An R111C Polymorphism in Wild Turkey Cardiac Troponin I Accompanying the Dilated Cardiomyopathy-related Abnormal Splicing Variant of Cardiac Troponin T with Potentially Compensatory Effects*

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Cardiac muscle contraction is regulated by Ca\(^{2+}\) through the troponin complex consisting of three subunits: troponin C (TnC), troponin T (TnT), and troponin I (TnI). We reported previously that the abnormal splicing of cardiac TnT in turkeys with dilated cardiomyopathy resulted in a greater binding affinity to TnI. In the present study, we characterized a polymorphism of cardiac TnI in the heart of wild turkeys. cDNA cloning and sequencing of the novel turkey cardiac TnI revealed a single amino acid substitution, R111C. Arg\(^{111}\) in avian cardiac TnI corresponds to a Lys in mammals. This residue is conserved in card...
assays using various TnT (6) and TnI (7) fragments, and the newly published crystallography structure of the partial human cardiac troponin complex (8) have identified the TnT-TnI interface structure provides valuable guidance in understanding the structure-function relationship of the troponin subunits.

In the present study, we characterize a unique polymorphism of cardiac TnI observed in wild turkey hearts. cDNA cloning and sequencing revealed a single amino acid substitution of Cys for Arg111 in the novel turkey cardiac TnI. Arg111 in avian cardiac TnI corresponds to Lys117 in human cardiac TnI and is conserved in cardiac and skeletal muscle TnIs across the vertebral phylum. This amino acid residue in the TnT-TnI interface provides valuable guidance in understanding the structure-function relationship of the troponin subunits.

From the chicken cardiac TnI cDNA library, the exon 5–8 region of chicken cardiac TnI cDNA was amplified by PCR using a pair of forward and reverse primers designed according to the published partial chicken cardiac TnI cDNA sequence (18). The 5′-region of chicken cardiac TnI cDNA was amplified from the library in a separate PCR using a reverse primer in the exon 7 region paired with T3 primer (in this a phage library, a forward binding site for the T3 primer is present flanking the 5′ end of the cDNA insert). The PCR-amplified cDNA fragments were cloned into the pcR4-TOPO vector using the TOPO TA cloning kit (Invitrogen) following the manufacturer’s instructions and sequenced using the dideoxy chain-termination method. Full-length chicken cardiac TnI cDNA was constructed by pasting restriction fragments of the 5′ and 3′ cDNAs.

cDNA encoding the turkey cardiac TnI was then cloned from domestic and wild turkey hearts. RT-PCR using primers synthesized according to the chicken cardiac TnI cDNA sequence. As described previously (9), turkey ventricular muscle RNA was isolated using the TRIzol reagent. 2 μg of the total cardiac RNA was used to synthesize cDNA from all poly(A)+ RNA by reverse transcription using an anchored oligo dT primer (5′-TTTTTTTTTTTTTTTTT-3′, where V = A, C, or G). Double-stranded cDNA encoding turkey cardiac TnI was then amplified by PCR with a forward primer corresponding to the region of the translation initiation codon (5′-GGGGCATATGGGCTGGAGGAGGAGGC-C-3′) and a reverse primer (5′-ATGCCCAACACGTGGCCTCTAAAGGGT-GA-3′) synthesized corresponding to a 3′ untranslated sequence 97–124 nucleotides downstream of the stop codon. Resultant PCR products were cloned into the pcR4-TOPO vector as above. Recombinant plasmid DNA was purified, and the cDNA insert was sequenced.

Construction of N-terminal Truncated Turkey Cardiac TnI—Polymerase chain reaction was used to generate a truncated cDNA encoding turkey cardiac TnI without the cardiac specific N-terminal region. A forward primer (5′-GGGGGATATGGGCTGGAGGAGGAGGC-C-3′) was synthesized corresponding to a Met codon prior to the turkey cardiac TnI codon for amino acid 23. This mutagenesis primer was used together with the T7 primer flanking the 3′ end of the cloned turkey cardiac TnI cDNA in the pcR4-TOPO vector sequence to generate the truncated cDNA template by PCR. The PCR products were digested with the restriction enzymes NdeI and ligated into the compatibly cut pAED4 vector. Recombinant plasmid DNA was purified, and the cDNA insert was sequenced as above to verify the construction.

