The genetics of congenital heart disease: a review of recent developments

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Purpose of review

As our understanding of the molecular regulation of cardiac development has progressed, an increasing number of genes that cause congenital heart disease when mutated are being identified. This review focuses on the progress made during the past year.

Recent findings

After *PTPN11* was identified as a Noonan syndrome disease gene, additional discoveries have made clear that mutations in other genes along the RAS signaling pathway can cause a spectrum of syndromes and possibly isolated congenital heart disease. Similarly, alterations of genes in other signaling and transcriptional pathways may contribute to the development of atrial septal defects and bicuspid aortic valves. Recently identified disease genes for syndromes associated with congenital heart disease are also reviewed. Finally, the possibility that somatic mosaicism may contribute to the development of congenital heart disease is discussed.

Summary

The recent knowledge about the molecular genetic causes of congenital heart disease is reviewed. In many instances, these gene discoveries are being rapidly translated into meaningful genetic testing, which is improving the diagnosis and prognostication for congenital heart disease in isolation or in the context of a syndrome. Ultimately, genetic information will be necessary for planning care as well as clinical research.

Keywords

bicupsid aortic valve, CHARGE syndrome, congenital heart disease, Noonan syndrome, somatic mosaicism

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Abbreviations

ASD	atrial septal defect
AVSD	atrioventricular septal defect
BAV	bicuspid aortic valve
CFC	cardiofaciocutaneous
CHD	congenital heart disease
MAPK	mitogen-activated protein kinase
TOF	tetralogy of Fallot
VSD	ventricular septal defect

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Introduction

Congenital heart disease (CHD) is the most common developmental defect, occurring in almost 3% of neonates if one includes the bicuspid aortic valve (BAV). Moreover, CHD contributes significantly to morbidity and mortality in this age group. The first success in palliating CHD was over 60 years ago when Blalock performed the first shunt operation on an infant with tetralogy of Fallot (TOF). Since then, and especially over the last three decades, the prognosis of neonates born with CHD has improved tremendously. This prolonged survival has brought new challenges – the emergence of co-morbidities and late mortality in adolescents and adults with CHD, as well as recurrences in their offspring. Clinical delineation of these issues and basic research of them is resulting in the identification of an increasing number of genes that cause CHD when mutated.

In this review, we present recent advances in identifying CHD genes and elaborating disease pathogenesis. A major theme is not surprising: defects in genes that encode proteins acting in particular signaling or transcriptional pathways cause similar phenotypes. This has been shown for Noonan syndrome and related phenotypes and the RAS/mitogen-activated protein kinase (MAPK) pathway. Disturbances of specific pathways may also play roles in the development of atrial septal defects (ASD) and bicuspid aortic valves. Further, we discuss recently identified disease genes for syndromes that include CHD and the possibility that somatic mosaicism contributes to the development of CHD.

Noonan syndrome and related disorders: SHP-2 and the RAS/MAPK pathway

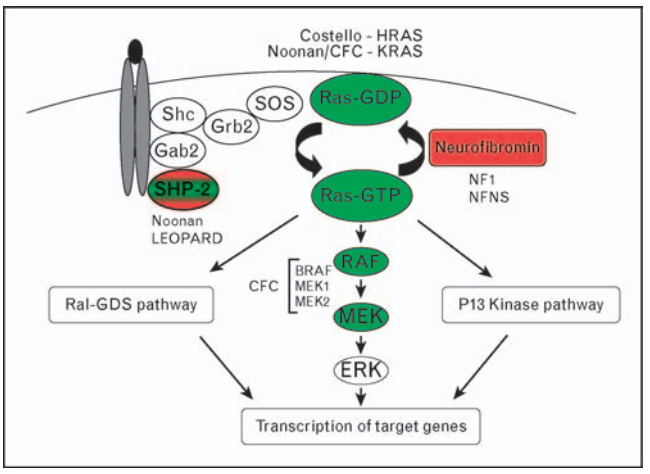
There is a spectrum of clinical syndromes that includes Noonan (MIM 163950), LEOPARD (MIM 151100),

Costello (MIM 218040) and cardiofaciocutaneous (CFC; MIM 115150) syndromes. Affected patients have a high incidence of heart disease (e.g., 80–90% in Noonan syndrome).

In Noonan syndrome, pulmonic stenosis, hypertrophic cardiomyopathy, atrioventricular septal defects (AVSD) and coarctation of the aorta are most common. Other features include facial dysmorphia, growth and mental retardation, as well as bleeding disorders, malignancies and skin lesions to a variable degree. In 2001, Tartaglia and co-workers [1] identified missense mutations in *PTPN11*, the gene encoding the nonreceptor-type protein tyrosine phosphatase SHP-2, as a cause of Noonan syndrome. Mutations engender gain of function and account for nearly 50% of Noonan syndrome cases. As SHP-2 is important for signal transduction via the RAS-MAPK pathway, it was hypothesized that patients with Noonan syndrome-related disorders might also have defects along this pathway (Fig. 1).

