

Blueprint Genetics

Test results

REFERRING HEALTHCARE PROFESSIONAL

REFERRING HEALTHCARE PROFESSIONAL

HOSPITAL

BpG

PATIENT

NAME Core Panel, Anonym	DOB 1950-01-01	AGE 66	GENDER Male	ORDER ID 5239
PRIMARY SAMPLE TYPE DNA	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID		

SUMMARY OF RESULTS

TEST RESULTS

TNNI3 c.434G>A, p.(Arg145Gln) is pathogenic.

GENETIC VARIANTS

VARIANT TABLE: Genetic alterations

GENE	POS	ID	CODON	CONSEQUENCE	TRANSCRIPT	DNA	PROTEIN	GENOTYPE	1000G	CLASSIFICATION
TNNI3	19:55665513	rs397516349	cGg/cAg	missense	NM_000363.4	c.434G>A	p.Arg145Gln	HOM	-	

SEQUENCING PERFORMANCE METRICS

PANEL Core cardiomyopathy	GENES 72	EXONS 1439	BASES 304264	BASES > 15X 303878	MEDIAN COVERAGE 263	PERCENT > 15X 99.8
-------------------------------------	--------------------	----------------------	------------------------	---------------------------------	-------------------------------	---------------------------------

TARGET REGION AND GENE LIST

Blueprint Genetics Core cardiomyopathy panel (version 1.1, updated May 6, 2014) consists of genes associated with cardiomyopathy: ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CAV3, CRYAB, CSRP3, CTF1, CTNNA3, DES, DMD, DNAJC19, DNMT1L, DSC2, DSG2, DSP, DTNA, EMD, EYA4, FHL1, FHL2, FKTN, FXN, GATAD1, GLA, HFE, ILK, JPH2, JUP, LAMA4, LAMP2, LDB3, LMNA, MIB1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOM1, MYOZ2, MYPN, NEBL, NEXN, PDLIM3, PKP2, PLN, PRKAG2, PSEN1, PSEN2, RBM20, RYR2, SCN5A, SDHA, SGCD, TAZ, TCAP, TGFB3, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TRIM63, TTN, TTR, TXNRD2, VCL. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This diagnostic tool covers the majority of known cardiomyopathy mutations to date. The test is not validated to identify large CNVs. It should be used to diagnose mutations such as single nucleotide substitutions and small indels.

STATEMENT

CLINICAL HISTORY

The patient is a 66-year-old male with hypertrophic cardiomyopathy (HCM) diagnosed at the aged of 14 years. He has positive family history of HCM.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Core Cardiomyopathy Panel identified a homozygous missense variant c.434G>A, p.(Arg145Gln) (rs397516349) in *TNNI3*. It is predicted deleterious by *in silico* programs PolyPhen, SIFT and MutationTaster. There are three heterozygous carriers of the variant in the Exome Aggregation Consortium ExAC control cohorts, comprised of over 60,000 unrelated individuals (<http://exac.broadinstitute.org>).

TNNI3 c.434G>A, p.(Arg145Gln) has been reported in the literature in at least three patients affected with hypertrophic cardiomyopathy (HCM; PubMed: 9241277, 15607392, 23283745). In addition, it has been detected in clinical testing in at least four patients with HCM and listed in ClinVar as a likely pathogenic or pathogenic variant (ClinVar: SCV000059947.3, SCV000209169.1, SCV000253695.1, SCV000220055.1). Moreover, different missense variants in the same codon (Arg145Gly and Arg145Trp) have been described in patients with HCM (ClinVar). Functional studies have demonstrated that the variants at codon 145 affect contraction of the cardiac muscle (PubMed: 11735257).

