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# Cardiac phenotypes in chromosome 4q− syndrome with and without a deletion of the *dHAND* gene

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**Purpose:** Terminal deletions of chromosome 4q are commonly associated with cardiovascular malformations (CVMs). The *dHAND* gene (*HAND2,* heart and neural crest derivative express 2), a basic helix-loop-helix transcription factor expressed in the developing heart, maps to 4q33. A targeted deletion in mouse shows that *dHAND* plays an important role in heart development, suggesting that haploinsufficiency of *dHAND* in patients with 4q deletions may be causal for CVMs. The purpose of this study is to examine the possible association between *dHAND* haploinsufficiency with the CVMs that occur in patients with 4q terminal deletions. **Methods:** Fluorescence in situ hybridization (FISH) was performed with a human *dHAND* genomic probe on five patients with terminal deletion at 4q33 or 4q34. **Results:** Of the three patients with a deletion of the *dHAND* locus, two had CVM (both valvar pulmonic stenosis). Of the two patients without a deletion of the *dHAND* gene, one had a small atrial septal defect noted on autopsy. In one of the patients with breakpoint on chromosome 4 assigned to 4q34.2 by GTG-banding, FISH revealed deletion of the *dHAND* gene. **Conclusion:** The results suggest that cardiac phenotypes are very variable in patients with the terminal deletion of chromosome 4q and that haploinsufficiency of the *dHAND* is not necessarily associated with CVMs. The correct cytogenetic interpretation of terminal chromosome deletions might be augmented by FISH. ***Genet Med* 2002:4(6):464–467.**

***Key Words:*** *4q terminal deletions,* dHAND *gene, cardiovascular malformations*

Terminal deletions of the long arm of chromosome 4 are associated with a recognizable phenotype consisting of cleft palate, dysmorphic facial features (flat round face, micrognathia, short nose, apparent hypertelorism), upper extremity malformations, and growth and mental retardation. Over the past 30 years, 50 cases have been reported, 23 (41%) of which have had a cardiovascular malformation (CVM).[1–14] Compared with the 10% frequency in the Baltimore-Washington Infant Study, a case-control study of liveborn infants with a confirmed CVM in 1981–1989,[14] right ventricular outflow tract obstructive defects are more common (35%) in patients with 4q deletion.

Unlike interstitial deletions of 4q, which exhibit a more variable phenotype, terminal 4q deletions demonstrate a correlation between clinical phenotype severity and chromosomal breakpoint.[1,14,15] In particular, there appears to be a karyotype-phenotype correlation because CVM has been noted in almost two thirds of patients with the terminal deletions at 4q31, whereas only about a quarter of patients with the more distal deletions at 4q34 or q35 have CVM. It has been postulated that genes distal to 4q34 may play a critical role in producing a CVM,[14] though the molecular basis is unknown.

Recently, the *dHAND* gene, a basic helix-loop-helix transcription factor, was mapped to 4q33.[16] *dHAND* is expressed in the heart, aortic sac, and neural crest– derived tissue. A targeted deletion of *dHAND* in mouse embryos resulted in embryonic lethality due to right-sided heart failure and aortic sac malformation.[17,18] The expression pattern and phenotypes in mutant dHAND mice are consistent with the high frequency of CVMs in patients with terminal 4q deletions. The overrepresentation of right-sided CVMs is intriguing, which suggested that *dHAND* deficiency might cause the heart anomalies in 4q− syndrome and prompted us to investigate the correlation of *dHAND* deletion with CVMs in patients with terminal deletions involving 4q33-q34.

