

## ORIGINAL ARTICLE

# Mutation of Perinatal Myosin Heavy Chain Associated with a Carney Complex Variant

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## ABSTRACT

**BACKGROUND**

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Familial cardiac myxomas occur in the hereditary syndrome Carney complex. Although *PRKARIA* mutations can cause the Carney complex, the disorder is genetically heterogeneous. To identify the cause of a Carney complex variant associated with distal arthrogryposis (the trismus–pseudocamptodactyly syndrome), we performed clinical and genetic studies.

**METHODS**

A large family with familial cardiac myxomas and the trismus–pseudocamptodactyly syndrome (Family 1) was identified and clinically evaluated along with two families with trismus and pseudocamptodactyly. Genetic linkage analyses were performed with the use of microsatellite polymorphisms to determine a locus for this Carney complex variant. Positional cloning and mutational analyses of candidate genes were performed to identify the genetic cause of disease in the family with the Carney complex as well as in the families with the trismus–pseudocamptodactyly syndrome.

**RESULTS**

Clinical evaluations demonstrated that the Carney complex cosegregated with the trismus–pseudocamptodactyly syndrome in Family 1, and genetic analyses demonstrated linkage of the disease to chromosome 17p12–p13.1 (maximum multipoint lod score, 4.39). Sequence analysis revealed a missense mutation (Arg674Gln) in the perinatal myosin heavy-chain gene (*MYH8*). The same mutation was also found in the two families with the trismus–pseudocamptodactyly syndrome. Arg674 is highly conserved evolutionarily, localizes to the actin-binding domain of the perinatal myosin head, and is close to the ATP-binding site. We identified nonsynonymous *MYH8* polymorphisms in patients with cardiac myxoma syndromes but without arthrogryposis.

**CONCLUSIONS**

We describe a novel heart–hand syndrome involving familial cardiac myxomas and distal arthrogryposis and demonstrate that these disorders are caused by a founder mutation in the *MYH8* gene. Our findings demonstrate novel roles for perinatal myosin in both the development of skeletal muscle and cardiac tumorigenesis.

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**C**ARDIAC MYXOMAS ARE THE MOST COMMON primary cardiac tumors in adults; approximately 7 percent are components of the familial autosomal dominant multiple neoplasia syndrome called Carney complex (Online Mendelian Inheritance in Man [OMIM] number 160980).<sup>1-4</sup> Familial cardiac myxomas are associated with spotty pigmentation of the skin and other phenotypes, including primary pigmented nodular adrenocortical dysplasia, extracardiac (frequently cutaneous) myxomas, schwannomas, and pituitary, thyroid, testicular, bone, ovarian, and breast tumors. Cardiac myxomas do not develop in all patients with the Carney complex, but affected patients have at least two features of the complex or one feature and a clinically significant family history.<sup>5-7</sup> We<sup>7,8</sup> and others<sup>9</sup> have demonstrated that a mutation of the *PRKARIA* gene encoding the R1 $\alpha$  regulatory subunit of protein kinase A (PKA) causes the Carney complex. Another locus was hypothesized to be at chromosome 2 in band p16, but no gene was identified.<sup>6</sup>

We now describe a large kindred with the Carney complex that is linked neither to a mutation in *PRKARIA* nor to 2p16. Affected family members have typical manifestations of the Carney complex<sup>10</sup> as well as the trismus–pseudocamptodactyly syndrome.<sup>11</sup> The trismus–pseudocamptodactyly syndrome (also termed Hecht–Beals or Dutch–Kentucky syndrome; OMIM number 158300) is a hereditary distal arthrogryposis characterized by an inability to open the mouth fully (trismus) and pseudocamptodactyly in which wrist dorsiflexion, but not volarflexion, produces involuntary flexion contracture of distal and proximal interphalangeal joints. Such hand and jaw contractures are caused by shortened flexor muscle-tendon units; similar lower-limb contractures also produce foot deformity.<sup>12-14</sup> The trismus–pseudocamptodactyly syndrome is a morbid autosomal dominant trait with variable expressivity but high penetrance.<sup>2,13-17</sup> In these patients, trismus complicates dental care, feeding during infancy, and intubation for anesthesia, and the pseudocamptodactyly impairs manual dexterity, with consequent occupational and social disability. Many patients require surgical correction of contractures.

Recently, mutations in contractile proteins were shown to cause other syndromes of distal arthrogryposis: mutations in troponins I and T were found in type 2B,<sup>18,19</sup> and a mutation of  $\beta$ -tropomyosin was found in type 1.<sup>18</sup> A mutation in myosin IIa

causes inclusion-body myopathy, sometimes associated with joint contractures.<sup>20</sup> We sought to identify the trismus–pseudocamptodactyly syndrome associated with cardiac myxomas as a variant form of the Carney complex (CNC-TPC) in order to use genetic techniques to define the molecular causes of both cardiac tumorigenesis and skeletal deformity.

