HAND Proteins: Molecular Mediators of Cardiac Development and Congenital Heart Disease

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Congenital heart defects are the clinical manifestation of anomalies in embryonic cardiac development. Such defects occur in distinct regions or chambers of the heart. A molecular framework in which to consider cardiac development and congenital heart disease in a segmental fashion has begun to emerge. dHAND and eHAND are two related basic helix-loop-helix transcription factors that are expressed in a complementary fashion in the developing right and left ventricles, respectively. They are also expressed in the neural crest-derived cardiac outflow tract and aortic arch arteries. Targeted mutations of dHAND and eHAND in mice have revealed novel pathways of organogenesis in mesodermal and neural crest derivatives. dHAND mutants exhibit hypoplasia of the right ventricle, branchial arches, and aortic arch arteries. The distinct nature of cardiac defects in dHAND mutants provides an entry into dissecting molecular pathways governing morphogenesis of specific components of the heart. Congenital heart disease is considered as a defect in segmental development of the heart and the role of dHAND and eHAND in regulating such developmental pathways in normal and abnormal cardiogenesis is examined. (Trends Cardiovasc Med 1999;9:11–18). © *1999 Elsevier Science Inc.*

In one of the more interesting forms of malformation the septum of the ventricles is not only incomplete, but is found to deviate from its natural position, one of the ventricles being unduly developed, while the other is atrophied.

—Thomas B. Peacock, 1858

The human heart is highly susceptible to genetic and environmental influences during embryonic development, as manifested by the high incidence of congenital heart defects in human pregnancies (Hoffman 1995). In the western world, congenital heart disease (CHD) remains the leading cause of death during the first year of life. Although the embryology and physiology of most congenital heart defects are well understood, our understanding of the etiology of CHD remains poor. Recent identification of morphogens and regulatory factors involved in cardiogenesis have provided an entry into dissecting the developmental programs that control cardiac development and which might be disrupted in congenital heart disease (Olson and Srivastava 1996, Fishman and Chien 1998).

It is useful to establish a clinical, morphologic, and molecular framework in which to consider the potential etiologies of CHD. Heart defects seen in newborns rarely affect the heart globally nor do they generally affect myocardial contractility. Rather, CHD typically represents specific morphogenetic defects of individual chambers or regions of the heart with the remainder of the heart developing relatively normally. Such defects are usually compatible with the intrauterine circulation, where pulmonary and systemic circulations are not separated and thus result in adequate embryonic growth and development. A good example of this is seen in infants born with either a hypoplastic right or left ventricle, where the neighboring ventricle is morphologically, electrically, and physiologically normal (Gentles et al. 1997) (Figure 1C, D). Alternatively, development of the muscular component of the heart may proceed normally, but the vessels that arise from the heart may not make appropriate connections with specific cardiac chambers. Most commonly, the vessels fail to align themselves properly with the ventricles, as is seen where the aorta overrides the ventricular septum instead of the left ventricle (Figure 1A, B).

How might one explain such remarkably circumscribed cardiac defects on an etiologic basis? A prevalent hypothesis is that hemodynamic stimuli are necessary for appropriate cardiac chamber growth and development. In classic physiologic studies, interruption of blood flow across an atrioventricular valve during cardiogenesis in an animal model resulted in hypoplasia of the associated ventricular chamber (Harh et al. 1973). However, from a molecular and developmental biologist's view, the clinical observations above may also be explained by a model in which completely separable and independent developmental programs regulate growth of specific chambers and regions of the heart. In such a scenario, chamber hypoplasia or vascular malalignment/hypoplasia would be the primary event rather than a physiologic consequence of abnormal blood flow.

There are several potential methods to identify genes that may contribute to development of specific chambers of the heart and be involved in the pathogenesis of CHD. One method, which has been used successfully for some CHD where large kindreds are affected, involves genetic linkage analysis and subsequent positional cloning of mutated or deleted gene(s) in affected individuals (Basson et al. 1994 and 1997). Such studies in CHD have been limited by the paucity of large families with affected individuals. An alternate method would employ a systematic identification of genes involved in distinct steps of cardiogenesis. Sub-

