RESEARCH REPORT
RAPPORT DE RECHERCHE
ANTHROPOLOGIE XLIV/2 • 2006

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PHENOTYPE ASPECTS OF *Δ160* OF *TNNT2* GENE IN CZECH PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY

ABSTRACT: Hypertrophic cardiomyopathy is indicated by left and/or right ventricular hypertrophy, which is typically asymmetric and involves the interventricular septum. Ordinarily, the volume of the left ventricular is normal or reduced, while systolic gradients are common.
As a complex cardiac disease, familial hypertrophic cardiomyopathy (FHC) has unique pathophysiological characteristics and multifarious morphological, functional, and clinical features.
Troponin T is a regulatory protein found in striated muscles that forms a complex with troponin I (TnI) and troponin C (TnC) that, along with tropomyosin (TM), must be present for Ca^{2+}-dependent regulation of muscle contraction.
Exons 9 and 11 of TNNT2 gene, which are known to contain mutations associated with FHC, were analyzed by the polymerase chain reaction (PCR) and subsequently by DNA sequencing analyses, which were cross-sequenced.
The ΔGlu160 mutation was observed in patients with severe form of hypertrophic cardiomyopathy.

KEY WORDS: Hypertrophic cardiomyopathy – Familial hypertrophic cardiomyopathy – Troponin T – TNNT2 – Mutation

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) / Familial hypertrophic cardiomyopathy (FHC)

Hypertrophic cardiomyopathy (HCM) is predominated by the familial form with autosomal dominant inheritance. The disease is caused by mutations in sarcomeric contractile protein genes. Typical morphological changes include myocyte hypertrophy and disarray surrounding areas of increased loose connective tissue. Common effects of the disease include arrhythmias and premature sudden death (Palm *et al.* 2001, Anan *et al.* 1998, Lin *et al.* 2000).

Familial hypertrophic cardiomyopathy includes a group of hypertrophic cardiomyopathy (HCM) lacking a defined aetiology. It is also characterized by a high rate of morbidity and mortality and incomplete penetrance. Its prevalence is 1/500 in young adults. Patients with HCM exhibit protean clinical manifestations that vary from an asymptomatic course to that of severe heart failure and sudden cardiac death (SCD). HCM is the most common cause of SCD in young competitive athletes, accounting for approximately one-third of all SCD. Other clinical features of HCM include chest pain, dyspnoea, palpitations and syncope. Clinical manifestations of HCM are often present during the third and fourth decades of life. However, the age of onset is variable and partly dependent on the underlying mutation. HCM may manifest early in life or during puberty and growth spurt. All of the known causal genes for HCM encode contractile sarcomeric proteins. Mutations in sarcomeric proteins impart a variety of initial defects, such as impaired acto-myosin interaction, impaired activity of the myosin, impaired Ca^{2+} sensitivity and impaired sarcomere formation (Burhop *et al.* 2001). Although diverse mechanisms may be involved in the pathogenesis of HCM phenotypes, it appears that impaired cardiac myocyte contractile function (leading to activation of a variety of stress-responsive trophic and mitotic factors) is the primary abnormality.

Twelve genes have been identified in the aetiology of the pure form of FHC. All of these genes encode proteins of the cardiac sarcomere. Recently, mutations in the *PRKAG2* gene, coding for the γ2-subunit of the AMP-activated protein-kinase, were found to be responsible for familial Wolff-Parkinson-White-syndrome (WPW) with cardiac hypertrophy or for WPW with conduction defect and absence of cardiac hypertrophy. Around 150 mutations have been identified. Most are found in genes *MYH7*, *MYBPC3* and *TNNT2* (Keller *et al.* 2002).

Mutations leading to FHC are missense or frameshift mutations (Perry 1998). The molecular mechanisms are not yet determined. Missense mutations result in stable mutant proteins that act by a dominant negative effect on the structure or function of the sarcomere (poison peptide hypothesis). Frameshift mutations, however, result in unstable truncated proteins. They act as "null-allele" which incites haploinsufficiency of the "wild-type" protein (haploinsufficiency hypothesis).

