A Novel Splice Donor Site Mutation in the MYBPC3 Gene is Associated with Severe Neonatal Hypertrophic Cardiomyopathy

Baozhong Xin1, Erik Puffenberger2, John Tumbush1, J.R. Bockoven3, Heng Wang1
1Das Deutsch Center (DDC) Clinic for Special Needs Children, Middlefield, OH; 2The Clinic for Special Needs Children, Strasburg, PA; 3The Heart Center, Akron Children Hospital, Akron, OH

Abstract

Hypertrophic cardiomyopathy is inherited in an autosomal dominant pattern with incomplete penetrance and variable clinical presentations. Mutations in the myosin-binding protein C (MYBPC3) gene are one of the most frequent genetic causes of the disease. Patients with MYBPC3 mutations generally have a late onset and a good prognosis. We report here more than 20 Old Order Amish children with severe neonatal hypertrophic cardiomyopathy caused by a novel homozygous splice site mutation in the MYBPC3 gene. The affected children typically presented with signs and symptoms of congestive heart failure during the first three weeks of life. Echocardiography revealed hypertrophic non-obstructive cardiomyopathy. These children had a life span averaging 3 to 4 months. All patients died from heart failure before one year of age unless they received a heart transplant. A genome-wide autozygosity mapping study was performed in three patients using high-density single nucleotide polymorphism (SNP) genotyping arrays. The disease related gene was localized to a 4.6 Mb region on chromosome 1p11.2-p11.12. This region contained 37 characterized genes, including MYBPC3. Sequence analysis of the MYBPC3 gene in the probands identified a novel homozygous mutation, c.3330+2T>G, in the splice-donor site of intron 30, which resulted in skipping of the 140-bp exon 30 and a frame shift with a premature stop codon in exon 31 (p.Asp1064GlyfsX38). We speculate a fairly high prevalence of this phenotype in Old Order Amish communities and many individuals carrying a single c.3330+2T>G allele at risk for development of hypertrophic cardiomyopathy.

Introduction

Hypertrophic cardiomyopathy (HCM) is inherited as an autosomal dominant disease characterized by unexplained left ventricular hypertrophy. The prevalence of the condition is as high as 1 in 500 in the general population. A prominent feature of HCM is the remarkable clinical diversity, ranging from no symptoms to severe heart failure and sudden cardiac death. Genetic causes of HCM are also fairly diverse with more than 400 mutations identified in at least 9 genes encoding sarcomeric proteins.

Mutations in cardiac myosin-binding protein C (MYBPC3) gene are one of the most frequent genetic causes of HCM. In general, patients with HCM caused by MYBPC3 mutations have a more favorable clinical profile, characterized by a late onset and a relatively good prognosis. Homozygous mutation in the MYBPC3 gene causing severe HCM has not been reported to our knowledge. Here we describe a cohort of patients with severe HCM presenting in the neonatal period, caused by homozygosity for a novel mutation in the MYBPC3 gene.

Methods

Subjects: The 23 affected infants were Old Order Amish, with 21 from Ohio and 2 from New York. HCM was clinically diagnosed and confirmed by echocardiography in each patient. All patients died from heart failure before one year of age unless they received a heart transplant. DNA samples from the patients, their parents and siblings were acquired with informed consent.

Genotyping and mapping: High-density SNP genotyping arrays were used to map the disease locus in three affected individuals from three families (Fig. 1). A genome-wide autozygosity mapping localized the disease gene to a 4.6 Mb interval on chromosome 1p11.2-p11.12 (Fig. 2).

Mutation analysis: PCR primers were designed to amplify each of the 34 protein-coding exons and their flanking intronic sequences of MYBPC3. The cDNA was synthesized and amplified using primers covering exons 29 through 31. Direct sequencing was performed on an Applied Biosystems 310 Genetic Analyzer.

RNA Isolation and cDNA Amplification: Total RNA was isolated from whole blood. The cDNA was synthesized and amplified using primers covering exons 29 through 31.

Discussion

Homozygosity for the c.3330+2T>G mutation in the MYBPC3 gene is associated with a severe neonatal HCM. The identification of a single mutation in different families implies a founder effect for this severe condition. In families with shared genetic heritage, genome-wide SNP arrays allow disease gene mapping studies to be incorporated into routine diagnostic evaluations.

It remains concerning that many unidentified individuals in the community carrying a single c.3330+2T>G allele might be at risk of developing HCM. There is an urgent need to develop a practical strategy to deliver medical services to these individuals.

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