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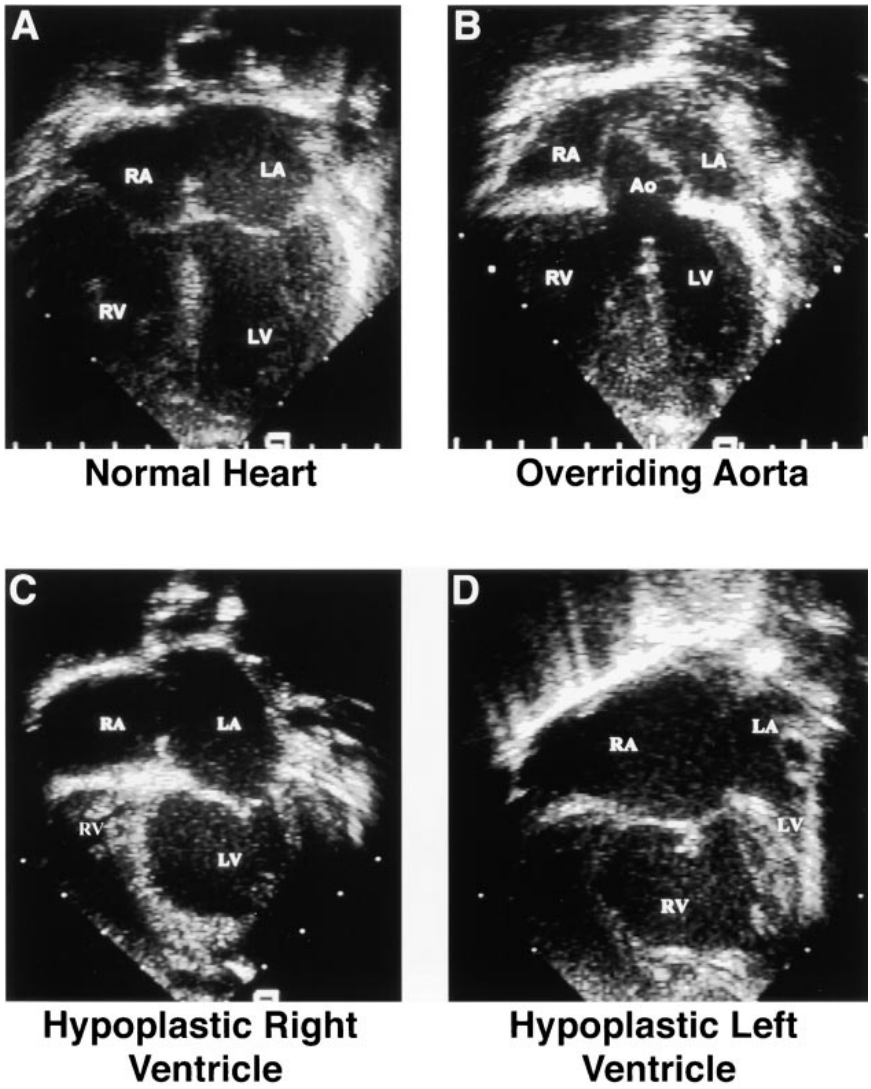

Figure 1. Segment-specific cardiac defects in human hearts. Two-dimensional ultrasound images of a normal heart **(A)** with four chambers including the right atrium (RA), left atrium (LA), right ventricle (RV), and left ventricle (LV). Malalignment of the aorta, seen in a variety of neural crest-related defects results in the aorta (Ao) overiding the ventricular septum **(B)** instead of connecting with the LV. Hypoplasia of the right **(C)** or left **(D)** ventricle can occur with the remaining ventricle being morphologically and functionally normal.

sequent mutation analysis of candidate genes in individuals with defects of corresponding steps of cardiogenesis may lead to a genetic basis for some types of CHD. In the latter approach, it would be necessary to dissect molecular pathways of cardiogenesis in a region-specific fashion.

The effort to understand cardiogenesis as a segmental process from a molecular perspective has only recently been possible. The *desmin* gene is expressed uniformly in the heart, but unique *cis* elements exist which govern expression specifically in the right ventricle (Kuisk et al. 1996). SM-22, a smooth muscle marker, is also expressed in the developing heart uniformly, but *cis* elements are present which regulate predominately right ventricular expression (Li et al. 1996). Similarly, a ventricular isoform of myosin light chain (MLC2V) is expressed in the right and left ventricles, but a stretch of 28 nucleotides in the regulatory region of the gene is responsible for right but not left ventricular expression (Ross et al. 1996). Finally, a regulatory sequence in the *myosin light chain 1F* gene (*MLC1F*) has been shown to control the gene's left but not right ventricular expression (Kelly et al. 1995). Although the trans-acting factors responsible for such segmental expression are unknown, the findings are consistent with a model where distinct regulatory pathways control development of each chamber of the heart.

The recent discovery of two members of the basic helix-loop-helix (bHLH) family of transcription factors, dHAND and eHAND, provides an entry into the molecular pathways regulating segmental development of the heart (Srivastava et al. 1995, Cserjesi et al. 1995, Cross et al. 1995, Hollenberg et al. 1995). dHAND and eHAND display complementary expression in the right and left ventricles, respectively (Srivastava et al. 1997). As such, they are the first and, to date, only transcriptional regulatory factors expressed in a ventricle-specific fashion. In addition, both genes are expressed in the mesodermal and neural crest components of the developing cardiovascular system. Functional studies have confirmed a chamber-specific role for the *dHAND* gene and have furthered efforts to dissect developmental pathways that govern right and left ventricle formation and cardiac neural crest development. Here the current knowledge of dHAND and eHAND's role in cardiac morphogenesis with respect to segmental pathways, mechanism of action, and human congenital heart disease is reviewed.

• Cardiac Morphogenesis: Mesodermal and Neural Crest Contributions

There are relevant anatomical and embryologic features of heart development that contribute to an understanding of the molecular pathways in which the *HAND* genes are involved. Two major cell types that contribute to the heart will be considered: lateral mesodermal cells and ectodermal cells derived from the neural crest. Both cell types have unique embryologic origins yet must coordinate with one another as the heart takes its final form.

