Exome sequencing identified mutations in CASK and MYBPC3 as the cause of a complex dilated cardiomyopathy phenotype

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Summary
Whole-exome sequencing for clinical applications is now an integral part of medical genetics practice. Though most studies are performed in order to establish diagnoses in individuals with rare and clinically unrecognizable disorders, due to the constantly decreasing costs and commercial availability, whole-exome sequencing has gradually become the initial tool to study patients with clinically recognized disorders when more than one gene is responsible for the phenotype or in complex phenotypes, when variants in more than one gene can be the cause for the disease. Here we report a patient presenting with a complex phenotype consisting of severe, adult-onset, dilated cardiomyopathy, hearing loss and developmental delay, in which exome sequencing revealed two genetic variants that are inherited from a healthy mother: a novel missense variant in the CASK gene, mutations in which cause a spectrum of neurocognitive manifestations, and a second variant, in MYBPC3, that is associated with hereditary cardiomyopathy. We conclude that although the potential for co-occurrence of rare diseases is higher when analyzing undefined phenotypes in consanguineous families, it should also be given consideration in the genetic evaluation of complex phenotypes in non-consanguineous families.

Introduction
The hereditary dilated cardiomyopathies (DCM) are characterized by enlargement of one or both ventricles of the heart, accompanied by left ventricular systolic dysfunction. DCM can be classified into isolated (or non-syndromic) and syndromic forms. Numerous genes that encode a variety of cardiomyocyte proteins have been identified for DCM in its syndromic and non-syndromic forms (McNally et al., 2013; Towbin 2014).

Heterozygous loss-of-function mutations in the X-linked CASK gene cause progressive microcephaly with pontine and cerebellar hypoplasia (MICPCH) and severe intellectual disability in females (Hackett et al., 2010; Burglen et al., 2012; Moog et al., 2015). In males, different CASK mutations have also been reported causing three phenotypic groups that represent a clinical continuum: (i) MICPCH with severe epileptic encephalopathy caused by hemizygous loss-of-function mutations; (ii) MICPCH associated with inactivating alterations in the mosaic state or a partly penetrant mutation; and (iii) syndromic/non-syndromic mild to severe intellectual disability with or without nystagmus caused by CASK missense and splice mutations that leave the CASK protein intact but likely alter its function or reduce the amount of normal protein (Moog et al., 2015).

Here we report an adult patient presenting with a complex phenotype consisting of severe dilated cardiomyopathy, hearing loss and developmental delay, in which exome sequencing revealed two variants, in the MYBPC3 and CASK genes, that are inherited from a healthy mother.

Methods
Whole-exome sequencing
Genomic DNA extraction, exome enrichment, sequencing and analysis were completed as described before (Reinstein et al., 2015). Briefly, genomic
DNA was extracted from peripheral leukocytes following standard protocols. Exome enrichment was achieved by the Nextera Rapid Capture Expanded Exome Kit (FC-140-1006) following manufacturer’s guidelines. Sequencing was performed using the Illumina HiSeq 2500 machinery to generate paired end reads of 150 bp with average coverage of 80X at the genetics and genomic medicine laboratory (Gene by Gene, Houston, Texas). Bioinformatic analysis was done by mapping the obtained fragments to the human reference genome (hg19) with the Burrows-Wheeler Alignment algorithm (BWA MEM), calling variants using SAMTOOLS (Li et al., 2009) and annotating them using SNPeff (Cingolani et al. 2012). The results were examined based on all modes of inheritance. Variants were filtered to generate a final list of rare functional variants (missense, nonsense, splice site variants and indels), and the removal of polymorphic variants that have minor allele frequency >0.01 in the Exome Variant Server (release ESP6500; 2014) or that have allele count >150 in ExAC database of Europeans (NFE). For validation and segregation analyses, PCR primers were designed to amplify the regions flanking the two mutations. PCR products were purified using magnetic-particle technology (Serady, Inc.). After purification, all fragments were sequenced using forward and backward internal primers to determine the noted regions. Sequencing was performed on a 3730x1 DNA Analyzer (Life Technologies), and the resulting sequences were analyzed with the Sequencher software (Gene Codes Corporation). Mutations were scored relative to the reference sequences deposited in the National Center for Biotechnology Information.

Results

Clinical report

The nuclear family is of Arab Muslim ancestry, and consists of parents and six children. The parents are non-consanguineous and healthy. The proband was presented to the emergency department at age 32 years with fever and shortness of breath. Blood count demonstrated leukocytosis and a chest x-ray, performed because of suspected pneumonia, demonstrated no pathogenic infiltrates but an enlarged cardiac silhouette. A follow-up echocardiogram demonstrated severe systolic dysfunction and the patient was transferred to the cardiac intensive care unit at a tertiary medical center with suspected fulminant myocarditis. Cardiac CT excluded coronary artery disease. As no obvious cause for his heart disease was established, a diagnosis of idiopathic dilated cardiomyopathy was given and a medical genetics consultation was requested. The proband was born at term and had mild–moderate gross developmental delay. He attended special education school and was diagnosed with sensorineural hearing loss at age 2 years. He did not have hospital admissions prior to the present one and no other medical complications. Ophthalmologic, neurologic and neuro-ophthalmologic examinations were normal and he had normal facial features (Fig. 1 (a)), and microcephaly (OFC: 53 cm; ~2 percentile). The proband has five additional siblings, aged 33, 29, 23, 22 and 8 years (Fig. 1 (b)); all are healthy, non-dysmorphic and have normal echocardiograms. Parents are in their early 60s and have normal echocardiograms.