LEOPARD syndrome, which closely resembles Noonan syndrome but includes lentigines, was found to be caused by specific *PTPN11* mutations. Unlike Noonan syndrome, these SHP-2 mutants have a loss of function and appear to have a dominant-negative effect [2•,3••]. This discrepancy between functional effect of mutations and phenotypic outcomes remains unexplained.

Figure 1 Schematic diagram showing the RAS-MAPK signal transduction pathway



The syndromes and their mutated proteins are as indicated: those with gain-of-function mutants are indicated in green and those with loss-of-function (neurofibromin) or dominant-negative effects (SHP-2) are shown in red. The double ovals in grey and black oval shown on the left represent a generic dimerized cell-surface receptor with its ligand. MAPK, mitogen-activated protein kinase; CFC, cardio-facio-cutaneous syndrome; NF1, neurofibromatosis type I; NFNS, neurofibromatosis-Noonan syndrome. This figure was modified from one kindly provided by Kevin Shannon (University of California at San Francisco). Reproduced with permission from Oxford University Press [46••].

No *PTPN11* mutation was found in Costello or CFC syndrome. Instead, heterozygous de-novo gain-of-function missense mutations in *HRAS* were identified in 83–100% of Costello syndrome cases [4••,5,6•–8•], and a correlation between genotype and risk of malignancy was observed [7•]. There is evidence that somatic mosaicism for *HRAS* mutations may play a role in the mutation-negative group [9•]. Most recently, sporadic *HRAS* mutations were linked to advanced paternal age and paternal origin, a common finding in autosomal dominant disorders [8•].

Gain-of-function mutations in *HRAS's* paralogue, *KRAS*, have been found in some patients with Noonan syndrome and CFC syndrome [10••,11••]. Overall, CFC syndrome appears to be caused more commonly by gain-of-function *BRAF*, *MEK1*, and *MEK2* mutations [12••,13••].

These findings signify that these formerly distinct syndromes can be classed as disorders of dysregulated RAS-MAPK signaling. They also have empowered genetic testing that is refining diagnostics and prognostication for affected children and families. A next step is to utilize this information to elaborate causes of isolated CHD. So far, only *PTPN11* has been screened for mutations in nonsyndromic patients with forms of CHD that commonly occur in Noonan syndrome [14,15]. Among more than 200 patients, a *PTPN11* mutation was identified in only one girl with a complete AVSD. Thus, it appears that *PTPN11* mutations play a minor role in the development of isolated CHD. It is possible, however, that mutations in genes encoding proteins elsewhere in the RAS-MAPK pathway will prove more important in the pathogenesis of isolated CHD.

Bicuspid aortic valve: NOTCH1

Bicuspid aortic valve is a highly heritable trait [16,17]. The aortic valve abnormality occurs either in isolation or in combination with other CHDs, especially coarctation of the aorta. Some patients with BAV also manifest ascending aortic aneurysm; while the latter was previously thought to result from hemodynamic derangements, some family members of individuals with BAV can have just aortic aneurysm [18].

Garg and co-workers [19] studied a large pedigree with autosomal-dominant BAV with early calcification of the valve, as well as other forms of CHD. They ultimately identified *NOTCH1* mutations as causative. Mutations found in two unrelated families were nonsense and frameshift defects, respectively, suggesting haploinsufficiency. They also showed that the premature aortic valve calcification was due, at least in part, to *NOTCH1* haploinsufficiency. Subsequently, Mohamed and colleagues [20] sequenced all *NOTCH1* coding exons and intron boundaries in 48 patients with nonfamilial

