

## Brief Clinical Report

# Ventricular Noncompaction and Distal Chromosome 5q Deletion

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We describe a 7 1/2-year-old girl with mildly unusual phenotype and complex heart disease including ventricular myocardial noncompaction. She was found to have a distal 5q deletion, del(5)(q35.1q35.3). Fluorescent in situ hybridization showed that this deletion included the locus for the cardiac specific homeobox gene, *CSX*. This suggests that some instances of ventricular myocardial noncompaction may be caused by haploinsufficiency of *CSX*. *Am. J. Med. Genet.* 85:419–423, 1999. © 1999 Wiley-Liss, Inc.

**KEY WORDS:** cardiac defects; cardiomyopathy; chromosome deletion; *CSX*; cytogenetic abnormality; fluorescent in situ hybridization

## INTRODUCTION

On occasion, identification of a chromosomal aberration in an individual with malformations can lead to testable hypotheses regarding single gene causes of isolated birth defects. We recently evaluated a girl with ventricular myocardial noncompaction (VMN) [Chin et al., 1990] as part of an apparently unique syndrome. She was found to have a chromosome 5q deletion. The clinical and cytogenetic findings in this child are described. Through fluorescent in situ hybridization (FISH) we demonstrated deletion of *CSX*, a heart-specific homeobox gene located on distal chromosome 5q [Turbay et al., 1996]. We postulate that the proposita's VMN may be due to haploinsufficiency of the *CSX* gene.

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## MATERIALS AND METHODS

High-resolution cytogenetic evaluation was carried out using standard methods.

The FISH study was carried out as follows. A genomic P1 clone of the human *CSX* gene was digested using HindIII and the 9.5-kb fragment was subcloned into the HindIII site of pBluescript SK (-). The plasmid was amplified in an *E. coli* XLI Blue MRF' strain (Stratagene, La Jolla, CA) and extracted using a QIAGEN (Valencia, CA) midi-prep column. Fluorescein-12-dUTP (NEN, Boston, MA) (1 nmol) was incorporated into 1 µg of probe DNA via nick translation. Five hundred nanograms of labelled DNA was ethanol precipitated along with 10 µg of human Cot-1 DNA (Gibco BRL, Grand Island, NY). The pellet was resuspended in a hybridization mixture of 2× SSC, 50% formamide, and 10% dextran sulfate. Chromosome spreads from lymphocytes were made. The slides were then treated with 2× SSC for 30 min at 37°C. The slides were dehydrated in a series of cold ethanol washes (70, 80, 95% ethanol sequentially) for 2 min each. Slides were denatured in 70% formamide/2× SSC at 70°C for 2 min then again dehydrated in 2-min cold ethanol washes (70, 80, 95 ethanol sequentially). Nine microliters of labelled *CSX* probe DNA was mixed with 4 µl of 5p15.2 cri-du-chat probe (Oncor, Gaithersburg, MD) which was used as an internal control. The probe mix was hybridized and washed as previously described [Wechsler et al., 1994]. The slides were detected with rhodamine-labelled anti-digoxigenin (Oncor) and counterstained with 17 µl DAPI (Gibco).

## RESULTS

### Clinical Report

The proposita was born at term by repeat cesarean section to nonconsanguineous, healthy parents. She had a birth weight of 2,920 g and a length of 48.3 cm. Apgar scores were reported as 9 at both 1 and 5 min. Neonatal problems included severe feeding difficulties secondary to abnormal oromotor function, and gastroesophageal reflux. She had an atrial septal defect and patent ductus arteriosus (both repaired in infancy). Infant photographs suggest facial hirsutism, marked synophrys, and downslanting palpebral fissures. Cytogenetic assessment at that time identified no abnormal-

ity. In infancy and early childhood she showed failure to thrive, slowing of linear growth, and moderate developmental delays. On the basis of her facial appearance, slow growth, and global developmental retardation, a diagnosis of deLange syndrome was suggested.

She was evaluated by us at age 7 1/2 years. Many of her unusual external findings had normalized. Morphologic abnormalities included bushy brows, mild synophrys, irregular lashes, and minimally downslanting of the palpebral fissures (Fig. 1). Growth, too, had normalized, with a height of 117 cm (25th centile). Habitus was gracile and she had generalized, asymptomatic joint hypermobility. The early and quite severe developmental delays have remitted and she has near-normal cognitive function.