Molecular investigation

A chromosomal microarray study demonstrated no copy number variations and no regions of loss of heterozygosity. Common mutations in Connexins 26 and 30 have been excluded. Next, we carried out a whole-exome sequencing study using DNA samples of the proband, his 33 year old healthy brother and their parents. The Rabin Medical Center Institutional Review Board provided local approval for this study, and all participants signed an informed consent. Exome results revealed two findings, discussed below.

The first finding was a heterozygous missense variant in the MYBPC3 gene: MYBPC3 rs371401403, c.2618C>T, p.Pro873Leu (chr11:47357547G>A, NM_000256). The second finding was a hemizygous missense variant in the CASK gene c.2126A>G, p. Lys709Arg (chrX:41401973 T>C, NM_003688). The variant in MYBPC3 has been reported several times before as disease causing in patients with dilated cardiomyopathy (Nanni et al., 2003). The amino acid change involves a highly conserved residue in the fibronectin type-3 domain (C7), which is required for the proper integration of MYBPC3 into the thick filament of the sarcomere. The variant in CASK was not found in any public database including the ExAC database that contains over 60 000 individuals. Most functional prediction tools suggested a damaging effect of this amino acid change (Polyphen2_HDIV:P; LRT:D; MutationTaster:D; FATHMM:D; MutationAssessor: N; SIFT:T) and evolutionary conservation measures among different species presented high values in this particular site (GERP+:++5.73, phyloP100way:7-65, Siphyl29way:15-21). Most importantly, there is a significant phenotypic similarity between the proband and previously reported individuals with CASK gene mutations. Validation and segregation analysis was completed using DNA samples of additional family members (Fig. 1(b)) and showed that both variants were transmitted from the mother. The variants in MYBPC3 and CASK were the only ones that were found to segregate as expected in the family. This analysis further identified three heterozygous female carriers for the CASK variant and three heterozygous female carriers for MYBPC3.
carriers for the MYBPC3 variant (Fig. 1(b)). Carriers for either the CASK or the MYBPC3 variants were cognitively intact while echocardiograms revealed normal heart function.

Discussion

We present a family with an apparently novel phenotype caused by two different Mendelian disorders identified by exome sequencing. The first is a CASK-related disorder caused by a novel variant in the CASK gene, mutations in which cause a spectrum of phenotypes that have recently been delineated in more details (Hackett et al., 2010; Moog et al., 2011; Burglen et al., 2012; Moog et al., 2015). The phenotype of our subject, a male patient with microcephaly, mild developmental delay and sensorineural hearing loss, and two asymptomatic female carriers, is consistent with phenotypic group (iii). The general phenotype of males in phenotypic group (iii) consists of mild to severe intellectual disability, microcephaly in some of them and an unknown proportion of brain anomalies, as brain imaging is not usually undertaken. Sensorineural hearing loss was rarely described in patients classified to this group. It is difficult to predict the pathogenicity of this novel variant; however, males with missense variants in CASK can have a relatively mild phenotype with mild to moderate intellectual disability and microcephaly. The missense variants found in group (iii) males are usually hypomorphic mutations (Hackett et al., 2010; Moog et al., 2011; Burglen et al., 2012; Moog et al., 2015). The identified CASK missense variant is compliant with X-linked recessive disorder, it is predicted to be damaging by most prediction tools, located in a highly conserved protein domain, segregating in the family, absent in large control cohorts, and most importantly, consistent with the proband phenotype, thus most likely is associated with the phenotype.

Isolated (non-syndromic) DCM of unknown cause has been shown to have a genetic basis in many cases and numerous genes have been identified for DCM in its non-syndromic forms. DCM may be asymptomatic for many years with onset that can occur in the fourth to sixth decade of life. A common clinical presentation, such as the one described in this report, is sudden heart failure symptoms elicited by a non-related insult (infection, strenuous activity etc.).
Mutations in the gene encoding cardiac myosin-binding protein C (MYBPC3) account for approxi-
mately 15% of cases of familial hypertrophic cardiomyopathy (HOCM) and up to 4% of cases of DCM (Frey et al., 2011; Marston et al., 2012; McNally et al., 2013). Of note, patients with MYBPC3 mutations have been reported to have a later age of disease onset (Niimura et al., 1998) compared with other genetic causes of HOCM. Only 58% of adults under the age of 50 years who had a mutation in the MYBPC3 gene (68 of 117 patients) had cardiac hypertrophy; disease penetrance remained incomplete through to the age of 60 years. Survival was generally better than observed in patients with HOCM caused by mutations in other genes encoding sarcomeric proteins. Similar data on patients with MYBPC3 mutations and dilated cardiomyopathy is not available due to the limited number of reported cases. Nevertheless, our observations in the reported family, that is, three heterozygote mutation carriers with normal echocardiograms and no cardiac symptoms, suggest that DCM penetrance in MYBPC3 mutation carriers is also incomplete.

In conclusion, though the identified CASK-related disorder is coincidental to the DCM syndrome and has no implications for the immediate medical treatment of the proband, its identification has important future prenatal implications for the extended family.

Declaration of interest

Doron M. Behar and Concetta Bormans are compensated and serve as the chief medical officer and the laboratory director of Gene by Gene, respectively.

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