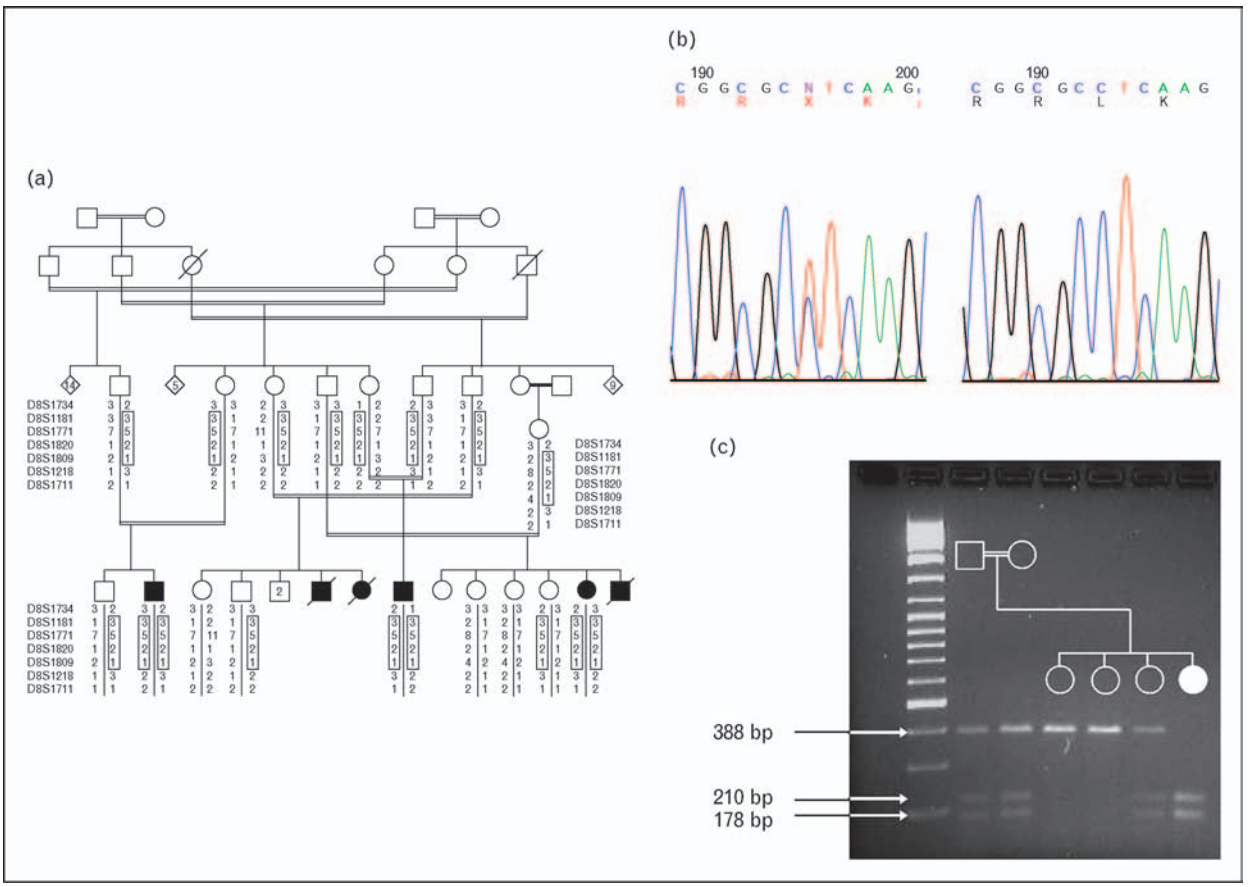
BAV. They identified two individuals harboring missense mutations, documenting that this gene is not a major cause of BAV.

HEY1 and 2, basic helix-loop-helix transcription factors, are direct transcriptional targets of NOTCH1. *Hey1/Hey2* double knockout mice exhibit absence or severe hypoplasia of major vessels in the embryo and yolk sac. Knockdown of *Hey*'s zebrafish homologue, *gridlock*, causes severe aortic maturation defects resembling aortic coarctation. These findings implicate the NOTCH-HEY pathway in valvulogenesis and vasculogenesis, suggesting that mutations in genes relevant for it will underlie left heart outflow tract defects. Manipulation of the pathway might provide a novel therapy to prevent or forestall valve calcification.

Conotruncal defects: NKX2.6

The most common genetic cause of conotruncal defects such as truncus arteriosus is a microdeletion of 22q11.2 as found in DiGeorge and velocardiofacial syndromes. Most conotruncal defects, however, do not arise in that context. Heathcote and colleagues [21] mapped an autosomal recessive form of truncus arteriosus to a region at chromosome 8p21 using a large consanguineous pedigree (Fig. 2). They then identified a homozygous missense mutation in *NKX2.6* resulting in an F151L substitution. This gene encodes a homeodomain-containing transcription factor. Phe151 resides in the homeodomain, and substitution of Phe residues in closely related transcription factors alters function. The prevalence and pathogenesis of *NKX2.6* mutations with respect to conotruncal defects remain to be determined.