Hospital admission was precipitated by refractory respiratory symptoms. Cardiac evaluation showed dilated cardiomyopathy, second-degree heart block, and sick sinus syndrome. A pacemaker was placed and cardiorespiratory function and symptoms improved. However, followup echocardiography showed characteristic changes of left VMN (Fig. 2).

#### Cytogenetic Assessment

We elected to repeat chromosomal evaluation. High-resolution G-banded studies showed a small, distal, probably interstitial deletion of chromosome arm 5q. Breakpoints were thought most likely to be at q35.1 and q35.3 (Fig. 3). Maternal chromosomes were apparently normal; the father was unavailable for testing.

#### Fluorescent in Situ Hybridization

The *CSX* (cardiac specific homeobox) gene maps to the juncture of bands 5q34 and 5q35.1 [Shiojima et al., 1995; Turbay et al., 1996], near the proximal breakpoint of the deletion in the proposita. Results of FISH using the P1 plasmid containing a *CSX* genomic clone are shown in Figures 4 and 5. All cells of the proposita showed absence of the *CSX* specific signal from one of the two 5 chromosomes, confirming that *CSX* is deleted.

#### DISCUSSION Distal 5q Deletions

Very few previous instances of distal 5q deletion have been reported, and in none have the putative breakpoints been identical to those identified here (Fig. 6).

Joseph et al. [1990] described a girl with a wide nasal base, hypertelorism, micrognathia, low-set ears, Dandy-Walker malformation and polymicrogyria, and complex congenital heart disease. The cardiac anomalies included an atrial septal defect, a ventricular septal defect, and a left superior vena cava. Following ligation of a patent ductus arteriosus she remained ventilator dependent, developed progressive cardiac wall thickening, and died at age 19 days of heart failure [Joseph et al., 1990] (P. Joseph, personal communication, 1998). Autopsy confirmed the presence of