## METHODS

### CLINICAL EVALUATION

A large family with familial cardiac myxomas and the trismus–pseudocamptodactyly syndrome (Family YK, hereafter referred to as Family 1 in this article) was identified and clinically evaluated along with two families with trismus and pseudocamptodactyly (Families MN and MBB, hereafter referred to as Families 2 and 3 in this article). Written informed consent was obtained from all participants according to the institutional review board of Weill Medical College. Participants were evaluated on the basis of one or more of the following: history, physical examinations, and transthoracic echocardiography; all evaluations were performed without knowledge of the participant's genotype. Samples of peripheral blood, saliva, or both were obtained.

### GENETIC ANALYSES

Peripheral lymphocytes were isolated, and lymphoblastoid lines were established by means of Epstein–Barr virus transformation.<sup>7,8</sup> Genomic DNA was isolated from lymphoblasts (QIAamp, Qiagen) and saliva (Buccal Cell Kit, Gentra). Initial genotyping was performed with the use of microsatellites (ABI Prism Linkage Mapping Set version 2, Applied Biosystems) on an automated sequencer (ABI 377) and analyzed with the use of GeneScan version 3.0 and Genotyper version 2.0 software. Other genotyping was performed with the use of phosphorus-32–labeled microsatellites<sup>7</sup> ascertained from Decode<sup>21</sup> and the Human Genome Project.<sup>22</sup> Linkage 5.1 software was used, with a penetrance of 0.90, a disease allele frequency of 0.001, and microsatellite allele frequencies specified in the Human Genome Project databases.<sup>22</sup>

### MUTATIONAL ANALYSES

Candidate-gene exons from the myosin heavy-chain genes (*MYH1*, *MYH2*, *MYH4*, and *MYH8*) were amplified by means of the polymerase chain reaction (PCR) with the use of flanking intronic primers

listed in Table 1 of the Supplementary Appendix (available with the full text of this article at [www.nejm.org](http://www.nejm.org)). Amplicons were subjected to bidirectional sequencing on an automated sequencer (ABI 3100) with the use of BigDye terminators (Applied Biosystems). *MYH8* internal-sequence primers were also used (listed in Table 2 of the Supplementary Appendix). The complete genomic segment of exons 4 through 25 of *MYH8* was amplified by PCR and sequenced to identify single-nucleotide polymorphisms. Such polymorphisms were subsequently genotyped by means of sequencing in all three families to establish *MYH8* haplotypes. *PRKARIA* was analyzed by denaturing high-performance liquid chromatography and sequenced as described previously.<sup>4</sup> Crystallography data for the head of the myosin heavy chain from chicken skeletal muscle (2MYS) were obtained from the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and modeled with the use of Cn3D version 4.1 and RasMol version 2.6 software.

## RESULTS

### IDENTIFICATION OF A CARNEY COMPLEX VARIANT

Family 1 is a large northern European (Belgian) white kindred<sup>10,11</sup> with 18 living members affected by CNC-TPC (Fig. 1). Penetrance of the disorder is complete, although expressivity is highly variable. Three affected family members (I-1, II-7, and III-19) have had cardiac myxomas, and all affected family members who were available for a complete examination had spotty skin pigmentation alone or in combination with cutaneous lesions. All affected family members except III-6 (who had findings on physical examination suggestive of acromegaly as well as spotty pigmentation and an eyelid tumor consistent with the presence of myxoma) and III-10 (who had facial lentigines) had evidence of distal arthrogryposis, including pseudocamptodactyly of the hands and feet, or trismus (or both) that improved symptomatically with aging. Two family members (III-2 and III-4) had required palliative hand surgery, and one (III-19) foot surgery.

### GENETIC HETEROGENEITY OF THE CARNEY COMPLEX

To determine the cause of CNC-TPC, we performed genetic linkage analyses in Family 1. Linkage to *PRKARIA* was excluded; lod scores less than  $-2.0$  were obtained with the use of chromosome 17q24

microsatellites, including an intragenic *PRKARIA* microsatellite<sup>9</sup> (lod score =  $-10.6$ ,  $\theta=0$ ). Sequence and denaturing high-performance liquid chromatographic analysis of the *PRKARIA* gene in Family 1 demonstrated no variations. Analyses involving chromosome 2p16 microsatellites (D2S337: lod score =  $-6.5$ ,  $\theta=0$ ; D2S391: lod score =  $-8.0$ ,  $\theta=0$ ) also excluded linkage. Lymphoblasts derived from one family member (I-1) had a normal 46,XY karyotype. Thus, we concluded that the Carney complex is genetically heterogeneous and that a mutated gene at a novel locus causes CNC-TPC in Family 1.