The muscular portion of the heart is derived from mesodermal precursor cells that become fated to form distinct chambers of the heart prior to the first heartbeat (Yutzey and Bader 1995). Such mesodermal cells give rise to a straight heart tube that is patterned in an anterior-posterior fashion to form the four chambers of the heart (Figure 2) (Srivastava and Olson 1997). Rightward looping of the heart tube begins to establish left-right asymmetry of the heart and is critical for proper atrio-ventricular and ven-

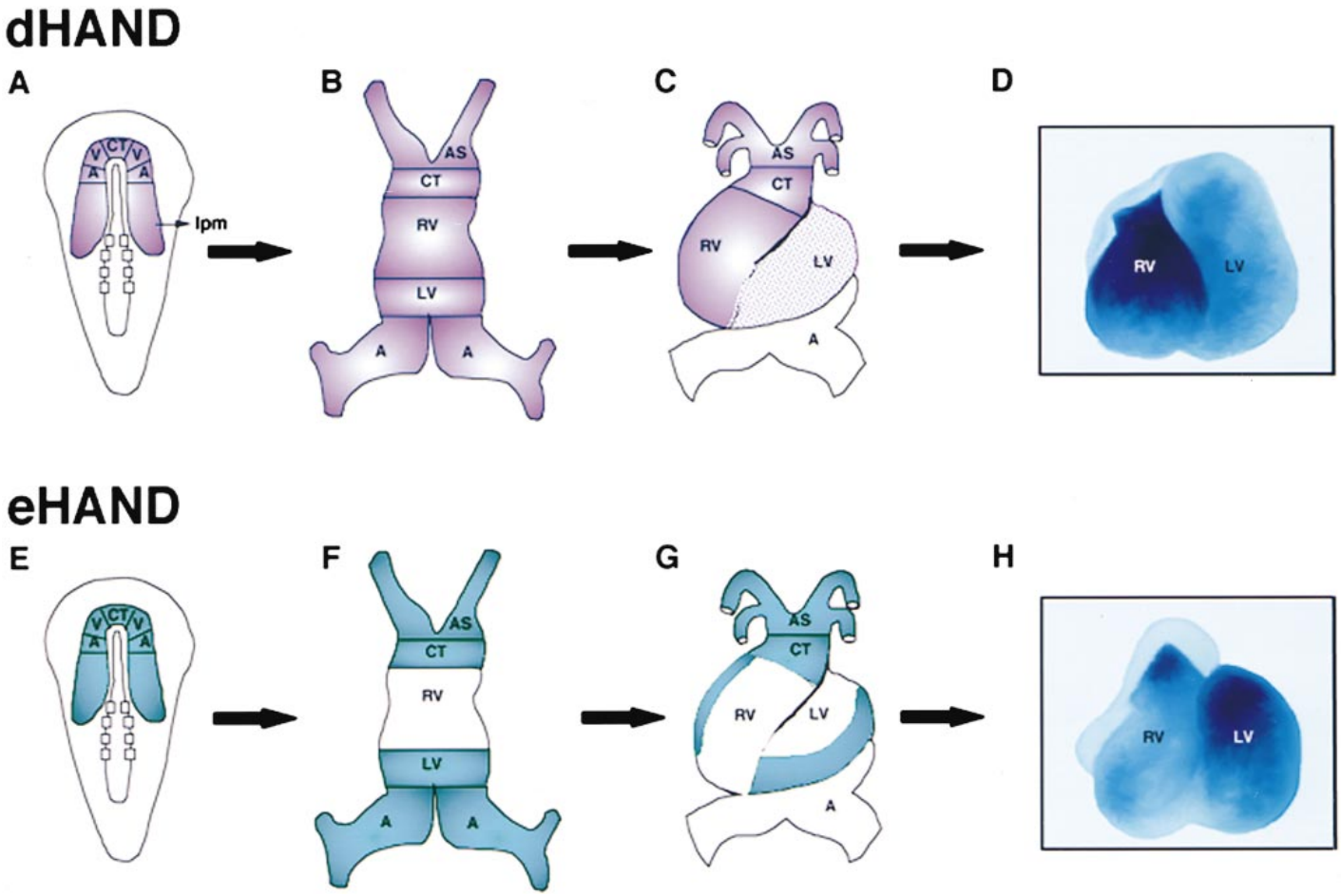

Figure 2. Summary of dHAND and eHAND expression patterns during mouse cardiogenesis. dHAND (purple) and eHAND (blue) are uniformly expressed in cardiac progenitor cells **(A, E)** and in the left and right lateral plate mesoderm (lpm) but become restricted to the future right and left ventricle-forming regions, respectively, as the heart tube loops. dHAND is expressed throughout the linear heart tube **(B)** but becomes predominately right sided after looping **(C)**. eHAND is expressed in the conotruncus (CT) and left ventricle (LV)-forming regions of the linear heart tube **(F)**; the anterior-posterior interrupted pattern becomes left-right by virtue of cardiac looping **(G)**. Expression is non-concentric and is along the outer curvature of the heart **(G)**. RNA in situ hybridization with isolated E10.0 mouse hearts shows expression of dHAND in the right ventricle **(D)** and eHAND in the left ventricle **(H)** (frontal views). Both genes are expressed in the aortic sac (AS) which gives rise to the aorta and pulmonary arteries, but are down-regulated in the myocardium of the heart once formation is complete. RV, right ventricle; A, atria.