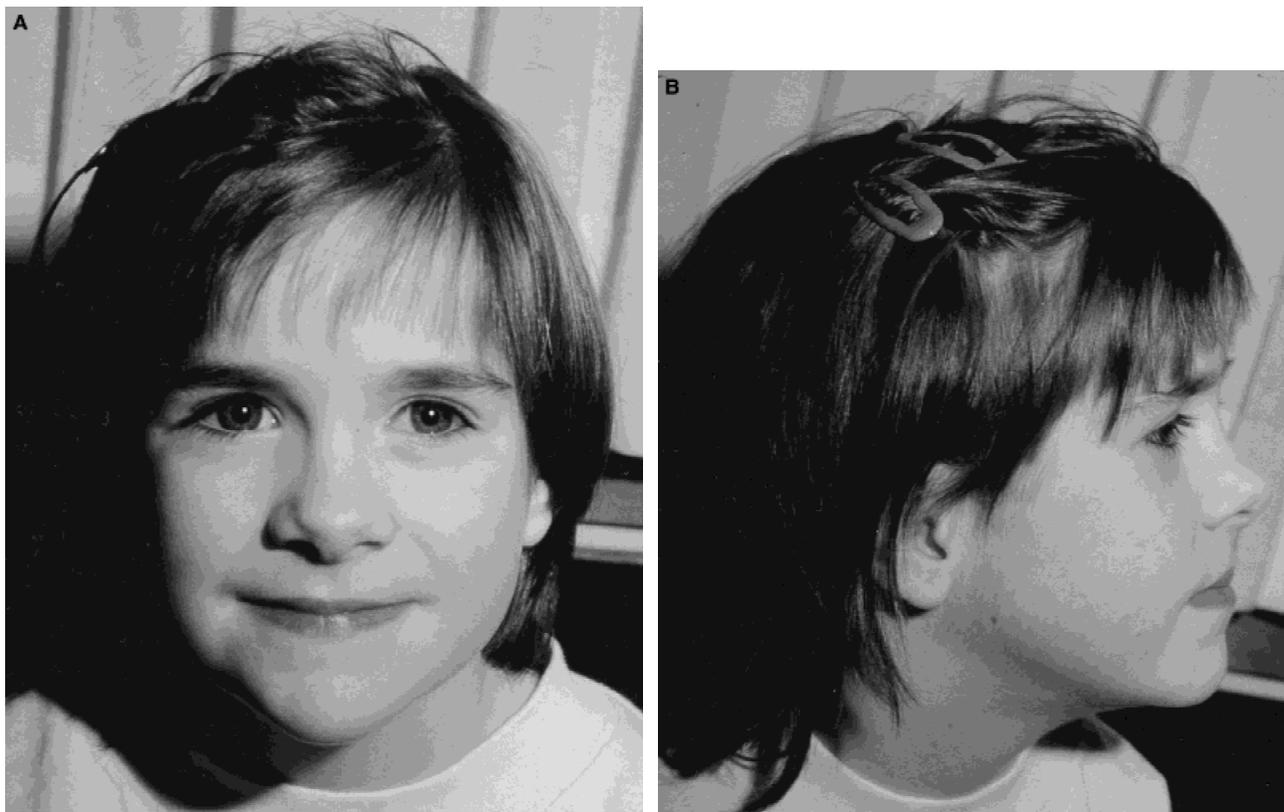


Fig. 1. A,B: Facial photographs of the proposita at 7 1/2 years of age, showing subtle minor anomalies.

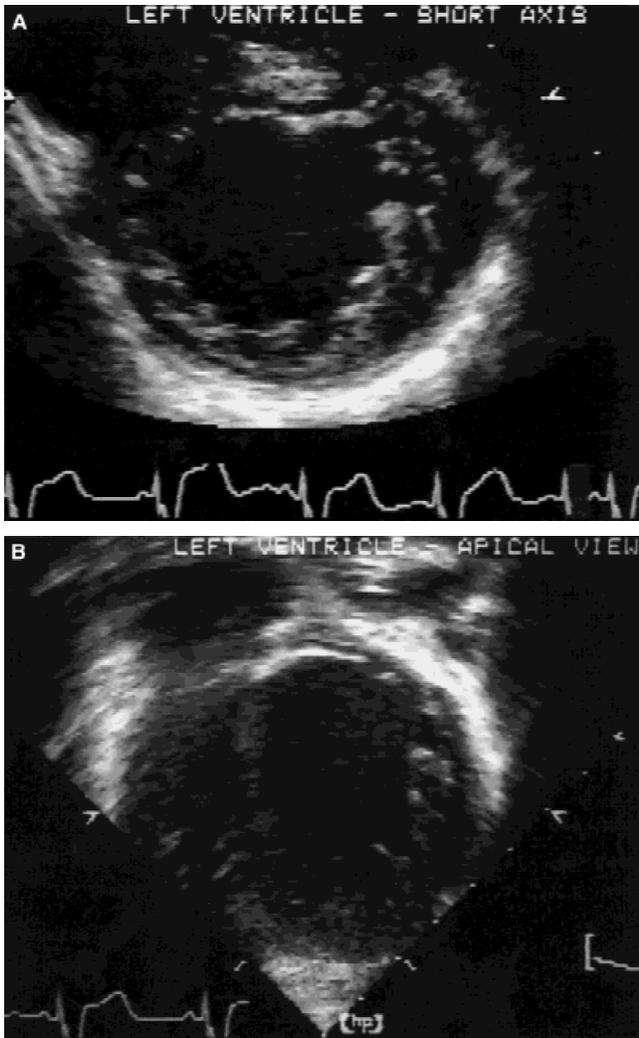


Fig. 2. Echocardiography of the proposita. **A:** Left ventricle in short axis. Note the prominent, deep trabeculations and primitive appearance of the myocardium. **B:** Left ventricle in an apical, four-chamber view, again showing deep trabeculations and a primitive appearance of the myocardium.

hypertrophic cardiomyopathy [Joseph et al., 1990]; no comment was made about trabecular characteristics of the ventricles (P. Joseph, personal communication, 1998). She was found to have a de novo 5q deletion, apparently encompassing the entire q35 band [46,XX,del(5)(q35qter)].

Kleczkowska et al. [1993] published a description of a 9-month-old girl with macrocephaly, broad nasal bridge, anteverted nares, epicanthic folds, downslanting fissures, seventh nerve paresis, micrognathia, bifid uvula, nuchal redundancy, short fingers, syndactyly, and clenched fingers, and marked delay, hypertonicity, and seizures. Neuroimaging showed communicating hydrocephalus. Except for a patent ductus arteriosus, no cardiac anomaly was noted. Chromosome analysis showed a de novo 5q deletion, apparently 46,XX,del(5)(q35.1qter).