Expression and Purification of N-terminal Truncated Turkey Cardiac TnI—The N-terminal truncated turkey cardiac TnI (TcTnI-ND) was expressed in E. coli from the cDNA construct for functional characterization. As described previously (9), BL21(DE3)pLYS S E. coli cells were transformed with the recombinant pAED4 plasmid and cultured in liquid media containing ampicillin and chloramphenicol. The culture was induced at mid-log phase with isopropyl-thiogalactoside and cultured in liquid media containing ampicillin and chloramphenicol. After 3 additional hours of induction, the bacterial cells were harvested and lysed by three passages through a French cell press in 5 mM EDTA, 15 mM imidazole, 0.5% Triton X-100, and 0.5 mM phenylmethylsulfonyl fluoride. The cell lysate was centrifuged at 10,000 g for 10 min to remove debris. The supernatant was then applied to a 5 ml Ni-NTA resin, washed with 10 mM imidazole, 250 mM NaCl, pH 8.0, and eluted with 500 mM imidazole, pH 8.0. The protein fraction was then dialyzed against 20 mM Tris-HCl, pH 8.0, and concentrated using an Amicon YM-10 membrane. Following dialysis, the fraction was then loaded onto a 1 ml HiTrap Blue column equilibrated in a buffer containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 1% dithiothreitol. The protein fraction was eluted with a linear gradient of 150 mM NaCl in an equilibration buffer containing 50 mM Tris-
Cardiac Troponin I Polymorphism in Wild Turkeys

RESULTS

A Novel Cardiac TnI in the Heart of Wild Turkeys. A, SDS-PAGE of total muscle homogenate resolved on 14% Laemmli gel with an acrylamide:biacylamide ratio of 180:1 demonstrates the integrity of the cardiac muscle samples. B, Western blot using the anti-cardiac and slow TnT mAb CT3 demonstrates that, unlike the single cardiac TnT band found in adult human, mouse, and chicken hearts, the adult wild turkey hearts express an additional low molecular mass cardiac TnT (arrow) identical to that of the DCM-related ΔE8 cardiac TnT reported previously in the domestic turkey. The ΔE8 cardiac TnT is distinguishable from slow skeletal muscle TnT detected by CT3 in turkey mixed fiber. C, Western blot of cardiac muscle homogenates resolved on 12% Laemmli gel with an acrylamide:biacylamide ratio of 29:1 using the anti-TnI mAb TnI-1 demonstrates that all mammalian and avian species examined express a single cardiac TnI in the adult heart. In contrast to the similarly migrating single cardiac TnI in the chicken and domestic turkey heart, an additional cardiac TnI of slow SDS-gel mobility was detected in the heart of wild turkey #1, whereas the heart of wild turkey #2 expressed only the slow migrating cardiac TnI (arrow). This slow migrating cardiac TnI is also distinct from the fast and slow skeletal muscle TnIs. D, the slow migrating cardiac TnI was proportionally incorporated into the wild turkey cardiac myofilaments, as shown by the mAb TnI-1 Western blot on extensively washed myofibrils. E, expression of cloned cDNA encoding chicken cardiac TnI. Western blot performed as in C on total protein extracted from E. coli transformed with the chicken cardiac TnI cDNA-expressing plasmid confirmed that the protein encoded is recognized by the anti-TnI mAb TnI-1 and is of identical size to that of the cardiac TnI in adult chicken heart. Low and high molecular mass bands in the E. coli extract were also recognized by mAb TnI-1, indicating degradation and aggregation, respectively, of chicken cardiac TnI in bacterial cells.
the wild turkey hearts is distinct from fast or slow skeletal muscle TnI, as its SDS-gel mobility was different from that seen in a mixed-fiber skeletal muscle of a newly hatched domestic turkey and the fast skeletal muscle of a 15-day-old domestic turkey (Fig. 1C). The slow migrating cardiac TnI was proportionally incorporated into the myofibrils as shown by the Western blot of extensively washed wild turkey cardiac myofibrils (Fig. 1D).