triculo-arterial alignment. Subsequent growth and maturation of individual chambers result in the mature four-chambered heart in higher vertebrates. The factors that regulate each of these steps during cardiac mesoderm development are likely be involved in the pathogenesis of CHD related to chamber malformations [reviewed in Olson and Srivastava (1996)].

A unique population of migratory neural crest cells, known as the cardiac neural crest, arises from the level of the mid-otic placode to the third somite and invades the aortic sac and the bilaterally symmetric aortic arch arteries as they emerge from the developing heart (Waldo et al. 1998). They are involved in formation of the truncus arteriosus and subsequent septation of the truncus into the aorta and pulmonary artery, and formation of the conotruncal portion of the ventricular septum (Kirby and Waldo 1990). After septation of the aorta and pulmonary artery, the vessels rotate in a twisting fashion to achieve their final connection with the left and right ventricles, respectively. Neural crest cells also contribute to development of the aortic arch arteries that undergo extensive remodeling to form the ascending aorta, proximal subclavian, carotid, and pulmonary arteries. Disruption of the cardiac neural crest in chick embryos resulted in defects of the cardiac outflow tract and aortic arch (Kirby and Waldo 1995). Determining how neural crest cells are instructed to migrate, differentiate, proliferate, and remodel is fundamental to understanding the pathogenesis of a variety of conotruncal and aortic arch defects.

- **Expression Pattern of dHAND and eHAND**

dHAND and eHAND share high homology within their bHLH regions and are encoded by genes with similar intron-exon organization, suggesting that the genes arose by duplication of an ancestral *HAND*-like gene. In the chick (Srivastava et al. 1995), dHAND and eHAND are coexpressed in a bilaterally symmetric pattern throughout the precardiac mesoderm, linear and looped heart tube, lateral mesoderm and certain neural crest-derived structures. Antisense experiments in the chick suggest that dHAND and eHAND play redundant roles in cardiac

development beyond the stage of cardiac looping (Srivastava et al. 1995). Disruption of dHAND and eHAND mRNA in combination, but not alone, resulted in arrest of cardiac development just after the heart began to loop in the rightward direction.

In contrast to their apparently homogeneous expression throughout the developing heart in the chick, dHAND and eHAND exhibit distinct segmental expression patterns during cardiogenesis in the mouse (Figure 2). Both genes are initially expressed in the precardiac mesoderm. dHAND is also expressed throughout the linear heart tube, but becomes restricted predominantly to the future right ventricular compartment during cardiac looping (Srivastava et al. 1995 and 1997). By contrast, eHAND expression is restricted to the anterior and posterior segments of the straight heart tube, which are fated to form the conotruncus and left ventricle, respectively, but is undetectable in the intervening right ventricle-forming region (Srivastava et al. 1997, Biben and Harvey 1997). The interrupted AP pattern of expression is maintained as the heart loops and becomes a distinct LR cardiac asymmetry by virtue of the morphogenetic movements of cardiac looping with expression of eHAND in the left, but not the right, ventricle. The spatially distinct expression patterns of *dHAND* and *eHAND* make them candidate genes for controlling segmental development of the heart tube.

