagnostic criteria, based upon family history of AC and/or sudden death, ECG depolarization/conduction/repolarization abnormalities, arrhythmias of RV origin, global and/or regional dysfunction and structural alterations of the RV, and fibro-fatty replacement of the RV myocardium at pathological analysis (58). In the absence of a single gold standard, the diagnosis was achieved by major or minor criteria (2 major, or 1 major and 3 minor, or 4 minor).

A revision of the diagnostic criteria was recently accomplished, by introducing quantitative other than qualitative diagnostic parameters, including cardiac magnetic resonance and genetic testing (59). The application of the diagnostic criteria greatly
contributed to the early detection in young subjects affected by silent AC at the screening for
sport eligibility, thus resulting in a sharp decline (nearly 90%) of sudden death during sport
activity (60).

As far as the treatment, while curative therapy is still far away in the absence of precise
knowledge of the pathogenesis of cardiomyocyte injury, an algorithm for antiarrhythmic drug
therapy in AC patients was first introduced by Thomas Wichter (61). Endocardial catheter
ablation was performed by Hugh Calkins, although limited by a high rate of arrhythmias
recurrence during the follow-up (62). More recently, Francis Marchlinski demonstrated the
superiority of epicardial catheter ablation vs the endocardial approach (63).

Prevention of sudden death is now feasible with the introduction of implantable
cardioverter defibrillator (ICD). Up to 20-25% of patients survived from cardiac arrest in a
follow-up of 48 months, thanks to appropriate electric shocks with cardioversion of
ventricular fibrillation to sinus rhythm. ICD implantation is indicated for both secondary (64)
and primary (65) prevention.

Other fascinating contributions came from pathobiology and genetics. Cristina Basso,
by studying a large series of heart specimens, disclosed that AC is not a congenital heart
disorder (i.e. lesion present at birth). It is a genetically determined myocardial dystrophy with
acquired cell death occurring with time, mostly during adolescence (66). It was considered a
sort of cell suicide, due to apoptosis, as demonstrated through TUNEL and electron
microscopy studies by Marialuisa Valente (67). Focal myocardial inflammation was found in
nearly 75% of cases, however Fiorella Calabrese ruled out viral infections by enterovirus (68).
Thereafter, AC was added in the list of cardiomyopathies in the WHO classification (69). By
the way, the disease was reported to spontaneously occur also in animals, both in cats (70)
and dogs (71).
In 1994, linkage analysis studies in families with AC, carried out by Alessandra Rampazzo and GianAntonio Danieli, led to identify the first gene locus in chromosome 14 (72). Thereafter, several loci were demonstrated in other chromosomal sites (73), suggesting genetic heterogeneity (multiple genes, similar phenotypic expression). The candidate genes were first searched for in the cytoskeleton, as in Duchenne and Becker muscular dystrophies.

The enlightening inspiration to solve the genetic puzzle came to the scholars of the Naxos disease (Nikos Protonotarios and Adalena Tsatsopoulou), who perceived that desmosomes are in common to heart and skin and that a cell junction defect might explain their cardiocutaneous syndrome. Other researchers, in previous investigations, by studying mice with targeted mutation of plakoglobin, a γ-catenin of the desmosome localized in chromosome 17q21, showed that the knock-out of this gene resulted in devastating cardiac lesions in the embryos, with disappearance of the desmosomes and spontaneous cardiac rupture (74). Based upon these observations, the Naxos group carried out the linkage analysis in their families and identified the Naxos disease locus exactly in the same chromosome 17q21 (75). Thereafter, gene sequencing proved that the molecular defect was a deletion of plakoglobin (76).

At the same time, in Equador, Luis Carvajal Huerta reported another recessive cardiocutaneous disease, characterized by dilated cardiomyopathy, wooly hair and palmoplantar keratosis (77). The gene defect was proven to be a mutation of desmoplakin, another protein of the desmosome (78). A child of the affected family died suddenly, due to arrhythmic cardiac arrest and the heart specimen was sent by the wife of Dr. Carvajal (who meanwhile passed away) to Jeff Saffitz in St. Louis for pathological study. Gaetano Thiene was asked by Dr Saffitz to go to St. Louis and examine the heart. It was a biventricular cardiomyopathy, with extensive left ventricular dilatation and mural thrombosis. The RV disclosed the typical aneurysms in the triangle of dysplasia with translucent thin wall and the
histology showed mostly fibrous replacement (79). Thus, another defective gene of desmosome (desmoplakin) was found to be responsible of a new recessive cardiocutaneous syndrome.