Figure 2 Mutation analysis in a consanguineous family inheriting truncus arteriosus in an autosomal recessive manner



(a) Pedigree of the family. Haplotypes are shown for the interval between markers D8S1734 and D8S1711. The proposed linked haplotype is boxed and is homozygous between markers D8S1181 and D8S1809 in the three individuals with truncus arteriosus. DNA was not available from deceased affected individuals. (b) Electropherograms showing partial sequence of *NKX2.6* exon 2 from an unaffected parent (left) and an affected child (right). The parent is a carrier for the thymine-to-cytosine transition, whereas the affected individual is homozygous for cytosine at position 451. This translates into the F151L missense mutation described in the text. (c) Agarose gel showing *Bsa*HI digest of samples from the Kuwaiti family with partial pedigree above the lanes. The 451T>C mutation creates a *Bsa*HI restriction site in exon 2 of *NKX2.6*, which allows the enzyme to cleave the 388-bp PCR product into fragments of 210 and 178 bp. The gel shows two heterozygous parents who have children of all three genotypes. The 388-bp PCR product is completely digested in the lane containing the affected child, confirming that this individual is homozygous for the mutant sequence. Reproduced with permission from [21].

Mutations in *NKX2.6*'s homologue, *NKX2.5*, are found in 4% of patients with TOF and ASDs with or without atrioventricular block. *Nkx2.6* is expressed in posterior myocardial progenitors, sinus venosus, dorsal pericardium, and outflow tract myocardium during development in the mouse. Mice lacking Nkx2.6 have normal cardiac development, which has been attributed to expanded expression of *Nkx2.5* [22]. Heathcote *et al.* [21] hypothesized that the F151L NKX2.6 mutant protein attenuates that compensation due to a dominant-negative effect, although several plausible mechanisms remain to be explored.

CHARGE syndrome: CHD7

CHARGE syndrome is an acronym for a combination of developmental defects that include colobomata, heart anomalies, choanal atresia, retardation of growth and development, and genital and ear anomalies. It occurs sporadically in most cases but can be inherited in an autosomal dominant fashion. Incidence is approximately 1 : 12 000. CHARGE syndrome is caused by mutations in the *CHD7* gene, a member of the chromodomain helicase DNA-binding protein gene family [23]. *CHD7* mutations have been described in approximately 70% of affected individuals [24•,25•,26••]. Nonsense and frameshift mutations predominate, making haploinsufficiency the likely mechanism. CHARGE syndrome-causing mutations are scattered throughout the *CHD7* gene and are without apparent correlation between allele and phenotype (Fig. 3). Somatic mosaicism has been described in one unaffected mother [25•]. There is a positive correlation between *CHD7* mutations and the presence of heart defects (92% compared with 71%), colobomata, and facial asymmetry [26••]. The range of CHDs is broad and includes conotruncal and left-sided obstructive lesions, but also isolated patent ductus arteriosus (PDA) and septal defects [24•,25•].

Atrial and ventricular septal defects

ASDs and VSDs usually occur as isolated defects but can also be components of complex CHD and/or arise in the context of genetic disorders, including Holt-Oram (MIM 142900), Rubinstein-Taybi (MIM 180849), Okihiro (MIM 607323) and Townes Brocks (MIM 107480) syndromes, as well as many chromosomal anomalies.

Septal defects as part of a clinical syndrome

The following are genetic syndromes that include septal defects as a component.

Holt-Oram syndrome

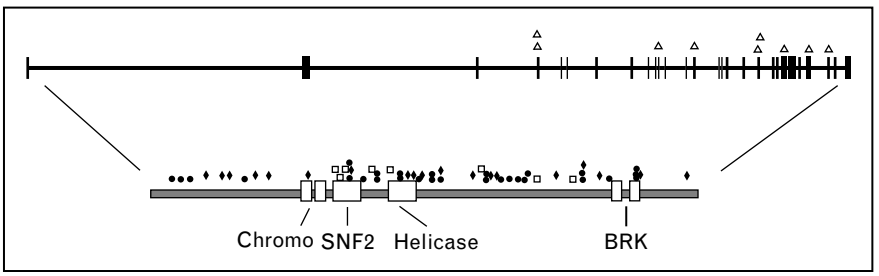
Holt-Oram syndrome is the classic heart-hand syndrome. Up to 74% of the patients have point mutations in *TBX5* that lead to haploinsufficiency. In a mouse model, gene expression dosage correlates with the severity of cardiac defects [27]. Borozdin and co-workers [28•] used real-time PCR to screen a mutation-negative cohort of 102 patients with Holt-Oram syndrome for submicroscopic *TBX5* deletions, and identified two. Another theoretical cause of Holt-Oram syndrome would be mutations in genes encoding TBX5 coactivators. Murakami *et al.* [29] found that the WW domain protein WWTR1 is a critical coactivator for TBX5. Known Holt-Oram syndrome truncation mutants had markedly decreased WWTR1 binding activity and were unable to transactivate TBX5 target genes. Thus far it is unknown if *WWTR1* mutations play a role in Holt-Oram syndrome or related disorders.