## MATERIALS AND METHODS

We studied five patients (three new, and two reported previously[14,19] with structural rearrangements of the long arm of

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chromosome 4 in which the breakpoint involved q33-q34) (Table 1). Routine cytogenetic analysis was performed on cultured lymphocytes treated with phytohemagglutinin as a mitogen. A BAC clone containing the whole human *dHAND* gene was isolated by standard procedures.[20] The presence of all exons of *dHAND* in the BAC clone was confirmed by polymerase chain reaction (PCR) amplification and sequencing. Briefly, exons 1 and 2 of *dHAND* were amplified by PCR. The following primer pairs were used:

Pair 1, forward (ex1F11): ACGCTGGGGCGCGTGGAG
Reverse (R10): GGCCAGCAGGTCCATGAGGTAGG

Pair 2, forward (F1): ACGTACCCGCCGACACCAAACTCT
Reverse (RP1): CCACCGCCTGCCGCCCCCTGGTA

Pair 3, forward (ex2F1): CCTCCCCGCCGGCTAGGGTAGC
Reverse (ex2R1): GCGCCTTGGCCCCTGCTCACTC

PCR products were purified with QIAquick columns (Qiagen, Inc., Valencia, CA) following the manufacturer's manuals. Each exon was sequenced by using an ABI 377 automated sequencer (Applied Biosystems) with primer RP1, ex2F1, and the following five primers:

FP1: TGAAGCGCCGAGGCACCGCCAACC
FP3: CGCGGAGGGCGAAATGAGTCTGGT
EX1R11: GCCGCTGGCATACTCGGGGCTGTA
R9: TGGCCAGGCGCAGGGTCTTGATTT
EX2F2: GGGGCTGGGACTCCCCCATCGTAT.

Sequence comparison from the PCR fragments with the DNAstar and BLAST program indicated that the sequence was identical with the human *dHAND* sequence in GenBank (accession number: NM_021973). The BAC DNA containing the entire *dHAND* coding sequence was labeled with digoxigenin-11-dUTP as described by Zhao et al.[21] Fluorescence in situ hybridization (FISH) was performed as described previously.[22] Digoxigenin labeled probe was detected by using the Oncor Kit (Oncor, Gaithersburg, MD) according to the manufacturer's instructions. A minimum of 10 metaphase cells were examined for each specimen.

## RESULTS

Metaphase karyotypes and FISH results are shown in Table 1. Patient 1, who inherited a der(4)t(4;13)(q33;q12) from her mother, was monosomic for 4q33→qter and monosomic for 13pter→q12. The remaining four patients were monosomic for a segment of chromosome 4 due to terminal deletion of 4q. FISH analysis revealed deletion of *dHAND* in Patients 1, 2, and 3 (Fig. 1). Valvar pulmonic stenosis was diagnosed in Patients 1 and 2. Patient 1 also had partial anomalous pulmonary venous return. No CVM was present in Patient 3. Although the breakpoint in Patient 2, described by Tsai el al,[14] was assigned to 4q34.2 by GTG-banding, FISH revealed deletion of *dHAND* gene indicating cryptic rearrangement of 4q in this patient including the *dHAND* locus at 4q33. Further FISH studies are warranted to better characterize this rearrangement.

*dHAND* was not deleted in Patients 4 and 5 (data not shown), who were a father and daughter, respectively, both of whom had an apparently similar deletion of 4q with a breakpoint assigned to 4q34.2 by GTG-banding, distal to the *dHAND* gene (Fig. 2). Patient 5 was referred for prenatal diagnosis because of a positive serum profile for Down syndrome and a family history of two previous abnormal pregnancies. The previous abnormalities included a fetus with a diaphragmatic hernia and a fetus with anencephaly. No cytogenetic studies were preformed on the first infant; the fetus with anencephaly had normal chromosomes. Analysis using standard cytogenetic methods revealed a small, apparently terminal deletion of chromosome 4 [46,XX,del(4)(q34.2)]. The pregnancy continued and was complicated by the development of intrauterine atrial flutter that was treated prenatally with procainamide and digoxin. At birth the female infant had no dysmorphic features. An echocardiogram revealed a patent ductus arteriosus with no other structural defects. The postnatal