There are several stages leading to the determination of the FHC phenotype. Initially, factors which vary the penetrance, such as age and gender, impact the phenotype. Next, the phenotype relies upon the responsible mutation. The penetrance and clinical presentation of mutations in *TNNT2* differ greatly. Whereas some mutations result in subclinical hypertrophy associated with high sudden cardiac death (SCD) – risk, for example, others are completely penetrant but without a high risk for arrhythmic events. Third, the phenotype is influenced by the complexity of the genotype. Approximately 8% of the affected families have a complex genotype with homozygous, double or compound heterozygous mutations. Finally, about 25% of genotypically affected patients do not develop a FHC phenotype. Genetic and/or environmental factors may account for the absence of the phenotype in such cases.

TNNT2 gene

Troponin T binds both TnI and TnC in the Tn complex, as well as TM and possibly actin, on the thin filament. The elongated shape of the Tn

complex and its position encompassing both the C-terminal third or more of TM is due to TnT. The elongated N-terminal end of TnT is oriented toward the C-terminus of TM along with the head-to-tail overlap region. The C-terminal half of TnT binds to TnI, to TnC and the middle of TM in a Ca^{2+}- sensitive manner.

The *TNNT2* gene induces a number of cTnT isoforms by alternative splicing. The composition varies from foetal to adult. Furthermore, it may be altered in heart failure (Perry 1998). The principal isoform in the normal adult heart consists of 288 amino acids. The residue numbers of human cTnT are provided below with respect to this isoform. TnT is composed of an extended amino-terminal portion (T1; residues 1-187 in the human cardiac protein) which is found next to Tm alongside the thin filament and a globular carboxy-terminal domain (T2; residues 188–288) which binds to Tm near Cys 190, as well as the amino-terminal portion of TnI and an unidentified domain on TnC. It appears as though TnT is relevant to the distribution of the inhibitory effect of the Tn complex, via Tm, to the seven actin monomers with which it interacts, in the absence of Ca^{2+}, and in the removal of this inhibitory effect from all seven actin monomers, as well as activating the actomyosin ATPase, in the presence of Ca^{2+}. In general, mutations in *TNNT2* gene are associated with mild left ventricular hypertrophy, but a relatively poor prognosis. Despite the significance of the casual mutations, none of the clinical, electrocardiographic or echocardiographic manifestations of HCM are specific to a certain mutation or gene. It is also clear that the causal mutations do not fully explain the degree of variability in the phenotype of HCM; there is a significant variability in the phenotype of HCM among individuals with the same mutation. This finding indicates that other genetic factors (modifier genes) and environmental factors play important roles in modifying HCM phenotypes.

MATERIALS AND METHODS

Study patients

181 Czech probands with HCM/FHC were enrolled in this study. The study group consisted of 24 families with FHC and probands without FHC history but with HCM diagnosis. The clinical diagnosis was based on echocardiography.

Genetic studies

DNA was isolated from peripheral blood lymphocytes by use of phenol-chloroform extraction. Exon 9 and 11 of *TNNT2* gene, which are known to contain mutations associated with FHC, were analyzed by the polymerase chain reaction (PCR) and subsequently by DNA sequencing analyses, which were cross-sequenced. Oligonucleotide primers were used to amplify exons 9 and 11, they were synthesized based on the determined DNA sequences of the normal human *TNNT2* gene as it appears in published sequences.

RESULTS

The ΔGlu160 mutation was observed in patients with severe forms of hypertrophic cardiomyopathy, left ventricle obstruction (gradient 70 mm Hg) and 70% of hypertrophy extent (Čapek 2003). Typically, troponin T mutations cause hypertrophic cardiomyopathy with sub-clinical findings (Sweeney *et al.* 1998). Thus, this unusual result is significant in that it was clinically detected.

CONCLUSION

181 patients were screened for mutations in exons 9 and 11 of the *TNNT2* gene. The ΔGlu160 mutation was observed in patients with severe forms of hypertrophic cardiomyopathy. This region is responsible for binding troponin T to α-tropomyosin (Čapek 2003). This mutation may lead to functional and structural effects on the troponin T protein (Watkins *et al.* 1995, Richard *et al.* 2003).

Another important finding of this study is that the frequency of mutations in the *TNNT2* gene of the Czech patients with FHC diagnosis does not correspond to the frequency of mutations in this gene in other European countries and the United States of America which have been empirically determined at a frequency of 15–20% (Burhop *et al.* 2001).

ACKNOWLEDGEMENTS

This study was supported by MŠMT ČR (Ministry of Education, Youth and Sports of the Czech Republic) grant No. LN00B107.

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