**Cloning of the 5’ Region of Chicken Cardiac TnI cDNA—** Based on the 3’ partial sequence of chicken cardiac TnI cDNA reported by Hastings et al. (18), we cloned a full-length chicken cardiac TnI cDNA by PCR from a unidirectional chicken cardiac cDNA library. Expression of the cloned cDNA in E. coli yielded a protein recognized by the anti-TnI mAb TnI-1 with identical size to cardiac TnI in the chicken heart (Fig. 1E). Sequence of the full-length chicken cardiac TnI cDNA has been submitted to GenBank™/EBI with the accession number AY463242. The physical properties of the protein deduced from the cDNA sequence demonstrate that the chicken cardiac TnI is of similar molecular weight to that of human and mouse cardiac TnI. The fact that the chicken cardiac TnI (208 amino acids, M_r = 23,627, isoelectric point (pI) = 9.96) exhibits a significantly faster SDS-gel migration rate than that of the similar-sized human (210 amino acids, M_r = 24,037, pI = 9.87) and mouse (211 amino acids, M_r = 24,258, pI = 9.57) cardiac TnI (Fig. 1C) demonstrates that SDS-gel mobility is a sensitive indicator of differences in TnI primary structure.

**A Single Nucleotide Mutation Results in a Cys Substitution for Arg^{111} in Wild Turkey Cardiac TnI—** Based upon the full-length chicken cardiac TnI cDNA sequence, we were able to use RT-PCR to clone cDNAs encoding the fast and slow migrating turkey cardiac TnIs. Sequencing analysis revealed that, with the substitution of Cys for Arg^{111} in wild turkey cardiac TnI, the wild-type cardiac TnI bands of different SDS-gel mobility (Fig. 1A) represent a polymorphism caused by two nucleotide transversions in wild turkey cardiac TnI gene, resulting in a substitution of Cys for Arg^{111}. A cDNA cloning and sequencing revealed the primary structures of the slow and fast migrating turkey cardiac TnIs. The deduced amino acid sequence of the wild-type turkey cardiac TnI was found to be identical to the chicken cardiac TnI, with Arg at position 111. A class of cDNA cloned from both wild turkey #1 and #2 corresponding to the slow migrating cardiac TnI differs from the wild type by a single G → T nucleotide transversion, resulting in the substitution of Cys for Arg^{111} (outlined by the box). The initiation site of TnI-ND is indicated in the sequence. B, the protein primary structural map of cardiac TnI and sequence alignment for the region flanking Arg/Cys^{111} demonstrates that this position is conserved (Arg or Lys) in all TnI sequenced to date (26–30). The highlighted residues in the human cardiac TnI sequence are residues that contact with TnT in the crystal structure of troponin (8).

![Figure 2](http://www.jbc.org/)
cardiac TnI (TcTnI-ND) containing either Arg or Cys at position 111 for functional characterization. The N-terminal truncation was designed according to a naturally occurring cardiac TnI proteolytic fragment originally found in a rat model of simulated microgravity (14). cDNAs were constructed accordingly, encoding amino acids 23–208 and containing Arg or Cys at position 111 (TcTnI-ND-Arg111 and TcTnI-ND-Cys111). B, 12% Laemmli gel with an acrylamide:bisacrylamide ratio of 29:1 and mAb TnI-1 Western blot of turkey heart homogenates and E. coli-expressed TcTnI-ND-Arg111 and TcTnI-ND-Cys111 demonstrate similar shifts in gel migration rate because of the presence of wild-type Arg111 or the Cys111 substitution in both intact and N-terminal-truncated cardiac TnIs.

The calculated molecular mass and pI are 21,070 Da and 10.05 for TcTnT-ND-Arg111 and 21,017 Da and 9.83 for TcTnT-ND-Cys111. Expression of the TcTnI-ND cDNA constructs in E. coli yielded a high level expression of proteins that were readily detectable by the TnI-1 mAb (Fig. 4B). The TcTnI-ND-Arg111 and TcTnI-ND-Cys111 proteins also clearly exhibit the fast and slow SDS-gel migration rates observed in the intact turkey cardiac TnI variants (Fig. 4B). Large scale expression of TcTnI-ND-Arg111 and TcTnI-ND-Cys111 in E. coli yielded good quantities of protein, demonstrating that the removal of N-terminal 22 amino acids significantly improved the compatibility of avian cardiac TnI to the bacterial host cell. The TnI purification methods developed in this study are highly effective in obtaining high quality materials for functional characterization (Fig. 5).