**Table 1**
Summary of patients with terminal deletion

| Pt. | Sex | Age (yr) | Metaphase karyotype | *dHAND* Deletion | *dHAND* CVM[a] | Malformations Cleft | Malformations Digital |
|---|---|---|---|---|---|---|---|
| 1 | F | 5 | 45,XX,der (4)t(4;13)(q33;q12)mat | + | PSV, PAPVR | + | − |
| 2[b] | M | 6 | 46,XY,del (4)(q34.2) | + | PSV | + | + |
| 3[c] | F | 3 | 46,XX,del (4)(q33) | + | − | − | − |
| 4[d] | M | 30s | 46,XY,del (4)(q34.2) | − | − | − | − |
| 5[d] | F | ND[e] | 46,XX,del (4)(q34.2)pat | − | ASD | − | − |

[a] CVM, cardiovascular malformation; PSV, pulmonic stenosis valvar; PAPVR, partial anomalous venous return; ASD, atrial septal defect.
[b] Reported by Tsai et al.[14]
[c] Reported by Engelen et al.[19] and Evers et al.[6]
[d] Patients 4 and 5 are father and daughter, respectively.
[e] ND, neonatal death.

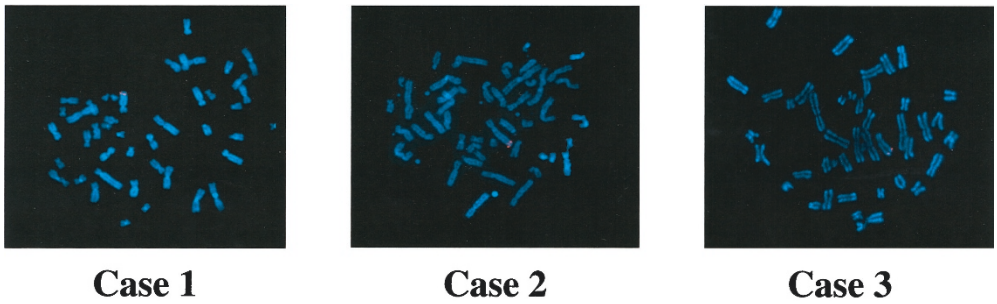


**Fig. 1** FISH analysis of Patients 1–3. BAC DNA containing the human *dHAND* gene was labeled with digoxigenin-11-dUTP.[21] Hybridization signals were detected with antidigoxingenin coupled with rhodamine supplied in the Oncor Kit according to the manufacturer's instructions. Hybridization signal specific for *dHAND* is present on only one chromosome, number 4 (10 metaphase cells were examined for each specimen).

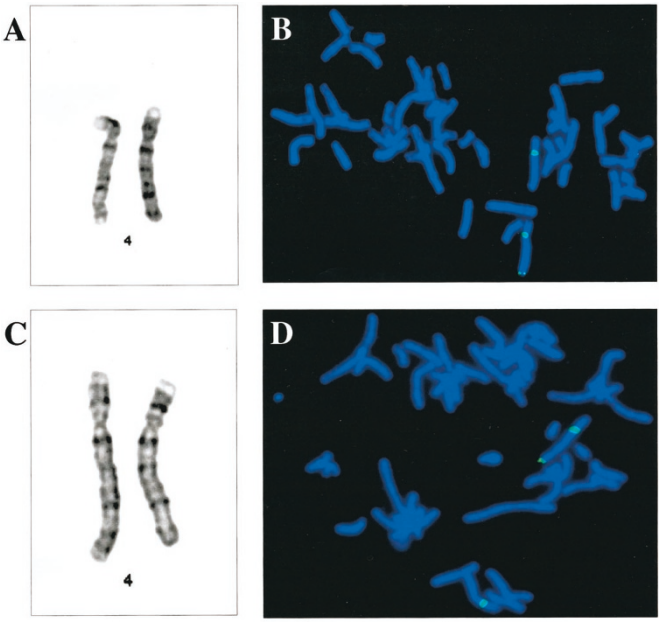


**Fig. 2** GTG-banded chromosome 4 homologs of Patients 4 and 5 are shown in A and C, respectively. B and D: FISH on metaphase chromosomes of Patients 4 and 5, respectively, with CEP4 (at centromere) and TelVysion 4q (at 4q35) probes (Vysis Inc., Downers Grove, IL) performed according to the manufacturer's recommendations. Hybridization signals specific to the CEP4 probe are present on both chromosome 4 homologs. Hybridization signal specific to the TelVysion 4q probe is present on only one chromosome, number 4 (10 metaphase cells were examined per specimen).