Okihiro and Townes-Brocks syndrome

Okihiro syndrome and Townes-Brocks syndrome are phenotypically related syndromes with CHD as a variable feature. The most common cardiac defects in Okihiro syndrome are VSD, ASD and TOF. The same lesions, as well as pulmonary atresia and truncus arteriosus, are seen in Townes-Brocks syndrome. Okihiro syndrome and Townes-Brocks syndrome are caused by mutations in the *SALL* family of zinc finger transcription factor genes, *SALL4* and *SALL1*, respectively. De-novo *SALL1* mutations are mostly of paternal origin without evidence of an age effect [30•]. There is evidence that haploinsufficiency for SALL1 correlates with a mild Townes-Brocks syndrome phenotype, whereas a dominant-negative effect is required for the full phenotype [31•]. Recently, it was shown that 20% of *Sall4*+/− heterozygous mice have VSDs as well as other cardiac

Figure 3 Genomic and protein map of *CHD7*, indicating the spectrum of mutations in CHARGE syndrome

On the protein map, blackened circles signify nonsense mutations, blackened diamonds signify frameshift mutations, unblackened squares signify missense mutations, and the unblackened circle specifies a mutation with an in-frame deletion of 3 aa. On the genomic sequence, triangles represent splice-site mutations. Reproduced with permission from [26••].



malformations [32•], and that *Sall1/4* double heterozygotes have an even higher incidence of VSDs (70%). The authors also demonstrated that truncated Sall1 disturbs Sall4 localization in heterochromatin, and proposed that some aspects of Townes-Brocks syndrome could result indirectly from SALL4 inhibition.

Nonsyndromic septal defects

The following are genes that cause isolated septal defects when mutated.

MYH6

Ching and colleagues [33] identified myosin heavy chain 6 (*MYH6*) missense mutations in a large family with an autosomal dominant form of ASD. *MYH6* mutations had previously been described only in late-onset hypertrophic cardiomyopathy. *MYH6* is expressed at high levels in the developing atria, and its transcription is activated by TBX5, the Holt-Oram syndrome disease gene. Known Holt-Oram syndrome TBX5 mutant proteins significantly reduced activation of the MYH6 promoter. Moreover, Garg and co-workers [34] had found earlier that GATA4 mutants that cause ASDs lead to decreased transactivation of MYH6. Overall, these results establish a link among three ASD genes.

CITED2

CITED2 is a transcriptional cofactor that interacts with CREB-binding protein (CREBBP) and EP300, and coactivates transcription factors such as the neural crest cell-related TFAP2A. Mice lacking Cited2 die *in utero* and have noncardiac as well as cardiac malformations, including ASD, VSD and conotruncal defects. Mutations in three genes encoding binding partners of CITED2 cause genetic syndromes that are associated with CHD. *CREBBP* and *EP300* mutations are known to cause Rubinstein-Taybi syndrome [35,36] and *TFAP2B* is the Char syndrome disease gene [37]. The cardiac phenotype of the former includes septal defects, PDA and also complex CHD, whereas PDAs predominate in Char syndrome. Sperling and colleagues [38] investigated *CITED2* as a candidate gene for isolated CHD in humans. They used denaturing high performance liquid chromatography to screen 392 patients with various forms of nonsyndromic CHD for mutations in *CITED2*, and found three missense mutations. In vitro, these mutations significantly reduce the capacity of CITED2 to transrepress HIF1A, and one of the mutant CITED2 proteins significantly diminishes TFAP2C coactivation. Although only 1% of the study group carried mutations leading to amino acid changes, these results should encourage investigation of other genes along that pathway as candidate genes for isolated CHD.

Somatic mutations

Somatic mosaicism refers to the condition in which a mutation arises after fertilization such that only a subset of cells or tissues harbors the defect. Aside from occupying a major role in the pathogenesis of many cancers, somatic mosaicism has been shown to underlie some cases of certain genetic disorders [39]. This genetic mechanism has been raised theoretically as relevant for CHD, particularly when isolated. Many of the genes that control cardiac development also play critical roles in the development of other organ systems. As such, germline inheritance of mutations for these genes may not be compatible with survival of the developing embryo and, in any case, would not likely cause only CHD. Moreover, mutations in relevant genes are found in a very small percentage of isolated CHD, suggesting that investigators might be examining the wrong portions of genes (i.e., the coding region) or the wrong tissue (the vast majority using DNA obtained from peripheral blood).