![Fig. 3. R111C at the TnI-TnT interface in troponin complex.](image)

The TnI-TnT interface structure corresponding to the region flanking the R111C polymorphism is modeled in this figure. The helices are depicted as ribbons: green for TnI, and red for TnT. Hydrogens are not shown for clarity. Backbone atoms are colored green, and side chain atoms are colored according to their element type (carbon, gray; oxygen, red; nitrogen, blue; sulfur, yellow). The human cTnI crystallographic structure is shown in A. Residues 114–117 were then changed from the original human cardiac to the turkey cTnI and a normal mode analysis was performed with the low mode search of Macromodel 7-2 (Schrodinger, Inc.) using an OPLS-AA force field and a GBSA solvent model. Only the four residues illustrated were allowed to vary during the conformational search. All other residues were frozen in the crystallographic position. The lowest energy homology models are shown in B (Arg117) and C (Cys117). Backbone carbonyls to which hydrogen bonding was observed during the normal mode vibrations are colored purple based on 794 structures from the Arg117 simulation and 973 structures from the Cys117 simulation. Note that hydrogen bonding of Cys117 to Ile114 (Met108 in turkey cardiac TnI) was observed but not in the lowest energy structure. The difference between the alpha carbons of the homology models and the original crystal structure was 0.4 Å RMSD.
To validate the use of the N-terminal truncated turkey cardiac TnI for the functional characterization of the R111C substitution, we compared the TcTnI-ND-Arg_{111} and TcTnI-ND-Cys_{111} to the intact chicken cardiac TnI by ELISA epitope conformation analysis. The results in Fig. 6 demonstrate that the binding affinity and maximal binding (inset) of mAb TnI-1, with its epitope in the C-terminal region of TnI, to TcTnI-ND-Arg_{111} and TcTnI-ND-Cys_{111} were identical to that of the intact chicken cardiac TnI. These results indicate that the removal of the N-terminal 22 amino acids does not destroy the global structure of cardiac TnI.

To further verify that the N-terminal truncated cardiac TnI is representative for the intact cardiac TnI in binding to cardiac TnT, we compared TcTnI-ND-Arg_{111} and the intact chicken cardiac TnI in ELISA solid-phase protein binding experiments. The results in Fig. 6 demonstrate that there is no detectable difference between the intact chicken cardiac TnI (Arg_{111}) and TcTnI-ND-Arg_{111} in binding affinity or maximal binding (inset) to wild-type turkey cardiac TnT. Therefore, under the experimental conditions, removal of the N-terminal domain from turkey cardiac TnI did not affect binding to cardiac TnT under the assay conditions.

**Substitution of Cys for Arg_{111} in Turkey Cardiac TnI Alters the Binding to Cardiac TnT**—The TcTnI-ND-Arg_{111} and TcTnI-ND-Cys_{111} proteins were examined for the effect of the R111C substitution within the TnI-TnT interface on the binding of TnI to the turkey cardiac TnT variants. To validate the solid-phase TnT binding assay, we first demonstrated that saturated amounts of the anti-TnT mAb CT3 bound to the WT and \( \Delta E8 \) turkey cardiac TnT purified from \( E. \) coli expression used in this study.

**Fig. 5.** Purification of N-terminal truncated turkey cardiac TnI expressed in \( E. \) coli. A, SDS-PAGE on 12% Laemmli gel with an acrylamide:biacrylamide ratio of 29:1 and Western blots using the anti-TnI mAb TnI-1 demonstrate the high level expression of TcTnI-ND-Arg_{111} and TcTnI-ND-Cys_{111} in large cultures of \( E. \) coli and the effective purification profiles (see “Experimental Procedures” for details). B, Coomassie Blue R250-stained 12% Laemmli SDS-PAGE gel with an acrylamide:biacrylamide ratio of 29:1 demonstrates the purity of the chicken cardiac TnI prepared from ventricular muscle, TcTnI-ND-Arg_{111}, and TcTnI-ND-Cys_{111}, and the WT and \( \Delta E8 \) turkey cardiac TnT purified from \( E. \) coli expression used in this study.