Desmoplakin became immediately a candidate gene also for the dominant variant of AC. The genetic screening in Padua in some of Nava’s families lead to the identification by Alessandra Rampazzo of desmoplakin as the disease causing gene, in the form of missense mutations (80). This proved that both autosomal and recessive AC were desmosomal diseases. Genotype-phenotype correlations, carried out by Barbara Bauce, disclosed that the desmoplakin variant of the disease was featured by extensive left ventricular involvement, as to suggest that the disease, being biventricular, should be better called AC (81). By performing contrast enhanced cardiac magnetic resonance in genotyped AC patients, Sen Chowdhry confirmed that the disease is wider than previously thought, with predominant left ventricular and biventricular forms, besides the classical RV AC (82).

Missense mutation of the gene encoding ryanodine receptor 2, in charge of the Ca++ release from smooth sarcoplasmic reticulum, was associated by Natascia Tiso to a variant of AC with polymorphic ventricular tachyarrhythmias (83). Since the same defective gene was proven by Silvia Priori (84) to be related to catecholaminergic polymorphic ventricular tachycardia first described by Philip Coumel in 1978 (85), the nosographic entity by Tiso was then considered the same as the one reported by Coumel.

All the other genes, which encode for desmosomal proteins, were subsequently investigated in patients affected by familial dominant (non-syndromic) AC, and found to be responsible of the same phenotype: plakophillin-2 by Brenda Gerull (86), desmoglein-2 by Kalliopi Pilichou (87) and desmocollin-2 by Paul Syrris (88), the latter confirmed soon after by Giorgia Beffagna (89). Thus, both dominant and recessive variants of AC were eventually nosographically identified as cell junction (desmosome) diseases (90, 91).
Electron microscopy studies, carried out by Cristina Basso in genotyped patients with AC, revealed abnormalities of the desmosomes. They appeared less numerous, short, pale, fragmented, as to hypothesize that disruption of intercalated disc was the final common pathway of a genetically determined, progressive cell death (92).

The discovery of the defective genes, although limited to 50% of affected families, opened new avenues. Genetic screening, for early diagnosis and detection of healthy carriers as well as reassurance of non-carriers, entails a tremendous impact on primary prevention of arrhythmic complications and life-style, by including sport activity disqualification and genetic counseling for disease recurrence in sibs and offsprings with the dilemma of procreation (93).

Experimental animal models (knock out, overexpression, knock in mice) are opening new avenues to understanding disease pathogenesis and identify targets for therapy (94-97). After plakoglobin, desmoplakin and plakophillin transgenic mice, Kalliopi Pilichou recently generated a transgenic mice with overexpression of mutated desmoglein-2 (97), the same defective gene previously detected in an AC Italian proband (87). The recapitulation of the disease in the mice was quite convincing: dilatation and aneurysms at echo of both ventricles, tachyarrhythmias, sudden death, fibrous replacement of the ventricular myocardium at histology. Interstingly enough, the animals were normal at birth and cell death occurred with time after a few weeks, in the shape of cell necrosis (oncosis) at electron microscopy, thus confirming that the disorder is a genetically determined cardiomyopathy and not a congenital heart disease (98).

Depletion of plakoglobin signal at intercellular junctions was found by Angeliki Asimaki in AC patients, whatever the defective gene, and may be considered a biomarker for the diagnosis at endomyocardial biopsy (99).
Generation of knock-in transgenic mice will contribute to the understanding of the mechanistic events, as to find etiological (not merely symptomatic) therapeutic interventions. Prevention of disease onset and progression will be possible only when the underlying biological and molecular phenomena will be better understood.

European and American teams continue to be committed in the study of the disease. At the turn of the last millennium, following a series of meeting of experts from both sides of the Atlantic Ocean (Fig.9,10), it became evident that the expertise of scientists and clinicians should merge into an “army” for the fight against the calamity of sudden death due to AC. An International Registry was considered mandatory in order to collect study material and concentrate efforts on this rare disorder (100).