Borlaz and colleagues have attempted to address the hypothesis that somatic mosaicism is important in isolated CHD using the Leipzig heart collection [40–45]. The collection includes 68 formalin-fixed hearts of patients with ASDs, VSDs, and AVSDs, some in the context of complex CHD, which were collected between 1954 and 1982. The controls were 10 normal formalin-fixed

Table 1 Somatic mutations in patients with congenital heart disease

	Number of patients with mutations					
	ASD ($n=16$)	VSD ($n=29$)	AVSD ($n=23$)	Total ($n=68$)	Mutations per patient	Mutations in controls
NKX2.5 [40,41]	15	28	23	66	1–14	2[a]
N19S	*0*	*18*	*0*			
K183E	*7*	*0*	*22*			
A281V	*5*	*24*	*5*			
D299G	*6*	*9*	*21*			
TBX5 [42]	2	0	5	13	1–2	0
GATA4[b] [43]				47	1–4	0
C292R	*6*	*19*	*6*	31		
HEY2[c] [45]	N/A	0	2	2	1–2	0

[a] Different from mutations found in congenital heart disease; mutations identified in formalin-fixed hearts.
[b] Screened exons 3–4 only.
[c] Screened exons 2–5 only.
ASD, atrial septal defect; VSD, ventricular septal defect; AVSD, atrioventricular septal defect; N/A, not applicable.

hearts collected contemporaneously, as well as six normal frozen hearts and 50 lymphocytic DNAs. The authors screened multiple transcription factors with relevance for cardiac development, such as *NKX2.5*, *TBX5*, *GATA4*, and *HEY2*, for mutations, and found many in diseased tissue (Table 1). Some patients carried multiple missense mutations, which were mainly absent in unaffected cardiac tissue of the same patients. Certain *NKX2.5* and *GATA4* mutations appeared to be specific for VSD and AVSD, respectively [40,41,43]. Some of the mutant proteins were expressed in yeast and found to have variable degrees of decreased or absent transcriptional activity. The presence of multiple mutations synergistically reduces transactivation capacity of NKX2.5, suggesting a gene-dosage effect [44]. In normal hearts, only two amino acid-changing *NKX2.5* mutations were found, which were not present in hearts with CHD, and could thus be polymorphisms. Screening of formalin-fixed hearts for mutations in genes unrelated to cardiac development did not show an increased susceptibility to mutations [45].

The vast absence of mutations in the controls and the functional alterations in the mutant proteins argue strongly for the presence of somatic mosaicism. Nonetheless, independent confirmation of this is needed before this exciting discovery gains widespread acceptance.

Conclusion

During the last year, significant advances have been made in identifying and characterizing gene defects that contribute to CHD, either in isolation or in the context of a syndrome. It is becoming clear that mutations in genes encoding proteins functioning within specific signaling or transcriptional pathways cause similar phenotypes. An important example of this is Noonan syndrome and related disorders and the RAS/MAPK signaling cascade [46••]. While the relevant developmental pathways are generally complex, resulting in numerous plausible candidate genes, technological gains in high throughput resequencing are empowering gene discovery efforts for CHD. The discovery of new genetic defects causing CHD is changing the way we diagnose and predict CHD. So far, epidemiological data have been used to estimate the recurrence risks in offspring of individuals with CHD. As more disease genes for CHD are identified, we are achieving greater accuracy in predicting recurrence risks and determining the status of at-risk fetuses. Ultimately, genetic information will be crucial for planning care as well as clinical research.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 225–226).

1 Tartaglia M, Mehler EL, Goldberg R, *et al.* Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001; 29:465–468.

2 • Tartaglia M, Martinelli S, Stella L, *et al.* Diversity and functional consequences of germline and somatic *PTPN11* mutations in human disease. Am J Hum Genet 2006; 78:279–290.
Noonan syndrome-causing *PTPN11* mutations engender less gain-of-function than those causing leukemia.

3 •• Kontaridis MI, Swanson KD, David FS, *et al. PTPN11* (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects. J Biol Chem 2006; 281:6785–6792.
Unlike in Noonan syndrome, Leopard syndrome-causing *PTPN11* mutations have a dominant-negative effect. Thus the pathogenesis of the two disorders is distinct.

4 •• Aoki Y, Niihori T, Kawame H, *et al.* Germline mutations in *HRAS* proto-oncogene cause Costello syndrome. Nat Genet 2005; 37:1038–1040.
The authors show for the first time that germline mutations in *HRAS* cause Costello syndrome.

5 Estep AL, Tidyman WE, Teitell MA, *et al.* HRAS mutations in Costello syndrome: detection of constitutional activating mutations in codon 12 and 13 and loss of wild-type allele in malignancy. Am J Med Genet A 2006; 140:8–16.

6 • Gripp KW, Lin AE, Stabley DL, *et al.* HRAS mutation analysis in Costello syndrome: genotype and phenotype correlation. Am J Med Genet A 2006; 140:1–7.
There are five known recurrent Costello syndrome-causing *HRAS* mutations, and no apparent genotype–phenotype correlation.