A 15-month-old boy described by Stratton et al. [1994] was identified prenatally as having a small 5q deletion. Postnatally he had macrocephaly, flat nasal bridge, telecanthus, epicanthic folds, anteverted nares, retrognathia, nuchal redundancy, short fingers, hypotonicity, mild developmental delay, and an atrial septal defect. To the time of the report, the primary health issue was recurrent respiratory problems. Efforts to obtain followup information, particularly regarding cardiologic status, were unsuccessful (R. Stratton, personal communication, 1998; L.D. Immken, personal communication, 1998). Cytogenetic studies demonstrated a de novo 5q deletion, apparently 46,XY,del5(q35.3qter) [Stratton et al., 1994].

One of us (R.M.P.) has recently evaluated a girl with macrocephaly, hypertelorism, asymmetric epicanthic folds, small nose with anteverted nares, downturned corners of the mouth, microretrognathia, pectus carinatum, single transverse crease of the left hand, short fingers, and abnormally small third fingernails. No structural cardiac anomaly was present. In addition, this patient experienced catastrophic intracerebral hemorrhages of unknown cause and died of progressive respiratory failure at age 29 days. Postmortem evaluation also demonstrated absence of the olfactory bulbs. Cytogenetic evaluation showed del5(q35.1qter).

In spite of having overlapping deletions, these five cases share only a few of the clinical characteristics identified. No clear, definable phenotype is evident. However, note that, in addition to the proposita reported here, two others had atrial septal defects and one other had a cardiomyopathy.

### Ventricular Myocardial Noncompaction

Ventricular myocardial noncompaction is a rare cardiac abnormality [Chin et al., 1990]. It may arise secondary to outflow obstruction or may be an isolated and presumably primary anomaly [Chin et al., 1990; Ritter

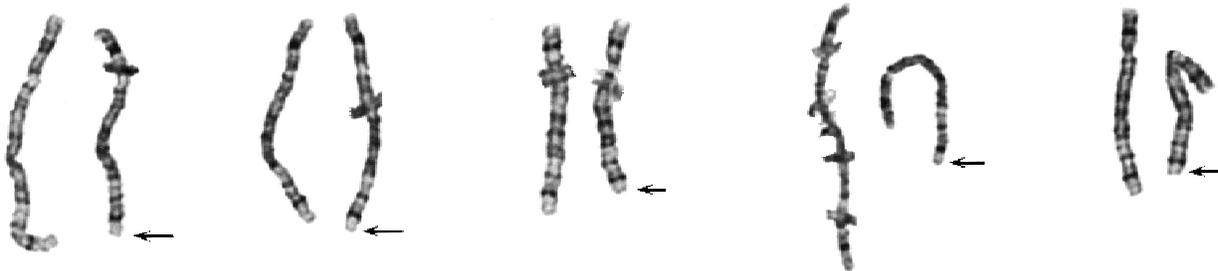


Fig. 3. Partial karyotypes of the chromosome 5 pairs from the proposita. In each instance the arrow indicates the deleted segment.



Fig. 4. Fluorescent in situ hybridization of a metaphase from the proposita. The Oncor 5p15.2 probe (internal control) is labelled with rhodamine and fluoresces red whereas the human CSX probe is labelled with FITC and fluoresces green.

et al., 1997]. Given that our proposita, in addition to the VMN, only had an atrial septal defect repaired in infancy and no evidence for left-sided outflow obstruction, her left VMN can be assumed to be of the primary type.

Primary VMN is thought to arise from an arrest of normal myocardial development. In the embryo a trabecular network fills much of the ventricles but normally becomes compacted with disappearance of large and deep intertrabecular spaces. With persistence of the embryonic trabecular myocardium, ventricular function is compromised. Clinical presentation may include left ventricular failure, arrhythmias, cardiomyopathy and/or embolization [Chin et al., 1990; Ritter et al., 1997].