**Fig. 6.** Deletion of the N-terminal 22 amino acids of turkey cardiac TnI does not alter C-terminal epitope conformation nor binding to TnT. A, ELISA epitope affinity titration curves and maximum binding levels (the \( A_{405} \) reading shown in inset) for mAb TnI-1 against a C-terminal epitope on TnI show no significant differences among the intact chicken cardiac TnI, TcTnI-ND-Arg_{111}, and TcTnI-ND-Cys_{111} immobilized on a microtiter plate. The results demonstrate that deletion of the N-terminal domain from turkey cardiac TnI does not disrupt the global structure of TnI. B, solid-phase protein binding curves demonstrate that there is no significant difference between intact chicken cardiac TnI (Arg_{111}) and TcTnI-ND-Arg_{111} in binding affinity to wild-type turkey cardiac TnT immobilized on a microtiter plate, as defined by the TnI concentration required for 50% of maximum binding. The maximal binding levels of intact chicken cardiac TnI and TcTnI-ND-Arg_{111} to wild-type turkey cardiac TnT were also similar (the \( A_{405} \) reading shown in inset). The results indicate that deletion of the N-terminal domain from turkey cardiac TnI did not affect binding to cardiac TnT under the assay conditions.
The significantly higher concentration of TcTnI-ND-Cys 111 relative to the DCM-related TcTnT-E8 abnormality suggests a compensatory effect. To verify the comparable coating of turkey cardiac TnT to the wild-type (WT) and exon 8-excluded (ΔE8) turkey cardiac TnT immobilized to the plate, B and C, solid-phase protein binding assays demonstrate the binding affinities of TcTnI-ND-Cys111 to WT and ΔE8 TcTnT were significantly lower than those of TcTnI-ND-Arg111, as shown by the higher concentrations required for 50% of maximum binding. D, although the binding curves confirm the increased TnI binding affinity of TcTnT-ΔE8 as compared with that of TcTnT-WT (9), the decreased binding affinity of TcTnI-ND-Cys111 for TcTnT suggests a compensatory effect.

DISCUSSION

Sensitivity of TnI SDS-gel Mobility to Structural Modifications—The turkey cardiac TnI R111C substitution in cardiac TnI alters binding affinity to WT and ΔE8 turkey cardiac TnT. A, to verify the comparable coating of turkey cardiac TnT to the microtiter plates of the protein binding assays, ELISA titration showed no significant difference between the levels of saturated binding of mAb CT3 to the wild-type (WT) and exon 8-excluded (ΔE8) turkey cardiac TnT immobilized to the plate. B and C, solid-phase protein binding assays demonstrate the binding affinities of TcTnI-ND-Cys111 to WT and ΔE8 TcTnT were significantly lower than those of TcTnI-ND-Arg111, as shown by the higher concentrations required for 50% of maximum binding. D, although the binding curves confirm the increased TnI binding affinity of TcTnT-ΔE8 as compared with that of TcTnT-WT (9), the decreased binding affinity of TcTnI-ND-Cys111 for TcTnT suggests a compensatory effect.2 These results indicate that similar amounts of the WT and ΔE8 TcTnT were immobilized on the microtiter plates.

Using the solid-phase protein binding assay, we demonstrated that the binding affinity of TcTnI-ND-Cys111 to TcTnT-WT was significantly lower than that of TcTnI-ND-Arg111 (Fig. 7B). The lowered binding affinity was reflected by the significantly higher concentration of TcTnI-ND-Cys111 required to reach 50% of maximum binding compared with that in TcTnI-ND-Arg111 (0.056 μM ± 0.006 versus 0.022 μM ± 0.004, p < 0.01), demonstrating a higher Kd during equilibrium binding. Likewise, TcTnI-ND-Cys111 exhibited decreased binding affinity to TcTnT-ΔE8, as compared with that of TcTnI-ND-Arg111 (Fig. 7C; the concentrations required for 50% of maximum binding were 0.044 μM ± 0.004 and 0.013 μM ± 0.001, respectively; p < 0.01). Fig. 7D summarizes the effects of the R111C substitution in cardiac TnI on binding affinities for the WT versus ΔE8 TcTnT. We showed previously that the DCM-related TcTnT-ΔE8 had a higher binding affinity to cardiac TnI (wild type) compared with the TcTnT-WT, correlating to an increased Ca2+ sensitivity in the activation of myofilament ATPase (9). This cardiac TnT abnormality was also detected in the TcTnI-ND binding experiments (Fig. 7D). By lowering the abnormally high binding affinity of TcTnT-ΔE8 to TnI, the cardiac TnI Cys111 polymorphism in wild turkey hearts may be compensatory to the DCM-related TcTnT-ΔE8 abnormality.

