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

EYAL REINSTEIN[1,2]*, SHAY TZUR[3,4], CONCETTA BORMANS[5] AND DORON M. BEHAR[5]

[1]*Medical Genetics Institute, Meir Medical Center, Israel*
[2]*Sackler School of Medicine, Tel Aviv University, Israel*
[3]*Laboratory of Molecular Medicine, Rambam Health Care Campus, Haifa, Israel*
[4]*Genomic Research Department, Emedgene Technologies, Tel-Aviv, Israel*
[5]*Genomics Research Center, Gene by Gene, Houston, Texas, USA*

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

* Corresponding author: Eyal Reinstein, MD, PhD, Medical Genetics Institute, Meir Medical Center, Kfar-Saba, Israel. Fax: +972-9-747-2648. E-mail: reinstein.eyal@gmail.com or eyalre1@clalit.org.il

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 (Seradyn, Inc.). After purification, all fragments were sequenced using forward and backward internal primers to determine the noted regions. Sequencing was performed on a 3730xl 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, SiPhy_29way: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
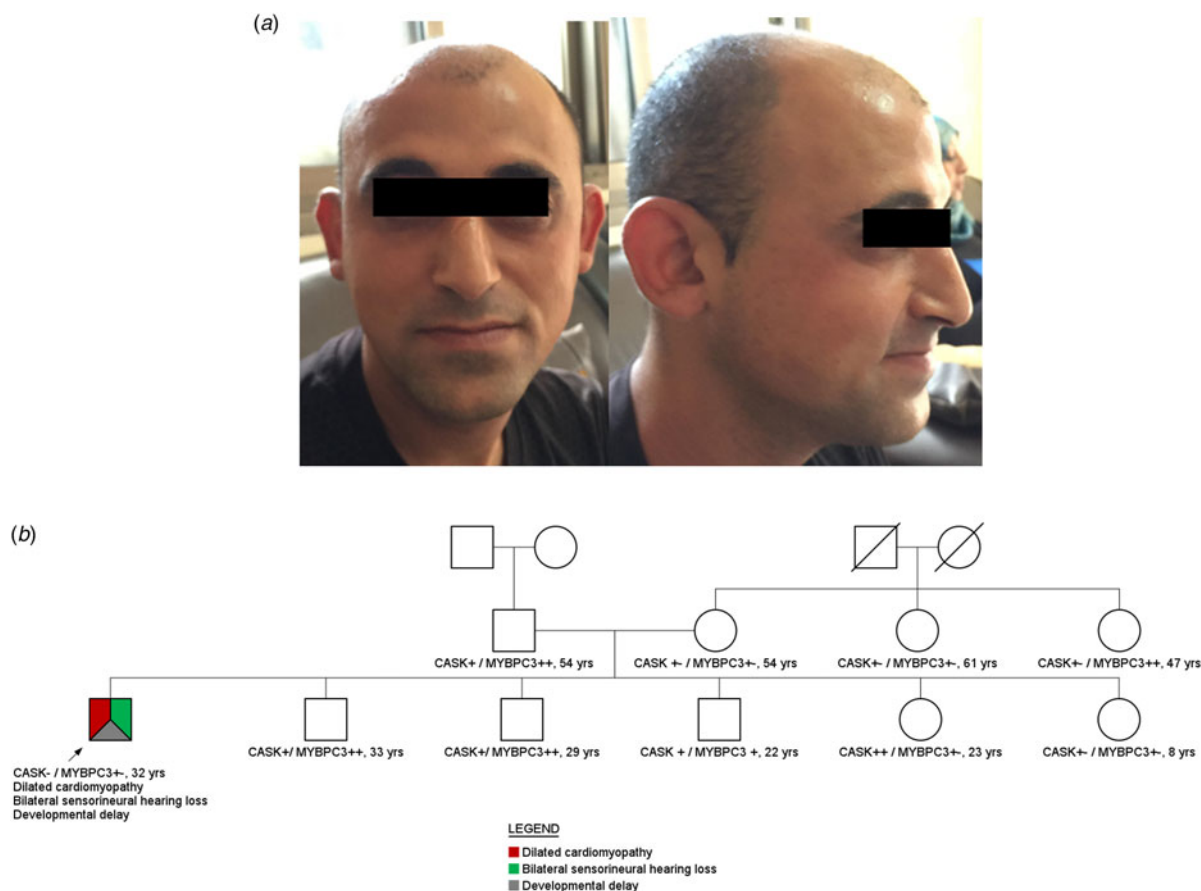
Fig. 1. (*a*) Facial features of the proband showing mild dysmorphic features including triangular face, microcephaly, and low-set protruding ears. (*b*) Pedigree of family showing the age of individuals and segregation of *CASK* and *MYBPC3* mutant alleles. Wild-type alleles are indicated by plus sign. Filled symbol indicates affected individual. Diagonal lines across symbols indicate deceased individuals.

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 approximately 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.