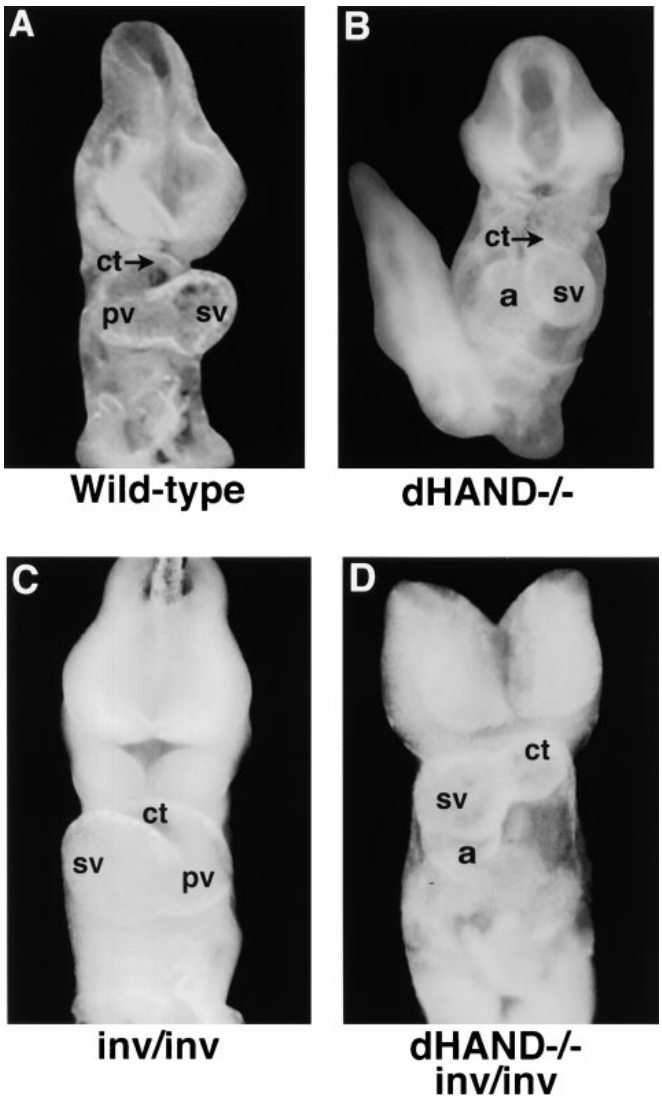

Figure 3. Pulmonary ventricle hypoplasia in *dHAND*-null and *dHAND*-null/*inv* embryos. Absence of the pulmonary (right) ventricle (pv) segment is evident in a frontal view of an E9.5 *dHAND*-null embryo **(B)** compared with a wild-type **(A)**. The conotruncus (ct) is directly connected to a left-sided systemic ventricle (sv). In situs inversus (inv) embryos, the heart tube loops in the leftward direction; in this case, the left-right (LR) orientation of the pv and sv are reversed **(C)**. Specificity of dHAND function to the pv is evident by hypoplasia of the pv in *dHAND/inv* mutants **(D)** in spite of its LR reversal. ct, conotruncus; a, atria. (Reproduced in part from Srivastava et al. 1997 and Thomas et al. 1998.)

- **Targeted Deletion of *dHAND* in Mice**

Significant insight into the role of the *dHAND* gene in cardiogenesis has come from mouse knockout studies. Heterozygote *dHAND*-null mice survive to reproductive age, but homozygous null mice die by embryonic day (E) 11.0, apparently from cardiac failure (Srivastava et al. 1997). *dHAND*-null embryos begin the process of cardiac looping in the rightward direction, but fail to develop the segment of the heart tube that forms the right ventricle (Figure 3A, B). This is consistent with the predominant expression of dHAND in the right ventricle-forming region. The atrial chamber moves dorsally as it should during cardiac looping in the mutant, suggesting that the process and direction of cardiac looping are initiated correctly. Thus cardiac looping is unaffected in the absence of dHAND, but it appears abnormal because of absence of the right ventricle segment of the looping heart tube. The left-sided ventricle, where dHAND is normally expressed at lower levels, forms in the mutant but lacks trabeculations, the finger-like projections of myocardium necessary for increasingly forceful contractions of the heart. Thus, dHAND is necessary for morphogenesis of an entire segment of the developing heart tube and for proper growth of other areas where dHAND is expressed at lower levels.

Antisense experiments in cultured chick embryos and gene-knockout experiments in mice both demonstrate an important role for the *HAND* genes, but differences in the expression patterns of dHAND and eHAND in chick and mouse embryos suggest how these genes might function. In chicks, dHAND and eHAND are coexpressed throughout the heart without segmental restriction and appear to have some degree of functional redundancy. By contrast, eHAND is not

expressed in the right ventricle-forming segment of the mouse heart and would therefore be unable to compensate for loss of dHAND in this segment. *dHAND*-null mice have a less severe defect in the left ventricle, where eHAND is expressed, suggesting some compensation by eHAND. This interpretation supports a model in which dHAND and eHAND play similar roles within spatially distinct regions of the heart. Alternately, dHAND and eHAND may confer the unique physiological properties of the right and left ventricles, respectively, and not be functionally similar in the mouse.

dHAND and eHAND are also expressed during development of the aortic sac and the bilaterally symmetric aortic arch arteries (I-VI) which arise from it (Srivastava et al. 1995, Cserjesi et al. 1995) (Figure 4A, B). Each aortic arch artery traverses a branchial arch and is remodeled during development to form the mature aortic arch and proximal pulmonary artery [reviewed in Olson and Srivastava (1996)]. Neural crest cells begin populating the aortic sac and aortic arches at approximately E9.0 in the mouse (Waldo et al. 1998) and undergo a process known as ecto-mesenchymal transformation. Mouse embryos lacking the *dHAND* gene form an aortic sac that becomes markedly dilated by E9.5–E10.0 (Figure 4C, D). Further analyses indicate that the aortic arch arteries involute and fail to remain patent, not allowing forward blood flow from the heart (Srivastava et al. 1997). The involution of aortic arch vessels is likely to have resulted in dilation of the aortic sac and severe cardiac failure. These findings suggest that the expression of dHAND in the aortic arches is necessary for maintenance of the neural crest-derived aortic arch arteries, consistent with the model of distinct pathways regulating formation and maintenance of aortic arch arteries (Waldo et al. 1996).