### GENETIC LINKAGE ANALYSIS OF CNC-TPC

We then performed a random genome search for the CNC-TPC disease gene in Family 1. After excluding 55 percent of the genome, we observed linkage to chromosome 17p12–13.1 with a maximum pairwise lod score of 4.14 (at the D17S1852 locus). Multipoint analysis yielded a peak lod score of 4.39, equivalent to odds of 24,500:1 that the CNC-TPC disease gene in Family 1 maps to this locus (Fig. 2). Haplotype analyses (not shown) suggested that the CNC-TPC disease gene localized to 4.5 Mb between microsatellites D17S1812 and D17S947.

### CANDIDATE-GENE ANALYSIS AND IDENTIFICATION OF AN *MYH8* MUTATION

Inspection of the CNC-TPC locus revealed a cluster of myosin heavy-chain genes, including extraocular, embryonic, perinatal, and three adult skeletal myosin heavy-chain genes.<sup>22–24</sup> Mutations in other sarcomeric genes produce arthrogryposis.<sup>18–20</sup> Therefore, with the exception of extraocular myosin, we considered these myosin genes ideal candidates for the CNC-TPC disease gene, and we sequenced their coding regions. No mutations were observed in embryonic or adult skeletal myosin genes.

In exon 16 of the *MYH8* gene encoding perinatal myosin heavy chain, bidirectional sequencing demonstrated a transition from G to A at nucleotide 2094 (G2094A) (Fig. 3A) that predicted an arginine to glutamine missense mutation at amino acid 674 (R674Q). The G2094A substitution was confirmed by typing a *TspRI* restriction-fragment-length polymorphism introduced by the mutation (Fig. 3B) and was shown to be present in all affected members of Family 1 and none of the unaffected members. G2094A was not present in 200 chromo-

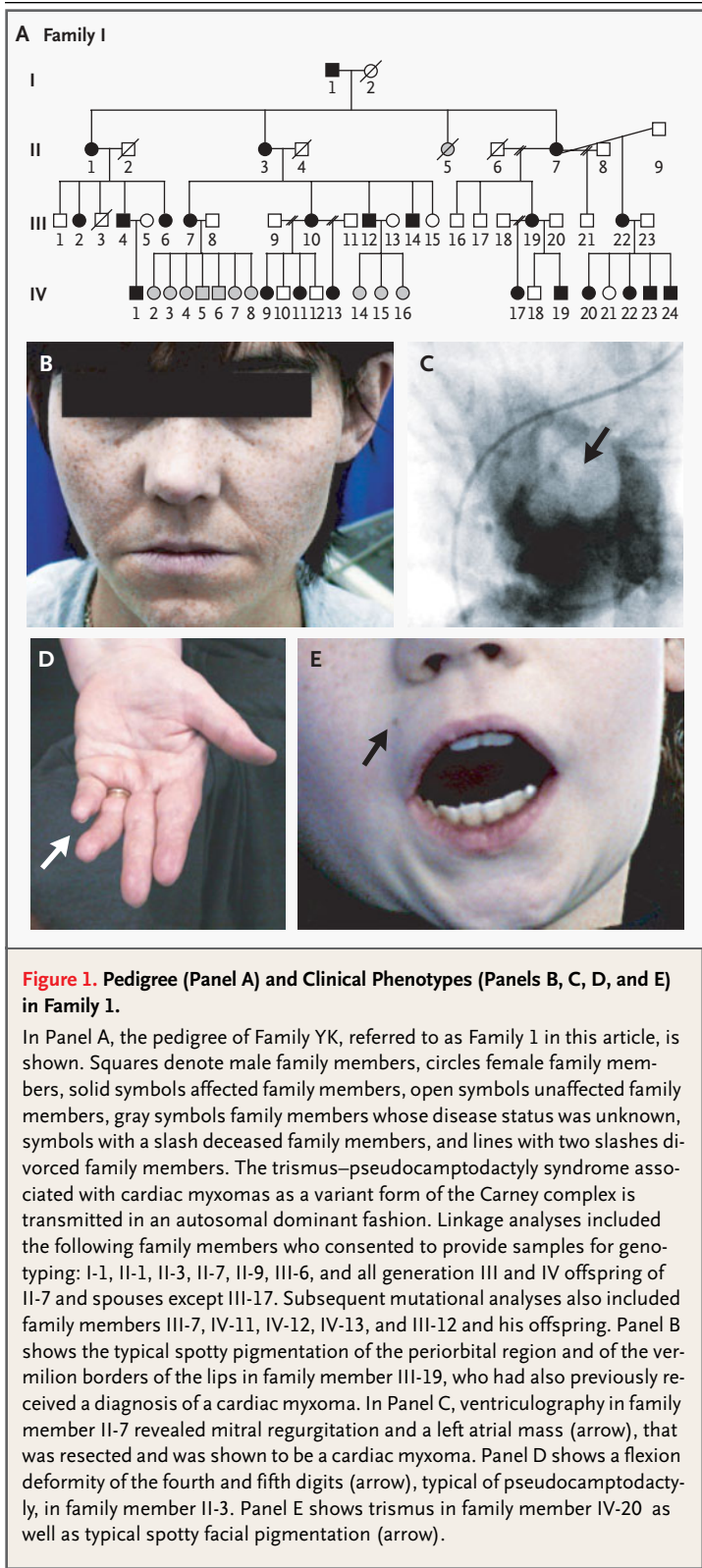
comes from ethnically matched, unrelated control subjects.