7 • Kerr B, Delrue MA, Sigaudy S, *et al.* Genotype-phenotype correlation in Costello syndrome: *HRAS* mutation analysis in 43 cases. J Med Genet 2006; 43:401–405.
There is a genotype–phenotype correlation with respect to the risk of developing malignancies.

8 • Zampino G, Pantaleoni F, Carta C, *et al.* Diversity, parental germline origin, and phenotypic spectrum of de novo *HRAS* missense changes in Costello syndrome. Hum Mutat 2007; 28:265–272.
Costello syndrome-causing HRAS mutations are associated with advanced paternal age.

9 • Gripp KW, Stabley DL, Nicholson L, *et al.* Somatic mosaicism for an HRAS mutation causes Costello syndrome. Am J Med Genet A 2006; 140:2163–2169.
First report of somatic mosaicism for an *HRAS* mutation causing Costello syndrome.

10 •• Schubbert S, Zenker M, Rowe SL, *et al.* Germline *KRAS* mutations cause Noonan syndrome. Nat Genet 2006; 38:331–336.
First report of *KRAS* mutations causing Noonan syndrome.

11 •• Carta C, Pantaleoni F, Bocchinfuso G, *et al.* Germline missense mutations affecting *KRAS* Isoform B are associated with a severe Noonan syndrome phenotype. Am J Hum Genet 2006; 79:129–135.
Identification of *KRAS* Isoform B missense mutations perturbing the conformation of the guanine ring-binding pocket of the protein in two patients with severe Noonan syndrome.

12 •• Niihori T, Aoki Y, Narumi Y, *et al.* Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome. Nat Genet 2006; 38:294–296.
First description of *KRAS* and *BRAF* mutations in CFC syndrome.

13 •• Rodriguez-Viciana P, Tetsu O, Tidyman WE, *et al.* Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science 2006; 311:1287–1290.
First report of mutations in *MEK1* and *2*, which are downstream of RAF, in CFC syndrome.

14 Sarkozy A, Conti E, Esposito G, *et al.* Nonsyndromic pulmonary valve stenosis and the PTPN11 gene. Am J Med Genet A 2003; 116:389–390.

15 Weismann CG, Hager A, Kaemmerer H, *et al.* PTPN11 mutations play a minor role in isolated congenital heart disease. Am J Med Genet A 2005; 136:146–151.

16 Cripe L, Andelfinger G, Martin LJ, *et al.* Bicuspid aortic valve is heritable. J Am Coll Cardiol 2004; 44:138–143.

17 McBride KL, Pignatelli R, Lewin M, *et al.* Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: Segregation, multiplex relative risk, and heritability. Am J Med Genet A 2005; 134:180–186.

18 Schievink WI, Mokri B. Familial aorto-cervicocephalic arterial dissections and congenitally bicuspid aortic valve. Stroke 1995; 26:1935–1940.

19 Garg V, Muth AN, Ransom JF, *et al.* Mutations in *NOTCH1* cause aortic valve disease. Nature 2005; 437:270–274.

20 Mohamed SA, Aherrahrou Z, Liptau H, *et al.* Novel missense mutations (p.T596M and p.P1797H) in *NOTCH1* in patients with bicuspid aortic valve. Biochem Biophys Res Commun 2006; 345:1460–1465.

21 Heathcote K, Braybrook C, Abushaban L, *et al.* Common arterial trunk associated with a homeodomain mutation of *NKX2.6*. Hum Mol Genet 2005; 14:585–593.

22 Tanaka M, Yamasaki N, Izumo S. Phenotypic characterization of the murine *Nkx2.6* homeobox gene by gene targeting. Mol Cell Biol 2000; 20:2874–2879.

23 Vissers LE, van Ravenswaaij CM, Admiraal R, *et al.* Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. Nat Genet 2004; 36:955–957.

24 • Aramaki M, Udaka T, Kosaki R, *et al.* Phenotypic spectrum of CHARGE syndrome with *CHD7* mutations. J Pediatr 2006; 148:410–414.
Phenotypic characterization of 17 CHARGE syndrome patients with *CHD7* mutations.

25 • Jongmans MC, Admiraal RJ, van der Donk KP, *et al.* CHARGE syndrome: the phenotypic spectrum of mutations in the *CHD7* gene. J Med Genet 2006; 43:306–314.
There is no genotype–phenotype correlation in CHARGE syndrome caused by *CHD7* mutations.

26 •• Lalani SR, Safiullah AM, Fernbach SD, *et al.* Spectrum of *CHD7* mutations in 110 individuals with CHARGE Syndrome and genotype-phenotype correlation. Am J Hum Genet 2006; 78:303–314.
Showed a positive correlation between the presence of *CHD7* mutations and heart defects.