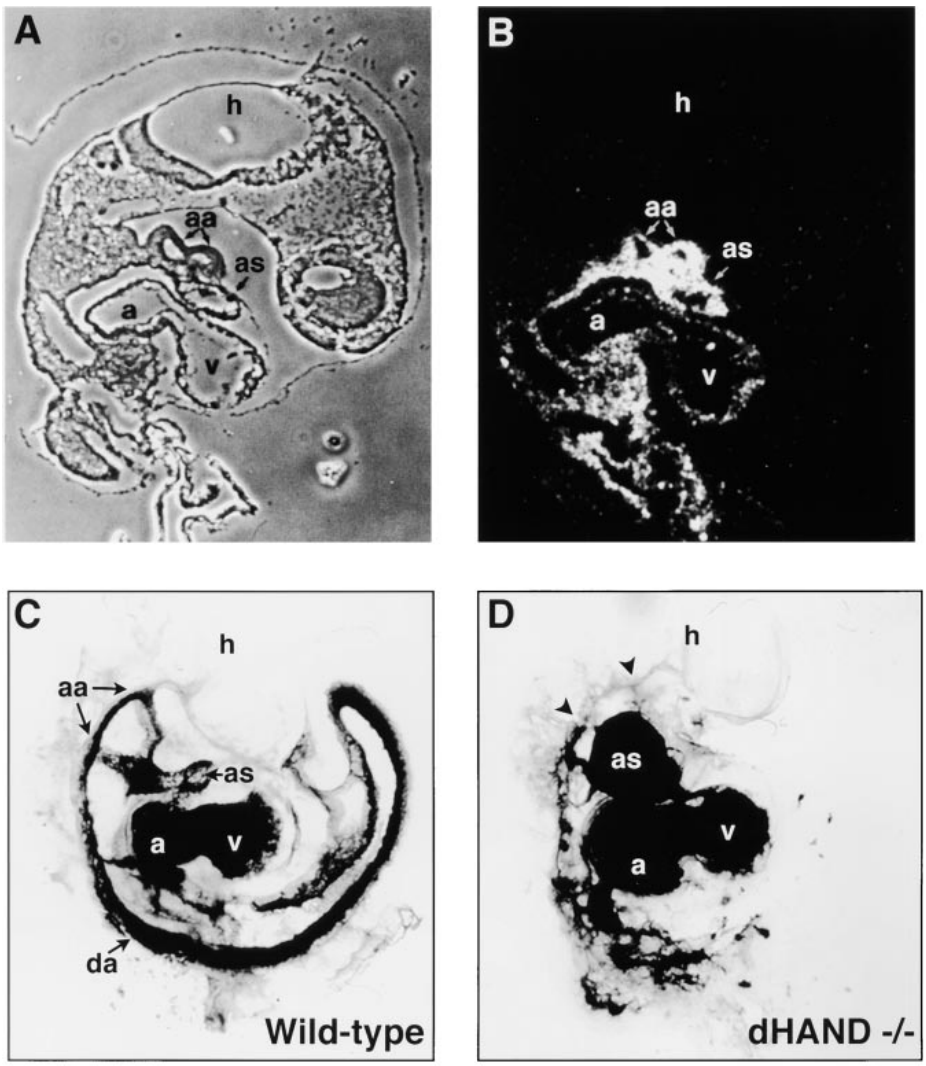

Figure 4. Role of dHAND in cardiac neural crest development. dHAND is expressed in the aortic sac (as) and aortic arch (aa) arteries of an E9.5 mouse embryo as seen by in situ hybridization in sagittal section **(B)**. **(A)** represents phase view of **(B)**. In **(C)** and **(D)**, hearts of wild-type and *dHAND*-null embryos were injected at E9.5 with India ink. Wild-type embryos showed normal development of the first and second aortic arch arteries which arose from the aortic sac. Mutant embryos showed dilation of the aortic sac and no evidence of a first or second aortic arch artery (*arrowheads*). Embryos were cleared for imaging of the vasculature. a, atrium; da, descending aorta; h, head; v, ventricle. (Reproduced in part from Srivastava et al. 1995 and 1997.)

- **Targeted Deletion of *eHAND* in Mice**

The *eHAND* gene has recently been disrupted in mice by two independent groups (Firulli et al. 1998, Riley et al. 1998). *eHAND*-null embryos had early embryonic lethality secondary to defects in placentation, making analysis of eHAND's role in cardiac morphogenesis ambiguous. Nevertheless, like dHAND, eHAND did not appear to be required for differentiation of cardiomyocytes based on expression of cardiac markers and chimeric analysis. Trophoblast rescue using tetraploid embryos allowed embryo growth to E10.5 with lethality secondary to cardiac insufficiency, although the cause of cardiac insufficiency (Riley et al. 1998) has not been defined. Understanding the precise role of eHAND in cardiac morphogenesis will be dependent upon more sophisticated tissue- and temporal-specific targeting strategies.