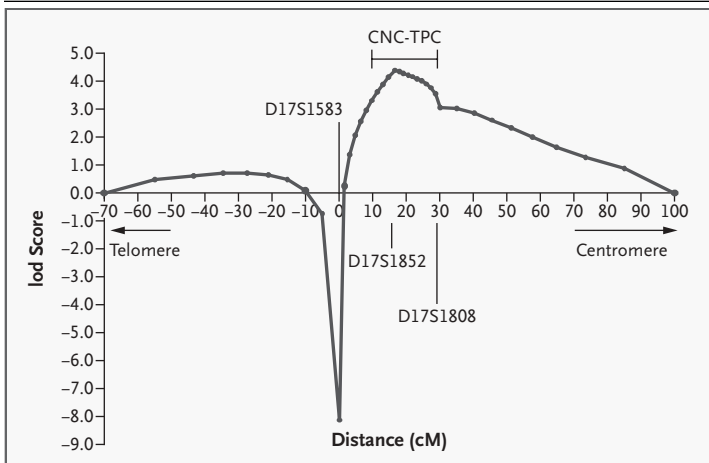
Perinatal myosin heavy chain is a myosin heavy-chain isoform predominantly expressed in skeletal muscle; it was initially cloned from cardiac-muscle complementary DNA<sup>25,26</sup> and was subsequently shown to be expressed in the embryonic myocardium in the chick<sup>27</sup> as well as in myofibroblasts from several tissues.<sup>28</sup> The R674Q variant is a non-conservative, charge-altering, amino acid change in a residue that is identical (Fig. 4A) in the subfragment 1 (head) domain of multiple human myosin isoforms (e.g., adult skeletal, adult cardiac, embryonic, IIa, and IIx/d isoforms) as well as in perinatal myosin heavy chain from other species, including mice, rats, horses, bulls, rabbits, pigs, chickens, frogs, drosophila, and fish (e.g., hawkfish and amberjack). The region surrounding R674 (residues 658 through 680) participates in the binding of myosin to actin.<sup>22,29</sup>

Detailed examination of the crystal structure of the evolutionarily conserved myosin head from chicken skeletal muscle (Fig. 4B) reveals that the R673 residue (orthologous to human R674) is located in the third of seven beta-pleated sheets.<sup>29</sup> These beta-pleated sheets interact to form the ATP-binding pocket, and R673 is adjacent to the P-loop of the neighboring beta-pleated sheet. This P-loop binds directly to ATP, and its structure may be disrupted by the substitution of arginine for glutamine. Given the cosegregation of the R674Q mutation in *MYH8* with CNC-TPC in Family 1, the absence of the variant in unaffected family members, and the predicted alteration in the conserved structure and function of perinatal myosin heavy chain, we concluded that the mutation causes CNC-TPC in Family 1.

#### CHARACTERIZATION OF THE *MYH8* FOUNDER MUTATION

To determine the contribution of *MYH8* mutation to isolated trismus–pseudocamptodactyly syndrome, we genetically analyzed two small branches of American kindreds (Families 2 and 3) (Fig. 5) previously described as affected only by the trismus–pseudocamptodactyly syndrome.<sup>15,17</sup> Genealogic analyses to the year 1850 revealed no interrelationships between these two families, although both reported Dutch ancestry. However, mutational analysis of *MYH8* indicated the presence of the same G2094A transition (R674Q mutation) in both fami-





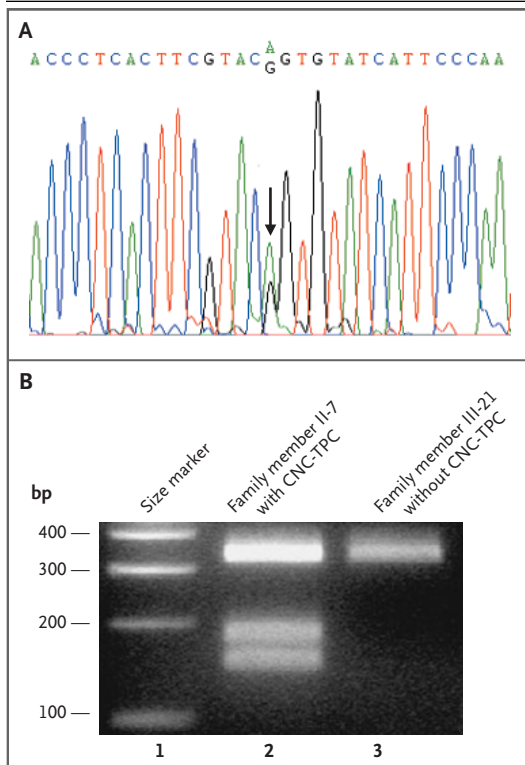
**Figure 2. Multipoint lod Score Analysis of CNC-TPC with the Use of Chromosome 17p Microsatellites in Family 1.**