27 Mori AD, Zhu Y, Vahora I, *et al.* Tbx5-dependent rheostatic control of cardiac gene expression and morphogenesis. Dev Biol 2006; 297:566–586.

28 • Borozdin W, Bravo Ferrer Acosta AM, Bamshad MJ, *et al.* Expanding the spectrum of *TBX5* mutations in Holt-Oram syndrome: detection of two intragenic deletions by quantitative real time PCR, and report of eight novel point mutations. Hum Mutat 2006; 27:975–976.
The authors describe for the first time that intragenic deletions in *TBX5* can cause Holt-Oram syndrome.

29 Murakami M, Nakagawa M, Olson EN, Nakagawa O. A WW domain protein TAZ is a critical coactivator for TBX5, a transcription factor implicated in Holt-Oram syndrome. Proc Natl Acad Sci U S A 2005; 102:18034–18039.

30 • Bohm J, Munk-Schulenburg S, Felscher S, Kohlhase J. SALL1 mutations in sporadic Townes-Brocks syndrome are of predominantly paternal origin without obvious paternal age effect. Am J Med Genet A 2006; 140:1904–1908.
SALL1 mutations are derived from the father, but advanced paternal age does not seem to play a role.

31 • Borozdin W, Steinmann K, Albrecht B, *et al.* Detection of heterozygous *SALL1* deletions by quantitative real time PCR proves the contribution of a SALL1 dosage effect in the pathogenesis of Townes-Brocks syndrome. Hum Mutat 2006; 27:211–212.
Haploinsufficiency of SALL1 causes a mild form of Townes-Brocks syndrome, but a dominant negative effect is required for the full phenotype.

32 • Sakaki-Yumoto M, Kobayashi C, Sato A, *et al.* The murine homolog of *SALL4*, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development. Development 2006; 133:3005–3013.
The authors suggest that some of the symptoms of Townes-Brocks syndrome caused by SALL1 truncations could be secondary to SALL4 inhibition.

33 Ching YH, Ghosh TK, Cross SJ, *et al.* Mutation in myosin heavy chain 6 causes atrial septal defect. Nat Genet 2005; 37:423–428.

34 Garg V, Kathiriya IS, Barnes R, *et al. GATA4* mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature 2003; 424:443–447.

35 Petrij F, Giles RH, Dauwerse HG, *et al.* Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 1995; 376:348–351.

36 Roelfsema JH, White SJ, Ariyurek Y, *et al.* Genetic heterogeneity in Rubinstein-Taybi syndrome: mutations in both the *CBP* and *EP300* genes cause disease. Am J Hum Genet 2005; 76:572–580.

37 Satoda M, Zhao F, Diaz GA, *et al.* Mutations in TFAP2B cause Char syndrome, a familial form of patent ductus arteriosus. Nat Genet 2000; 25:42–46.

38 Sperling S, Grimm CH, Dunkel I, *et al.* Identification and functional analysis of *CITED2* mutations in patients with congenital heart defects. Hum Mutat 2005; 26:575–582.

39 Youssoufian H, Pyeritz RE. Mechanisms and consequences of somatic mosaicism in humans. Nat Rev Genet 2002; 3:748–758.

40 Reamon-Buettner SM, Borlak J. Somatic *NKX2-5* mutations as a novel mechanism of disease in complex congenital heart disease. J Med Genet 2004; 41:684–690.

41 Reamon-Buettner SM, Hecker H, Spanel-Borowski K, *et al.* Novel *NKX2-5* mutations in diseased heart tissues of patients with cardiac malformations. Am J Pathol 2004; 164:2117–2125.

42 Reamon-Buettner SM, Borlak J. *TBX5* mutations in non-Holt-Oram syndrome (HOS) malformed hearts. Hum Mutat 2004; 24:104.

43 Reamon-Buettner SM, Borlak J. *GATA4* zinc finger mutations as a molecular rationale for septation defects of the human heart. J Med Genet 2005; 42:e32.

44 Inga A, Reamon-Buettner SM, Borlak J, Resnick MA. Functional dissection of sequence-specific *NKX2-5* DNA binding domain mutations associated with human heart septation defects using a yeast-based system. Hum Mol Genet 2005; 14:1965–1975.

45 Reamon-Buettner SM, Borlak J. *HEY2* mutations in malformed hearts. Hum Mutat 2006; 27:118.

46 •• Gelb BD, Tartaglia M. Noonan syndrome and related disorders: Dysregulated RAS-mitogen activated protein kinase signal transduction. Hum Mol Genet 2006; 15 (Spec No 2):R220–R226.
Describes the association of the RAS/MAPK signaling cascade with Noonan syndrome and related disorders.