It was then decided to apply for grants of the European Commission and the National Institute of Health. Two teams were created, one in Europe coordinated by Gaetano Thiene and one in North America coordinated by Frank Marcus. The two projects started by utilizing a similar database and sharing some Core Labs. The method was somewhat different: the European Registry enrolled patients who were previously diagnosed as well as new entries (101), whereas the North American Registry enrolled only newly diagnosed patients (102). Previously published diagnostic criteria were employed and protocols implemented accordingly. Genetic investigation was an integral part of both studies. Both projects were approved and funded by the European Commission and the NIH for 5 years, thus allowing the starting of a major interdisciplinary study of AC. The results exceeded the best expectations, culminating in the discovery of 5 disease genes, numerous publications in highly ranking cardiovascular journals and new diagnostic criteria. A monograph collected all these achievements (103) (Fig.11).

The progress in the knowledge of AC was possible thanks to an international tight collaboration and loyal competition. We like to quote here the late Lino Rossi's words “All of
them share the unique merit of a skillful and dedicated engagement in a scientific contest of vital importance which is not comparable to any sports competition; as such, the present overview concludes with the popular saying – who cares who came second? – here intended in an entirely positive, even laudative sense” (104).
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LEGENDS

Fig. 1  The book of Giovanni Maria Lancisi published in Naples in 1736.

Fig. 2  The drawings of the “parchment heart” of Osler, with paper-thin walls of both ventricles.

Fig. 3  The original picture of the Uhl’s anomaly.

Fig. 4  The heart at cardiac transplantation of one of the patients published by Dalla Volta in 1964. Note the huge dilatation of the right ventricle, both at gross and in vitro magnetic resonance, with paper-thin RV free wall.

Fig. 5  The autopsy report of a case of AC of a patient who died due to pulmonary embolism with adipositas cordis in the RV and foci of myocardial sclerosis in the left ventricle.

Fig. 6  A 26 year old physician who died during a tennis game in May 1979. A ventricular tachycardia, left bundle branch block morphology, had been recorded at the ECG during palpitations. The autopsy disclosed for the first time AC as a cause of sudden death in the young.

Fig. 7  Cartoon of Professor Lino Rossi stressing the connection between Venice and Naxos history.

Fig. 8  Dr Frank Marcus visiting the Patavian group in 1994. From left to right: Gaetano Thiene, Luciano Daliento, Marialuisa Valente, Beth Livolsi (a visiting nurse from the States, belonging to a family with AC), Gianfranco Buja, Frank Marcus, Bortolo Martini, Andrea Nava (courtesy of Dr Martini).

Fig. 9  A meeting in Baltimore in 1999 between American and European groups involved in the study of AC. From left to right - bottom: Jeff Towbin, Arthur Moss, Guy Fontaine, Cristina Basso, Thomas Wichter, Barbara Bauce, Frank
Marcus; top: Kathy Gear, Duane Sherrill, Hugh Calkins, Wojciech Zareba, Gaetano Thiene.

**Fig.10** Meeting in Naxos in 2003 of the European team. From left to right: Barbara Bauce, Guy Fontaine, Cristina Basso, Nikos Protonotarios, Gaetano Thiene, Katarzyna Wlodarska, Andrea Nava, Elzbieta Czarnowska, Thomas Wichter, Loizos Antoniades, GianAntonio Danieli, William McKenna.

**Fig.11** Meeting in Denver in 2007 of the European and American teams for the presentation of the book “Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia – Recent Advances” (103)

**Fig.12** Domenico Corrado, Barbara Bauce, Cristina Basso and Gaetano Thiene. Denver, May 2007
JOHANNIS MARIAE LANCISII
A Secretori Cubiculo, & Archiatri Pontificii
DE MOTU CORDIS
ET ANEURYSMATIBUS
OPUS POSTUMUM
IN DUAS PARTES DIVISUM.

NEAPOLI ANNO MDCXXXVIII.
Excedebat FELIX-CAROLUS MUSCA
SUPERIORUM FACULTATI.
Right ventricle, anterior aspect
Fig. 5

Pleura viscerale
Linfonodi
Bronchi
Arterie: tronco maggiore e ramificazioni (cfr. sterno)
Vene: dell'albero della polmonare

CUORE (gr. 450) con una flaccitudine ventricolare da commento patologico del gasso miocardico

Diametri: trasversale cm. 15, verticale cm. 11

Epicardio liscio, allunato, del fresco intenso

Spessore ventricoli: V.S. cm. 1,5 V.D. cm. 2,5

Cavità cardiache

Endocardio con piccola comparsa di rosso esudativo

Valvole: spostate e disomogenee della traiettoria di uscita, sforbide

Coronarie: placche nascoste, momentanea di miocardio

Miocardio: interesserà ed infilierà del miocardio e "sheath" cellulari di contenimento

Aorta
Arterie polmonare
Fig. 7