Family 1 genotypes at microsatellites D17S1583, D17S1852, and D17S1808 were used to determine multipoint lod scores at 17p12–13.1. D17S1583 is arbitrarily plotted at 0 cM, and other microsatellites are noted. An 18-cM genomic interval containing the CNC-TPC locus, based on 95 percent confidence intervals, is shown. A maximum multipoint lod score of 4.39 is observed at 16.6 cM.

lies. We studied 17p12–13 haplotypes in Families 1, 2, and 3 to determine whether they had a common genetic ancestry. Families 2 and 3 shared an extended haplotype of approximately 13 cM surrounding *MYH8* exon 16. However, Family 1 shared with Families 2 and 3 a more restricted haplotype of only 2.7 kb surrounding exon 16. Thus, although Families 2 and 3 may share recent genetic ancestry, Family 1 is, at a minimum, more distantly related, and the R674Q *MYH8* mutation is an ancient founder mutation.

**MYH8 VARIANTS AND ISOLATED CARNEY COMPLEX**

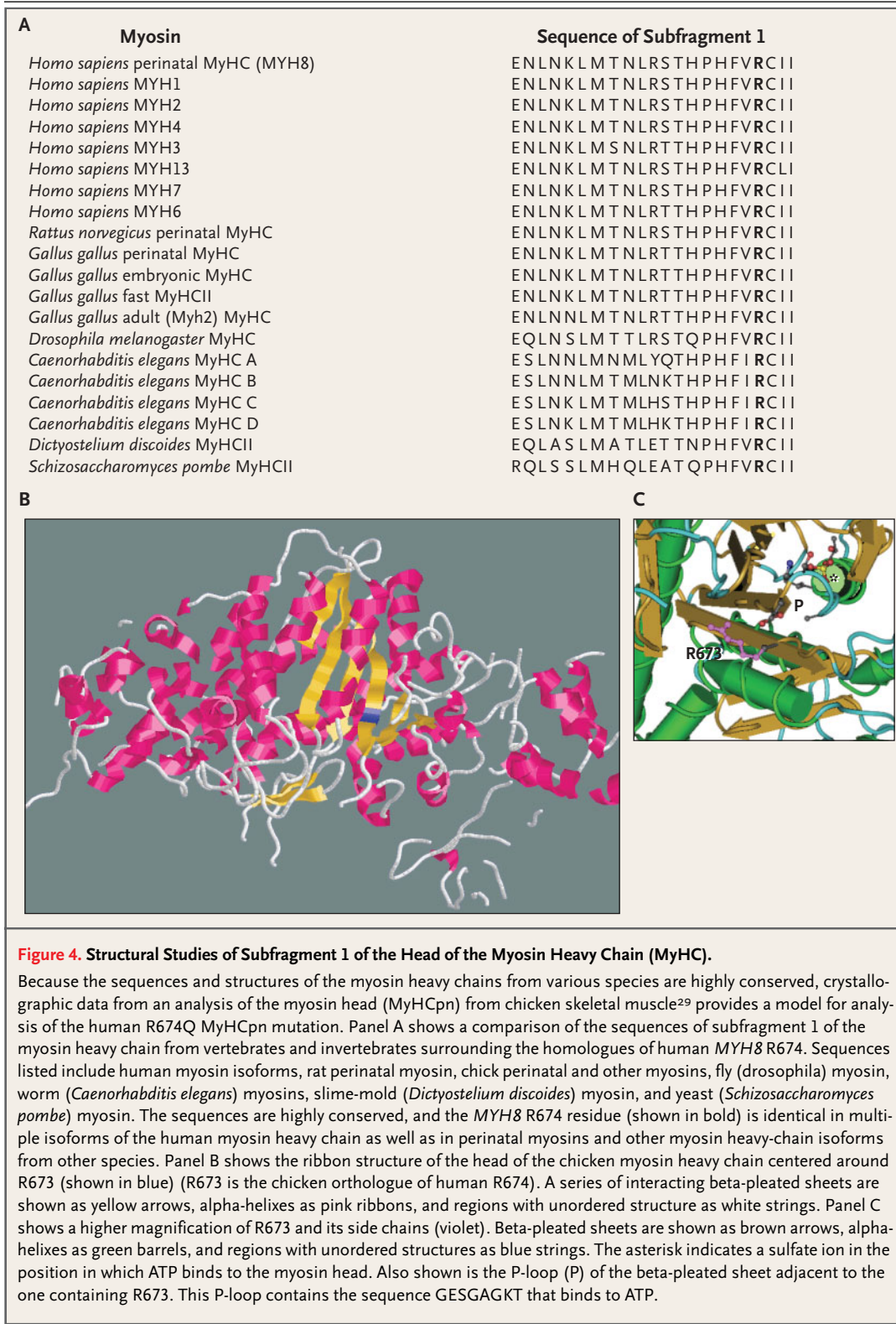
We also sought to determine the contribution of *MYH8* variation to familial cardiac myxoma. Therefore, we determined the *MYH8* sequence in 12 patients with the Carney complex or familial cardiac myxoma syndromes but without the trismus–pseudocamptodactyly syndrome. None had *PRKARIA* mutations. We identified eight *MYH8* sequence variants: C878T, A2464G, C2596A, C3343T, IVS17+45G→A, C5281T, T5147C, and T3759C. The first six did not alter encoded amino acid sequences and had previously been observed in normal subjects,<sup>22</sup> and we concluded that they represented nonpathogenic polymorphisms. Among the 12 pa-