Most isolated VMN arises in eumorphic individuals. However, it has been reported in one patient with Mel-

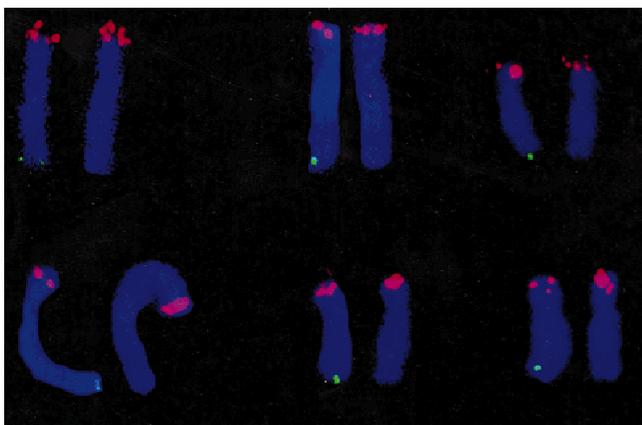


Fig. 5. Chromosome 5 pairs labelled as described in Figure 4. The deleted chromosomes 5 are situated on the right of each pair.

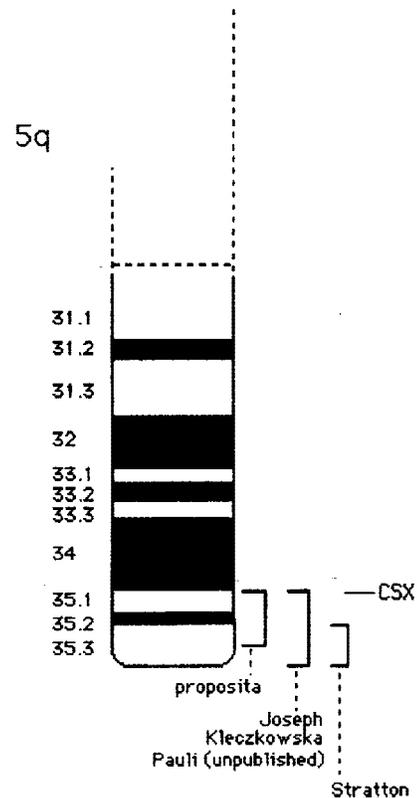


Fig. 6. Diagrammatic representation showing the putative deletion span for the five individuals with distal 5q deletions and the location of CSX. Names refer to the first author of the relevant publications.

nick-Needles syndrome [Wong and Bofinger, 1997] and in three other children with minor anomalies [Chin et al., 1990] in whom, unfortunately, chromosome evaluation was not completed (J.K. Perloff, personal communication, 1998).

Two patterns of familial recurrence have been recognized. Some families show apparent X-linked recessive inheritance [Chin et al., 1990; Bleyl et al., 1997a, 1997b] (F. Ichida, personal communication, 1998). Some of these (but not all; F. Ichida, personal communication, 1998) arise because of mutation of the G4.5 gene at Xq28, mutations of which can also cause Barth syndrome [Bleyl et al., 1997a, 1997b]. Others appear to show dominant transmission [Ritter et al., 1997] (F. Ichida, personal communication, 1998). A Japanese nationwide survey suggests that familiarity of VMN may be far more frequent than is generally recognized (F. Ichida, personal communication, 1998).

In addition to the X-linked G4.5 gene, at least one other candidate locus has been identified for VMN. Mutation in the FKBP12 gene in mice results in ventricular septal defects, dilated cardiomyopathy, and VMN [Shou et al., 1998]. The human homologue is on chromosome 20 [Shou et al., 1998].

Thus, the only two suggested loci for human VMN can not account for its appearance in a girl with a 5q deletion. Nonetheless, this co-occurrence of a very rare cardiac abnormality and an even rarer cytogenetic anomaly begs for an explanation.

## CSX

The *CSX* (cardiac-specific homeobox; also called *NKX2-5*) gene maps to very near 5q35.1 [Shiojima et al., 1995; Turbay et al., 1996]. FISH studies confirm that the *CSX* locus is deleted in the proposita.

*CSX* and its homologues (*tinman* in *Drosophila*, *Csx*/*Nkx2.5* in the mouse, and similar genes in all other investigated organisms with hearts) are homeodomain-containing transcription factors with highly restricted expression, limited for the most part to the heart [Komuro and Izumo, 1993; Bodmer and Venkatesh, 1998; Tanaka et al., 1998]. Absence of *tinman* expression in *Drosophila* causes failure of heart formation, while mouse *Csx* knockouts result in somewhat later arrest in cardiac development [Bodmer and Venkatesh, 1998; Tanaka et al., 1998].