course was complicated by the persistent atrial flutter that required cardioversion twice. The infant required increasing respiratory support and she became asystolic and unresponsive to resuscitation. The immediate cause of death was cardiac tamponade due to pneumopericardium post mechanical ventilation. An autopsy detected a small atrial septal defect (1.0 × 0.5 cm) and no other unexpected findings.

The father is physically and intellectually normal and has had a normal echocardiogram. Surprisingly, his chromosome studies revealed that he carried a chromosome 4 that appeared identical with that found in his daughter. In search of a cryptic translocation, FISH was performed with whole chromosome painting probe specific for chromosome 4. Both chromosome 4 homologs painted along their entire length and no other region of hybridization were detected. The terminal deletion was further confirmed by FISH with a 4q telomeric probe (Fig. 2C).

## DISCUSSION

CVMs are very common in 4q− syndrome with a high frequency of right ventricular outflow tract obstruction defects. The *dHAND* gene is highly expressed in the right side of a developing heart,[18] suggesting that deletions of *dHAND* gene might cause CVM in this syndrome. To examine the association of *dHAND* haploinsufficiency with congenital heart malformation in 4q− syndrome, we performed FISH with a human *dHAND* genomic probe on five patients with partial monosomy 4q with or without a CVM. Deletion of the *dHAND* gene was revealed by FISH in three patients. Among these three patients, two (Patients 1 and 2) have a CVM and one (Patient 3) has no CVM as reported previously.[6,22,23] Of the two patients (Patients 4 and 5) without deletion of the *dHAND* gene, one (Patient 5) had an atrial septal defect found at autopsy. These results indicate that *dHAND* is not the only factor causing congenital cardiac defects and that other genes distant to the *dHAND* gene are responsible for CVM, or factor(s) other than *dHAND* gene deletion might contribute to the congenital cardiac defects.

*dHAND* null-mutant mice show heart failure, absence of right-sided heart development, dilated aortic sac, and failure to form normal aortic arteries,[17,18,23] suggesting that *dHAND* is required for formation of the right ventricle of the heart. However, no CVMs were found in mice carrying a hemizygous mutation, indicating that haploinsufficiency of *dHAND* is not necessarily a cause for CVMs in 4q− syndrome.

Our data cannot exclude the possibility of an incomplete penetrance in *dHAND* deletion in CVM and the genetic background in this condition. Searching for the mutation of the *dHAND* gene in the right ventricular outflow defect will provide direct evidence. Thus far, we have found no mutation of the *dHAND* gene in patients with nonsyndromic right ventricular outflow trunk defects.

Another interesting finding in this study is that Patient 4, with a terminal deletion of 4q by karyotype analysis, confirmed

by FISH using telomeric probe, is physically and intellectually normal and has had a normal echocardiogram. He did not receive medical attention until his daughter was found to have an abnormal karyotype. Cryptic translocation was ruled out by FISH with whole chromosome painting probe specific for chromosome 4. Both chromosome 4 homologs painted along their entire length and no other region of hybridization was detected.

Finally, the breakpoint in Patient 2 was assigned by GTG-banding to 4q34.2, distal to the *dHAND* locus; however, FISH revealed deletion of the *dHAND* gene, indicating a more complex rearrangement of 4q in this patient. Therefore, FISH proves to be an invaluable technique for elucidating cryptic or complex chromosome rearrangements.

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