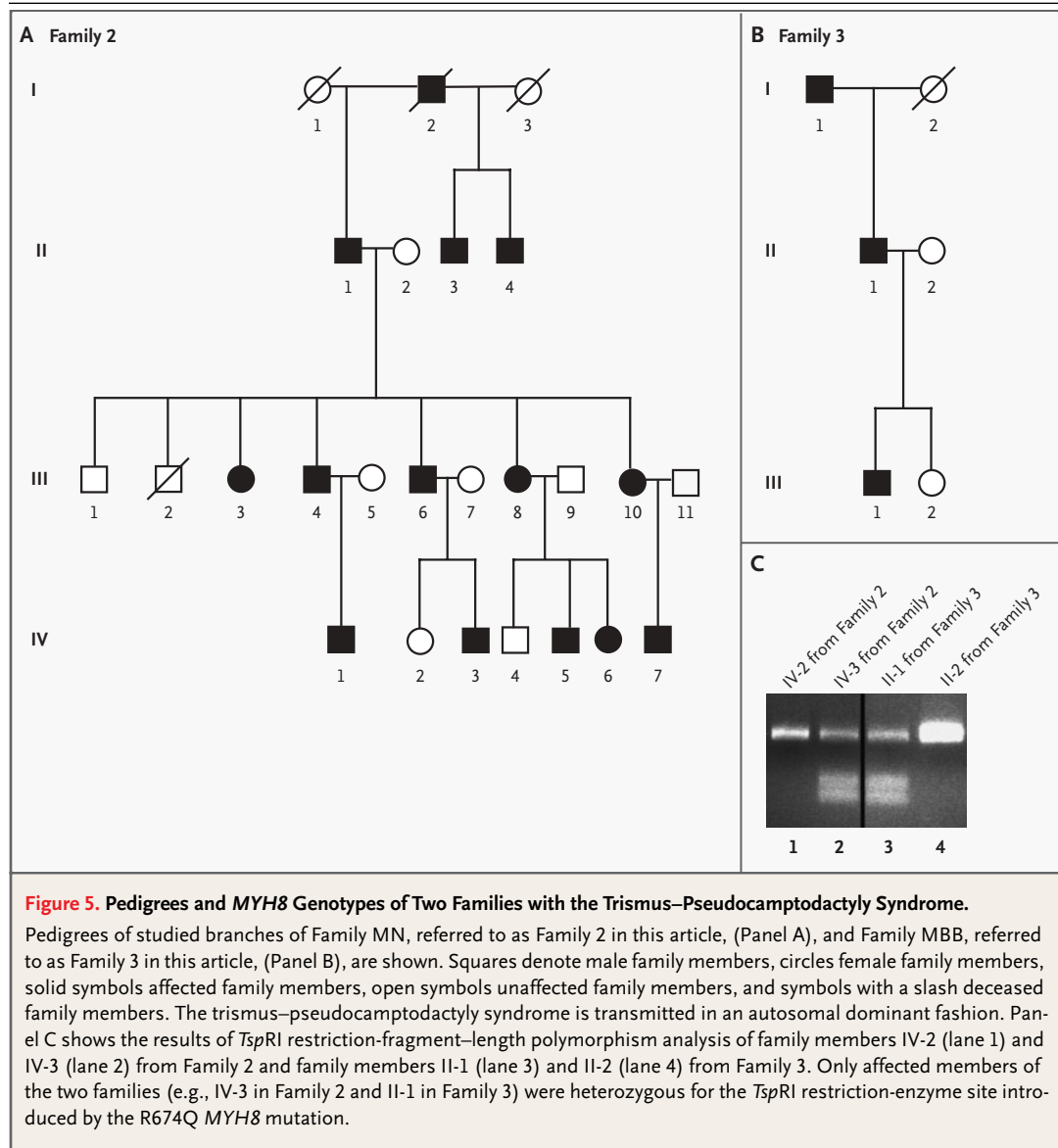


**Figure 3. Mutational Analysis of *MYH8* Exon 16 in CNC-TPC.**

In Panel A, exon 16 was amplified by means of PCR from genomic DNA isolated from affected members of Family 1 and was subjected to sequence analysis. A heterozygous transition from G to A (arrow) was noted at nucleotide 2094. This variant is predicted to encode an R674Q missense mutation in *MYH8*. Panel B shows the results of restriction-fragment–length polymorphism analysis of *MYH8* exon 16 in two members of Family 1. The 326-bp amplicon containing exon 16 was digested with the *TspRI* restriction enzyme and analyzed by agarose-gel electrophoresis. Lane 1 shows the DNA size markers, lane 2 family member II-7 with CNC-TPC, and lane 3 family member III-21 without CNC-TPC. In both family members, the analysis revealed a 326-bp product representing the wild-type *MYH8* allele. However, family member II-7 had two additional (183 bp and 143 bp) bands produced by scission of the mutant allele at the novel *TspRI* site introduced by the G2094A transition.

tients, 4 were T/T homozygotes at nucleotide 5147 and 8 were C/C homozygotes at nucleotide 5147. This variant predicted the substitution of arginine for tryptophan at position 1692 and was present in 95 of 186 chromosomes from ethnically matched, unrelated control subjects. One patient with multiple cardiac myxomas, spotty skin pigmentation,





and reversible cardiomyopathy was heterozygous for T3759C, and this transition predicted the missense substitution of threonine for an evolutionarily conserved residue, methionine at position 1229. However, we also observed this variant in 2 of 262 chromosomes from ethnically matched, unrelated control subjects.

#### DISCUSSION

We demonstrated that the R674Q mutation of the *MYH8* gene encoding perinatal myosin heavy chain causes a variant form of the Carney complex asso-

ciated with distal arthrogyposis, which includes typical spotty pigmentation of the skin, familial cardiac and cutaneous myxoma, and endocrinopathy. Arthrogyposis manifests as typical trismus–pseudocamptodactyly syndrome, with congenital contractures of the hands, feet, and jaw. As previously reported,<sup>12</sup> limb contracture in this syndrome, unlike other distal arthrogyposes, is a pseudocamptodactyly — that is, it is evident only on dorsiflexion of the wrist. The association of limb abnormalities with cardiac myxomas extends the broad spectrum of hereditary heart–hand syndromes, such as Holt–Oram and Char syndromes,