References

Burglen, L., Chantot-Bastaraud, S., Garel, C., Milh, M., Touraine, R., Zanni, G., Petit, F., Afenjar, A., Goizet, C., Barresi, S., Coussement, A., Ioos, C., Lazaro, L., Joriot, S., Desguerre, I., Lacombe, D., des Portes, V., Bertini, E., Siffroi, J. P., de Villemeur, T. B. & Rodriguez, D. (2012). Spectrum of pontocerebellar hypoplasia in 13 girls and boys with *CASK* mutations: confirmation of a recognizable phenotype and first description of a male mosaic patient. *Orphanet Journal of Rare Diseases* **7**, 18.

Cingolani, P., Platts, A., Wang le, L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X. & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)* **6**(2), 80–92.

Exome Variant Server (2014). NHLBI GO Exome Sequencing Project (ESP), Seattle, WA. Available at http://evs.gs.washington.edu/EVS/ (accessed 5 April 2016).

Frey, N., Luedde, M. & Katus, H. A. (2011). Mechanisms of disease: hypertrophic cardiomyopathy. *Nature Reviews Cardiology* **9**(2), 91–100.

Hackett, A., Tarpey, P. S., Licata, A., Cox, J., Whibley, A., Boyle, J., Rogers, C., Grigg, J., Partington, M., Stevenson, R. E., Tolmie, J., Yates, J. R., Turner, G., Wilson, M., Futreal, A. P., Corbett, M., Shaw, M., Gecz, J., Raymond, F. L., Stratton, M. R., Schwartz, C. E. & Abidi, F. E. (2010). *CASK* mutations are frequent in males and cause X-linked nystagmus and variable XLMR phenotypes. *European Journal of Human Genetics* **18**(5), 544–552.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics* **25**(16), 2078–2079.

Marston, S., Copeland, O., Gehmlich, K., Schlossarek, S. & Carrier, L. (2012). How do *MYBPC*3 mutations cause hypertrophic cardiomyopathy? *Journal of Muscle Research and Cell Motility* **33**(1), 75–80.

McNally, E. M., Golbus, J. R. & Puckelwartz, M. J. (2013). Genetic mutations and mechanisms in dilated cardiomyopathy. *Journal of Clinical Investigation* **123**(1), 19–26.

Moog, U., Bierhals, T., Brand, K., Bautsch, J., Biskup, S., Brune, T., Denecke, J., de Die-Smulders, C. E., Evers, C., Hempel, M., Henneke, M., Yntema, H., Menten, B., Pietz, J., Pfundt, R., Schmidtke, J., Steinemann, D., Stumpel, C. T., Van Maldergem, L. & Kutsche, K. (2015). Phenotypic and molecular insights into CASK-related disorders in males. *Orphanet Journal of Rare Diseases* **10**, 44.

Moog, U., Kutsche, K., Kortüm, F., Chilian, B., Bierhals, T., Apeshiotis, N., Balg, S., Chassaing, N., Coubes, C., Das, S., Engels, H., Van Esch, H., Grasshoff, U., Heise, M., Isidor, B., Jarvis, J., Koehler, U., Martin, T., Oehl-Jaschkowitz, B., Ortibus, E., Pilz, D. T, Prabhakar, P., Rappold, G., Rau, I., Rettenberger, G., Schlüter, G., Scott, R. H., Shoukier, M., Wohlleber, E., Zirn, B., Dobyns, W. B. & Uyanik, G. (2011). Phenotypic spectrum associated with CASK loss-of-function mutations. *Journal of Medical Genetics* **48**(11), 741–751.

Nanni, L., Pieroni, M., Chimenti, C., Simionati, B., Zimbello, R., Maseri, A., Frustaci, A. & Lanfranchi, G. (2003). Hypertrophic cardiomyopathy: two homozygous cases with "typical" hypertrophic cardiomyopathy and three new mutations in cases with progression to dilated cardiomyopathy. *Biochemical and Biophysical Research Communications* **309**(2), 391–398.

Niimura, H., Bachinski, L. L., Sangwatanaroj, S., Watkins, H., Chudley, A. E., McKenna, W., Kristinsson, A., Roberts, R., Sole, M. & Maron, B. J., Seidman, J. G. & Seidman, C. E. (1998). Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. *New England Journal of Medicine* **338**(18), 1248–1257.

Reinstein, E., Orvin, K., Tayeb-Fligelman, E., Stiebel-Kalish, H., Tzur, S., Pimienta, A. L., Bazak, L., Bengal, T., Cohen, L., Gaton, D. D., Bormans, C., Landau, M., Kornowski, R., Shohat, M. & Behar, D. M. (2015). Mutations in *TAX*1*BP*3 cause dilated cardiomyopathy with septo-optic dysplasia. *Human Mutation* **36**(4), 439–442.

Towbin, J. A. (2014). Inherited cardiomyopathies. *Circulation Journal* **78**, 2347–2356.