The Cardiac TnI Cys111 Allele in Wild Turkey Population—Arg111 in avian cardiac TnI is a conserved residue in all TnIs sequenced to date (Fig. 2B). In contrast, the Cys111 allele was readily detectable in the wild turkey population. Although the Cys111 allele can coexist with the Arg111 allele (wild turkey #1 is an Arg111/Cys111 heterozygote, as shown by both Western blot (Fig. 1C) and cDNA cloning/sequencing), a homozygous Cys111 individual was also found (wild turkey #2). Therefore, the Cys111 allele seems to have a high frequency in the wild turkeys. The fixation of this unique polymorphism in the turkey but not other species indicates a specific selection value. It is worth noting that the chicken and turkey are evolutionarily closely related species and that their wild-type cardiac TnI has identical amino acid sequences. According to the avian constraint hypothesis, functional constraint on avian proteins causes the reduction of genetic divergence and, therefore, avian protein structure generally has a lower tolerance to amino acid substitutions compared with that of other species (24). Therefore, the fixation and spread of the cardiac TnI Cys111 allele in wild turkeys indicates a strongly favored selection value. The relationship between their molecular mass and SDS-gel migration rate (23). Although mammalian and avian cardiac TnI are of similar molecular weight and amino acid composition, both chicken and turkey cardiac TnI migrate significantly faster than that of the mammalian cardiac TnI (Fig. 1C). This observation suggests that the molecular conformation and/or flexibility of TnI, even in the presence of SDS, is sensitive to minor structural alterations. A smaller SDS-gel mobility shift has also been observed in mouse cardiac TnI with a single amino acid substitution.2 This hypothesis is consistent with the nature of TnI as an allosteric regulatory protein.

2 J.-P. Jin and Z.-B. Yu, unpublished results.
domestic turkey has a high instance of DCM that causes round heart disease with considerable mortality (25). In contrast to the short inactive life span of the domestic turkey, the wild turkey population is under stringent natural selection. We reported previously that the abnormally spliced cardiac TnT variant in the domestic turkey may contribute to the pathogenesis of DCM (9). As shown in Fig. 1B, wild turkeys also express the exon 8-deleted cardiac TnT in cardiac muscle identical to that of domestic turkeys. The presence of this DCM-related cardiac TnT abnormality in the wild turkey would impose a specific selection pressure on functionally related genes. Therefore, it is logical to consider that the cardiac TnI Cys111 allele is of compensatory value to improve survival of the wild turkey population that otherwise would suffer from a high instance of DCM because of the presence of abnormal cardiac TnT.

Potential Compensatory Effect of the Turkey Cardiac TnI Cys111 Allele on the DCM-related TnT Abnormality—Protein binding experiments revealed that the R111C substitution lowered the binding affinity of turkey cardiac TnI to TnT, compensatory to the increased TnI-binding affinity of the DCM-related exon 8-deletion in turkey cardiac TnT (Fig. 7). On the other hand, the incorporation of the Cys111 cardiac TnI into the cardiac myofilaments was not affected, as shown by its proportional incorporation into the wild turkey cardiac myofibrils (Fig. 1D). This finding is consistent with the notion that the R111C substitution in cardiac TnI does not produce drastic effects on troponin structure (Fig. 3).

Supporting the decreased binding affinity of cardiac TnI to TnT, modeling the substitution of Cys for Lys117 in human troponin structure adds a hydrogen bond between Cys117 and Ile114 in the TnI-TnT coiled-coil structure (8, Fig. 3C). This might reduce the flexibility of the TnI structure that interfaces with TnT in the functionally important IT arm of troponin (8). We have demonstrated previously that the increased TnI-binding affinity is one of the primary functional changes produced by the DCM-related cardiac TnT splicing variant (9, 10). The reduced TnT-binding affinity of the wild turkey cardiac TnI Cys111 polymorphism is proposed, therefore, to obtain its selection value by compensating for the TnT-originated abnormality in TnT-TnI interaction and Ca2+-regulation of cardiac muscle contraction. It is worth noting that the binding affinities of cardiac TnI Cys111 for both wild type and ΔE8 cardiac TnT are reduced to levels lower than that between cardiac TnI Arg111 and wild-type cardiac TnT, which is the normal reference point (Fig. 7D). However, the significantly weakened binding between cardiac TnI Cys111 and TnT may minimize the effect of cardiac TnT abnormality on myocardial contraction. The biochemical and physiological mechanisms deserve further investigation.

In summary, as an example of the combined power of protein polymorphism analysis, molecular cloning, and protein structure-function characterization, the finding of the cardiac TnI Cys111 allele in wild turkeys with potentially compensatory effect on TnT abnormality demonstrates the closely related structure-function relationship between TnI and TnT and suggests a novel target for the treatment of TnT myopathies.

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