which are due to *TBX5* and *TFAP2B* mutations, respectively.<sup>30,31</sup> The highly variable expressivity of CNC-TPC is typical of such heart–hand syndromes.<sup>2,30,31</sup>

Two families (Family 2 and Family 3) reported to have isolated trismus–pseudocamptodactyly syndrome also had the R674Q *MYH8* mutation. Our findings suggest that not only is R674Q *MYH8* a founder mutation in families with northern European or Dutch ancestry but also, in at least some cases, the Carney complex and the trismus–pseudocamptodactyly syndrome are allelic. Although members of these two families have not had documented cardiac myxomas, family members have not consented to undergo cardiovascular imaging. Even Family 1 was initially described as having the trismus–pseudocamptodactyly syndrome alone,<sup>11</sup> and independent cardiovascular evaluations<sup>10</sup> revealed cardiac myxomas. Members of an unstudied branch of Family 2 affected by the trismus–pseudocamptodactyly syndrome also have histories of spotty skin pigmentation, pituitary tumor, and embolic stroke (potentially due to cardiac myxoma embolization). The true incidence, then, of cardiac myxomas and other manifestations of the Carney complex in families with apparently isolated trismus–pseudocamptodactyly syndrome remains to be defined.

Conversely, our data suggest that *MYH8* mutations are not a major cause of the Carney complex without the trismus–pseudocamptodactyly syndrome. No mutations were detected in patients with cardiac myxoma syndromes who did not have the trismus–pseudocamptodactyly syndrome. However, in this cohort, we identified nonsynonymous *MYH8* polymorphisms. Although these variants may not represent pathogenic mutations, since they are found in apparently normal persons, such polymorphisms may nevertheless be predisposing factors for cardiac myxoma. Like other neoplastic processes, cardiac myxomas require a second “hit” against a variant constitutional genetic background for the initiation of tumorigenesis and therefore have age-related incomplete penetrance.<sup>4</sup> Even in families with the Carney complex caused by characterized *PRKARIA* mutations<sup>4,7-9</sup> and in Family 1, which had the R674Q *MYH8* mutation, the cardiac myxoma phenotype exhibits incomplete penetrance. Thus, apparently normal persons carrying a rare *MYH8* polymorphism (e.g., M1229T) may still be at risk for cardiac myxomas.