Recently, heterozygous mutations in *CSX* in humans have been shown to account for some autosomal dominant occurrences of atrial septal defect plus atrioventricular conduction defect [Schott et al., 1998]. Thus, haploinsufficiency of *CSX* can cause cardiac anomalies in humans. Unexplained is the remarkably milder phenotype of the human mutations compared with those in other species [Schott et al., 1998].

The proposita described here had both an atrial septal defect and a cardiac conduction defect but, in addition, had VMN. This suggests that haploinsufficiency of *CSX* may cause some forms of VMN as well as the atrial septal defect plus atrioventricular conduction defect syndrome. This seems biologically plausible as well, since VMN is generally viewed as an arrest in the normal embryologic progression of heart morphogenesis. Deletions incorporating *CSX* or mutations in *CSX* per se should be sought in individuals with sporadic or non-X-linked familial VMN.

## REFERENCES

- Bleyl SB, Mumford BR, Brown-Harrison M-K, Pagotto LT, Carey JC, Pyscher TJ, Ward K, Chin TK. 1997a. Xq28-linked noncompaction of the left ventricular myocardium: prenatal diagnosis and pathologic analysis of affected individuals. *Am J Med Genet* 72:257-265.
- Bleyl SB, Mumford BR, Thompson V, Carey JC, Pyscher TJ, Chin TK, Ward K. 1997b. Neonatal, lethal noncompaction of the left ventricular myocardium is allelic with Barth syndrome. *Am J Hum Genet* 61:868-872.
- Bodmer R, Venkatesh TV. 1998. Heart development in *Drosophila* and vertebrates: conservation of molecular mechanisms. *Dev Genet* 22:181-186.
- Chin TK, Perloff JK, Williams RG, Jue K, Mohrmann R. 1990. Isolated noncompaction of left ventricular myocardium. A study of eight cases. *Circulation* 82:507-513.
- Joseph P, Kimm J, Kalyan-Raman UP, Nixon JP, Hiller J. 1990. Terminal deletion of the long arm of chromosome 5. *Am J Hum Genet* 47:A31.
- Kleczkowska A, Fryns JP, Van Den Berghe H. 1993. A distinct multiple congenital anomalies syndrome associated with distal 5q deletion (q35.1qter). *Ann Genet* 36:126-128.
- Komuro I, Izumo S. 1993. *CSX*: A murine homeobox-containing gene specifically expressed in the developing heart. *Proc Natl Acad Sci USA* 90:8145-8149.
- Ritter M, Oechslin E, Sütsch G, Attenhofer C, Schneider J, Jenni R. 1997. Isolated noncompaction of the myocardium in adults. *Mayo Clin Proc* 72:26-31.
- Schott J-J, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP, Maron BJ, Seidman CE, Seidman JG. 1998. Congenital heart disease caused by mutations in the transcription factor *NKX2-5*. *Science* 281:108-111.
- Shiojima I, Komuro I, Inazawa J, Nakahori Y, Matsushita I, Abe T, Nagai R, Yazaki Y. 1995. Assignment of cardiac homeobox gene *CSX* to human chromosome 5q34. *Genomics* 27:204-206.
- Shou W, Aghdasi B, Armstrong DL, Guo Q, Bao S, Charng M-J, Mathews LM, Schneider MD, Hamilton SL, Matzuk MM. 1998. Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. *Nature* 391:489-492.
- Stratton RF, Tedrowe NA, Tolworthy JA, Patterson RM, Ryan SG, Young RS. 1994. Deletion 5q35.3. *Am J Med Genet* 51:150-152.
- Tanaka M, Kasahara H, Bartunkova S, Schinke M, Komuro I, Inagaki H, Lee Y, Lyons GE, Izumo S. 1998. Vertebrate homologs of *tinman* and *bagpipe*: roles of the homeobox genes in cardiovascular development. *Dev Genet* 22:239-249.
- Turbay D, Burns Wechsler S, McQuate Blanchard K, Izumo S. 1996. Molecular cloning, chromosomal mapping, and characterization of the human cardiac-specific homeobox gene *hCSX*. *Mol Med* 2:86-96.
- Wechsler DS, Hawskins AL, Li X, Jabs EW, Griffin CA, Dang CV. 1994. Localization of the human *Mxi1* transcription factor gene (*MXI1*) to chromosome 10q24-q25. *Genomics* 21:669-672.
- Wong JA, Bofinger MK. 1997. Noncompaction of the ventricular myocardium in Melnick-Needles syndrome. *Am J Med Genet* 71:72-75.