- **Role of *HAND* Genes in Left-Right Asymmetry**

The complementary expression of dHAND and eHAND in the right and left ventricles, respectively, has produced some confusion regarding the role of the HAND proteins in left-right (LR) embryonic asymmetry. Work in recent years has identified numerous signaling molecules and transcription factors, including sonic hedgehog, nodal, activin, snail, and others, which are expressed in a LR

asymmetric fashion prior to heart formation and control embryonic LR asymmetry [reviewed in Levin (1997), Srivastava (1997)]. It has been suggested that eHAND may lie downstream of such a signaling pathway and may mediate LR signals that control the direction of cardiac looping (Biben and Harvey 1997, Sparrow et al. 1998). However, there is abundant information that is inconsistent with such a model. Cardiac looping is initiated in the appropriate rightward direction in *dHAND*- and *eHAND*-null embryos, suggesting that they are not required for rightward bending of the heart tube. A rightward shift of the caudal region of the heart tube was also seen in mice deficient in the cardiac homeobox gene, *Nkx2.5*, which down-regulates eHAND expression (Biben and Harvey 1997). These data are more consistent with the LR symmetry of dHAND and eHAND we observe in the linear heart tube (Thomas et al. 1998a) and suggest that the LR asymmetry observed after cardiac looping is merely the result of morphogenetic movements of the heart tube that convert an AP asymmetry to an LR asymmetry. As predicted by this model, we observed pulmonary and systemic ventricle specificity of expression of dHAND and eHAND in wild-type and situs inversus (inv) mice, independent of their LR orientation (Thomas et al. 1998a). dHAND function is also specific to the pulmonary rather than right-sided ventricle as evidenced by the hypoplastic left-sided pulmonary ventricle in *dHAND/inv* mutant embryos (Figure 3C, D). Thus, the *HAND* genes are involved in development of segments of the heart tube which give rise to specific chambers of the heart during cardiogenesis, rather than controlling the direction of cardiac looping by interpreting the cascade of LR embryonic signals.

• *HAND* Genes Mediate Signaling in Neural Crest-Derived Cells

Mice deficient in the signaling peptide, endothelin-1 (ET-1), which functions through a G protein-coupled cell surface receptor, had defects of neural crest-derived cells (Kurihara et al. 1994 and 1995). Specifically, defects were seen in tissues derived from the branchial arches, including craniofacial structures, and the cardiac neural crest. The *ET-1*-null phenotype is remarkably similar to the clinical condition known as DiGeorge syndrome, where affected individuals have craniofacial, conotruncal, and aortic arch defects and are thought to have abnormalities in neural crest migration or development (Van Mierop and Kutsche 1986). *HAND* gene expression is adjacent to ET-1 expression in the branchial arches and coincident with expression of ET-1's receptor, ET_A (Thomas et al. 1998b). There is a dramatic down-regulation of dHAND and eHAND in the branchial and aortic arches of *ET-1*-null embryos, suggesting that the *HAND* genes function in a common molecular pathway with ET-1. The *ET-1*-null phenotype is less severe than the *dHAND*-null phenotype where hypoplasia of the branchial and aortic arches is notable soon after neural crest invasion. As such, dHAND or eHAND may be mediators of ET-1 signaling and may play a role in the pathogenesis of the DiGeorge-like phenotype in *ET-1*-null mice.

• Mechanism of Action of dHAND

Dissection of the molecular pathways in which the *HAND* genes function has begun to clarify the developmental programs operative in distinct regions of the heart. The next challenge will be to determine the cell biology and mechanistic features of critical developmental steps during cardiac morphogenesis. Further analysis of the *dHAND*-null phenotype, has recently provided some insight into the cell biology of dHAND's developmental pathway. Careful tracing of cell fate indicates that dHAND is not required for specification of right ventricular cells nor is it required for differentiation of such cells (Thomas and Srivastava unpublished observation). Proliferation of cells is similarly unaffected. Instead, *dHAND*-null right ventricle cells undergo programmed cell death during expansion of the right ventricle (Thomas and Srivastava unpublished observation), similar to that seen in *dHAND*-null branchial arches (Thomas et al. 1998b). Whether dHAND regulates genes involved in an apoptotic pathway or functions by regulating survival factors independent of cell death pathways remains to be determined. Nevertheless, it appears that dHAND plays a fundamental role in regional organ growth rather than cell specification or differentiation. This is consistent with its expression in multiple embryonic lineages. Our most recent efforts have been directed at determining the downstream mediators of dHAND and have resulted in the cloning of numerous novel right ventricle- and branchial arch-specific genes that will provide another layer, and undoubtedly branches, in the dissection of segmental pathways of cardiogenesis.

• Clinical Implications of the HAND Pathways

Beyond understanding the basic biologic principles through which the HAND proteins and other members of their pathway act, the type of efforts described here should ultimately result in identification of genes that, when mutated or deleted, cause CHD. An example of this was recently seen where *Nkx2.5*, the homeobox gene upstream of eHAND, was found to be mutated in families with atrial septal defects and atrio-ventricular node defects (Schott et al. 1998). Based on our experiments in mice, it is likely that studies concerning the *HAND* genes will lead to an understanding of the pathogenesis of two important categories of human heart disease: those involving issues of ventricular growth and those involving the great vessels (pulmonary artery and aorta) arising from the heart.