How might mutations of perinatal myosin

heavy chain contribute to abnormal skeletal-muscle development as well as cardiac tumorigenesis? The functions of perinatal myosin heavy chain and its specific contributions to myogenesis are unknown. Mouse and avian studies have revealed that its expression is transient, beginning during limb-bud development and then declining after birth.<sup>24,27,32-34</sup> Using reverse-transcriptase PCR, we have observed similarly transient expression of perinatal myosin heavy chain in human embryonic skeletal muscle and heart (data not shown). At least in adult rodents and birds, its expression is dynamically regulated in response to disease. Although perinatal myosin heavy chain is not normally expressed in adult skeletal muscle, it is persistently expressed in both dystrophic avian muscle<sup>34</sup> and adult rodent and avian muscle that is regenerating after injury.<sup>24,34,35</sup> It is not known whether such expression is a marker of pathology or plays an active role in the response of muscle to injury, since regenerating muscle recapitulates the program of fetal gene expression. Nonetheless, the finding of normal embryonic and perinatal expression of perinatal myosin heavy chain in skeletal muscle suggests that mutant expression leads to arthrogryposis through developmental defects.

Perinatal myosin heavy chain is a component of the sarcomere, and mutation of the protein’s head may impair the interactions of myosin with other sarcomeric proteins in addition to altering ATP handling and cellular energetics. ATP hydrolysis by the sarcomeric myosin motor permits a sliding interaction of the myosin thick filament and actin thin filament to produce muscle contraction in cardiac and skeletal muscle. The R674Q mutation in perinatal myosin heavy chain localizes to a portion of the actin-binding domain and could alter myosin–actin interactions. Mutations of the homologous  $\beta$ -myosin heavy-chain gene (*MYH7*) cause hypertrophic and dilated cardiomyopathy.<sup>2,36</sup> Such mutations<sup>36</sup> occur most commonly in the myosin head and localize to actin-binding domains or, like the R674Q mutation in perinatal myosin heavy chain, flank ATP-binding sites. Consequently, these mutant myosins exhibit altered mechanics with increased ATPase activity and augmentation of the velocity of actin-filament movement.

Although an R671 mutation of *MYH7*, the perinatal myosin heavy-chain R674 homologue, has not been reported, mutations of nearby arginine residues (652 and 663) within the same actin-binding segment cause hypertrophic cardiomyopathy.<sup>2,36</sup> In

addition, the proximity of R674 to the P-loop involved in ATP binding further suggests that there is a potential perturbation of ATP binding to mutant perinatal myosin heavy chain. We hypothesize that altered perinatal myosin heavy chain mechanics may contribute to the dysmorphogenesis of fetal skeletal muscle and tendons, with ensuing jaw and limb arthrogyrosis. Since mutations of other skeletal isoforms of contractile proteins produce arthrogyrosis distinct from that characterizing the trismus–pseudocamptodactyly syndrome,<sup>18-20</sup> the specific type of congenital arthrogyrosis may result from unique spatiotemporal patterns of expression of sarcomeric protein.

The association of the perinatal myosin heavy-chain mutation with cardiac myxomas suggests that perinatal myosin heavy chain participates in the regulation of myxoma progenitor cells. It has been suggested that cardiac myxomas arise from poorly characterized, pluripotent, subendocardial reserve cells.<sup>4</sup> In other organs, such as liver and kidney, perinatal myosin heavy chain is expressed in myofibroblasts,<sup>28</sup> which are the predominant cellular component of granulation tissue and which play critical roles in injury-associated remodeling of several tissues, including skin, liver, kidney, and heart.<sup>37</sup> Myofibroblasts differentiate along several lineages and, in the heart and other organs, proliferate after ischemic injury. Given the expression of perinatal myosin heavy chain in myofibroblasts and our finding that it participates in the pathogenesis of cardiac myxomas, we hypothesize that genetic abnormalities in perinatal myosin heavy chain promote the proliferation of cardiac myofibroblasts, with consequent tumorigenesis.

Mutations in both *MYH8* and *PRKARIA* produce the Carney complex, which suggests that perinatal myosin heavy chain and PKA are involved in at least intersecting metabolic pathways regulating cardiac growth. Although myosin heavy chains are not known to be phosphorylated by PKA, other sarcomeric proteins in the actin–myosin complex (e.g., troponin I and myosin-binding protein C) are PKA substrates.<sup>38</sup> In addition, phosphorylation of upstream stimulatory factor 1 by PKA helps regulate myosin promoter activity.<sup>39</sup> The incorporation of mutant perinatal myosin heavy chain into the sarcomere could disrupt sarcomeric integrity and alter downstream signaling potentially mediated through PKA localized to Z bands. Therefore, we predict that sarcomere imbalances may induce not only cardiomyopathy but also cardiac tumorigenesis.

From a clinical perspective, the identification of an *MYH8* founder mutation in patients with CNC-TPC should prompt future assessment of the risk of cardiac myxomas in patients with the trismus–pseudocamptodactyly syndrome. For now, surveillance echocardiography of such patients may allow the diagnosis of cardiac tumorigenesis before symptoms occur. Preimplantation genetic diagnosis has already been applied to heart–hand syndromes,<sup>40</sup> and elucidation of molecular mechanisms underlying CNC-TPC will foster genetic approaches to the diagnosis and treatment of cardiac myxomas and arthrogyrosis.

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