Ventricular hypoplasia, left or right, is one of the more severe defects encountered in pediatric cardiology. In nearly all cases, a small remnant of the hypoplastic ventricular chamber is identifiable, suggesting that a population of cells had, at some point, been instructed to form the hypoplastic cardiac chamber (Figure 1C,D). The phenotype of *dHAND*-null hearts is remarkably similar to right ventricular hypoplasia seen in humans, suggesting that a single gene defect may be the cause of some ventricular hypoplasias, although many cases of ventricular hypoplasia are likely related to altered cardiac hemodynamics during embryogenesis. It is thus tempting to speculate that the developmental pathways regulated by dHAND and eHAND will lead to the underlying genetic defect in some humans with hypoplastic right and left ventricles. Indeed, we have identified a small group of unrelated children with CHD who have deletions or additions of the locus in which the human *dHAND* gene resides

(Srivastava, Strauss, and Watson unpublished observation).

The second large category of CHD in which the HAND pathway may be implicated involves development of the neural crest-derived vessels that arise from the heart. Such defects include persistent truncus arteriosus (failure of septation of truncus into pulmonary artery and aorta), interruption of the aortic arch such that the ascending aorta fails to communicate with the descending aorta, and a variety of defects where the pulmonary artery and aorta are not properly aligned with the right and left ventricles (Figure 1B). Neural crest defects of the heart can occur alone or in combination with defects of other cranial neural crest-derivatives, including the thymus, parathyroid gland, and craniofacial bones, all of which are derived from the branchial arches. Individuals with such a combination of cranial neural crest defects often have a microdeletion of one allele of chromosome 22q11 and have been referred to as having CATCH-22 (*c*ardiac defects, *a*bnormal facies, *t*hymic hypoplasia, *c*left palate, and *h*ypocalcemia associated with chromosome *22* microdeletion) syndrome (Driscoll 1993, Wilson 1993). This name encompasses the phenotype seen in DiGeorge and velo-cardio-facial syndromes.

We have previously postulated that the critical gene or genes on chromosome 22 will function in a molecular pathway involving dHAND or eHAND (Srivastava et al., 1995). Human *dHAND* and *eHAND* have been cloned and mapped, but neither resides on chromosome 22. However, recent efforts at identifying downstream target genes of *dHAND* have implicated a factor involved in degradation of ubiquitinated proteins, UFD1L (Yamagishi et al., 1999). *UFD1L* was previously cloned and mapped to 22q11 (Pizutti et al., 1997) along with nearly 30 other genes. In mice, *UFD1L* is expressed specifically in virtually all tissues affected in CATCH-22 (Yamagishi et al., 1999) and is part of a small 20 kb deletion in an individual who did not have a larger 2 Mb deletion but had all the phenotypic features of a 22q11 deletion. This suggests that a single gene can be responsible for most of the observed phenotype. The systematic analysis of a molecular pathway regulating neural crest cells utilizing techniques of molecular biology, developmental biology and human genetics led to identification of an important human disease gene. It is likely that this approach will be useful in identifying other genes responsible for human cardiac defects.

• Future Prospects

The studies reviewed here on the *HAND* genes exemplify the power of dissecting molecular pathways of organogenesis in the interest of basic science and human disease. However, many important questions remain in understanding the role of the HAND proteins in cardiogenesis and CHD. What are the target genes that lie downstream of these transcription factors and what are their mechanisms of action? What factors lie upstream of dHAND and eHAND and control the chamber-specific expression patterns observed? How do the pathways involving the HAND proteins interact, directly or indirectly, with pathways of other cardiac transcription factors including the MEF2 (Lin et al. 1997), GATA (Molkentin et al. 1997, Kuo et al. 1997), and Nkx families (Harvey 1996). Finally, will mutations in the *HAND* genes or other upstream or downstream factors be the cause of hypoplastic right and left heart syndromes or cardiac outflow tract and aortic arch defects? Further understanding of these and other factors will, it is hoped, provide a basis for improved genetic counseling and possible interventions in congenital heart disease.

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Conducting the Embryonic Heart: Orchestrating Development of Specialized Cardiac Tissues

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The heterogeneous tissues of the pacemaking and conduction system comprise the "smart components" of the heart, responsible for setting, maintaining, and coordinating the rhythmic pumping of cardiac muscle. Over the last few years, a wealth of new information has been collected about the unique genetic and phenotypic characteristics expressed by these tissues during cardiac morphogenesis. More recently, genetically modified viruses, mutational analysis, and targeted transgenesis have enabled even more precise resolution of the relationships between cell fate, gene expression, and differentiation of specialized function within developing myocardium. While some information provided by these newer approaches has supported conventional wisdom, some fresh and unexpected perspectives have also emerged. In particular, there is mounting evidence that extracardiac populations of cells migrating into the tubular heart have important morphogenetic roles in the inductive patterning and functional integration of the developing conduction system.
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