The *TNNI3* gene encodes a protein called cardiac troponin I (TnI), which is found solely in the heart muscle. Cardiac TnI is one of three proteins that make up the troponin protein complex of the titin filament in cardiac muscle cells. TnI is the inhibitory subunit; blocking actin-myosin interactions and thereby mediating muscle relaxation. Mutations in *TNNI3* have been previously associated with familial HCM, dilated cardiomyopathy (DCM) and familial restrictive cardiomyopathy (RCM; OMIM: *191044). It is estimated that about 2-7% of familial cardiomyopathy cases are caused by a mutation in the *TNNI3* (PubMed: 21533915). It has been demonstrated, that clinical expression of *TNNI3* mutations is heterogeneous within and between families with no apparent mutation or gene-specific disease pattern (PubMed:15607392). The majority of the *TNNI3* mutations (85%) have been identified in exon 7 and exon 8, encoding domains interacting with cardiac actin (*ACTC1*) and cardiac troponin C (*TNNC1*; PubMed: 9241277, 21533915). Six of the mutations reported (Arg141Gln, Arg145Trp, Arg157Val, Arg162Gln, Ser166Phe, and Lys183Del) appeared with a particularly high frequency and were identified in 116 of the 256 (45%) probands in recent review (PubMed: 26440512).

In general, patients with double mutations in sarcomere genes have more severe HCM phenotype and an earlier age at disease onset compared to heterozygotes (PubMed: 15519027). There is limited knowledge of homozygous or compound heterozygous *TNNI3* mutations. We have previously identified a homozygous missense variant Arg162Gln in a young adult patient with HCM and no family history (unpublished observation). Gray et al. found a homozygous variant affecting the same codon p.(Arg162Trp) from a HCM family with a consanguineous marriage, in which both homozygous carriers had HCM (PubMed: 23270746). The proband was a 17-year-old girl with NYHA-Class III-IV heart failure, mild LVH (septum 14 mm), extensive non-compaction, as well as delayed enhancement in MRI and atrial arrhythmias. Her brother was successfully resuscitated from cardiac arrest while being asymptomatic although echo revealed severe LVH after incident. The heterozygous parents were shown to be healthy. Another homozygous *TNNI3* mutation Ala2Val has been previously identified in a family with recessive dilated cardiomyopathy (PubMed: 15070570). The index patient underwent cardiac transplantation aged 28 years and his sister had NYHA class III heart failure at age of 29. Zheng et al. published another familial recessive HCM associated with compound heterozygous *TNNI3* variants (c.235C>T, p.(Arg79Cys) and c.470C>T p.(Ala157Val), of which the first one is classified as likely benign by two laboratories (ClinVar: SCV000059927.2 and SCV000220045.1) (PubMed: 26506446). The asymptomatic mother with upper normal septum thickness (11 mm) at age of 48 has only the Ala157Val mutation but is at the time of the study negative for HCM. Thus, two sons carrying both variants and diagnosed with HCM at early age may still have

autosomal dominant HCM.

By searching the literature, we have identified 33 published cases with homozygous or compound heterozygous mutations in other sarcomere gene *MYBPC3*, of which 11 cases carried two mutations that we classify as disease causing. Mutation localization in trans was confirmed in nine out of 11 cases (PubMed: 12951062, 20031619, 22907696 and 20378854). Of these patients, mean age at onset was 4.1 years. Three cases presented at neonatal phase, of which all died before age of two months (PubMed: 20031619 and 22907696). All patients with homozygous or compound heterozygous truncating pathogenic mutations in *MYBPC3* reported so far (n=21) were diagnosed with severe cardiomyopathy and/or died within the first few months of life (PubMed: 25335496). In contrast to heterozygous pathogenic mutations, biallelic truncating pathogenic *MYBPC3* mutations cause severe neonatal cardiomyopathy with features of HCM, DCM, LVNC, and septal defects in approximately 60% of the patients.

Mutation nomenclature is based on GenBank accessions NM_000363.4 (*TNNI3*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and well-established role of *TNNI3* c.434G>A, p.(Arg145Gln) as a disease causing mutation, we classify it as pathogenic. Homozygosity of this variant is considered as the major cause for early onset manifestation of the disease. Genetic counseling and family member testing is recommended. Disease caused by *TNNI3* mutations is primarily dominantly inherited. In this case there is 100% risk for the offspring to be heterozygous mutation carrier. BpG offers mutation testing for the family if requested.

CONFIRMATION

TNNI3 c.434G>A, p.(Arg145Gln) was confirmed by bidirectional Sanger sequencing.

STEP	DATE
Order date	Feb 09, 2016
Sample received	Feb 09, 2016
Reported	Feb 29, 2016

On Feb 29, 2016 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



Eija Seppälä, Ph.D.
Senior Geneticist



Tero-Pekka Alastalo, MD, Ph.D.
Chief Medical Officer



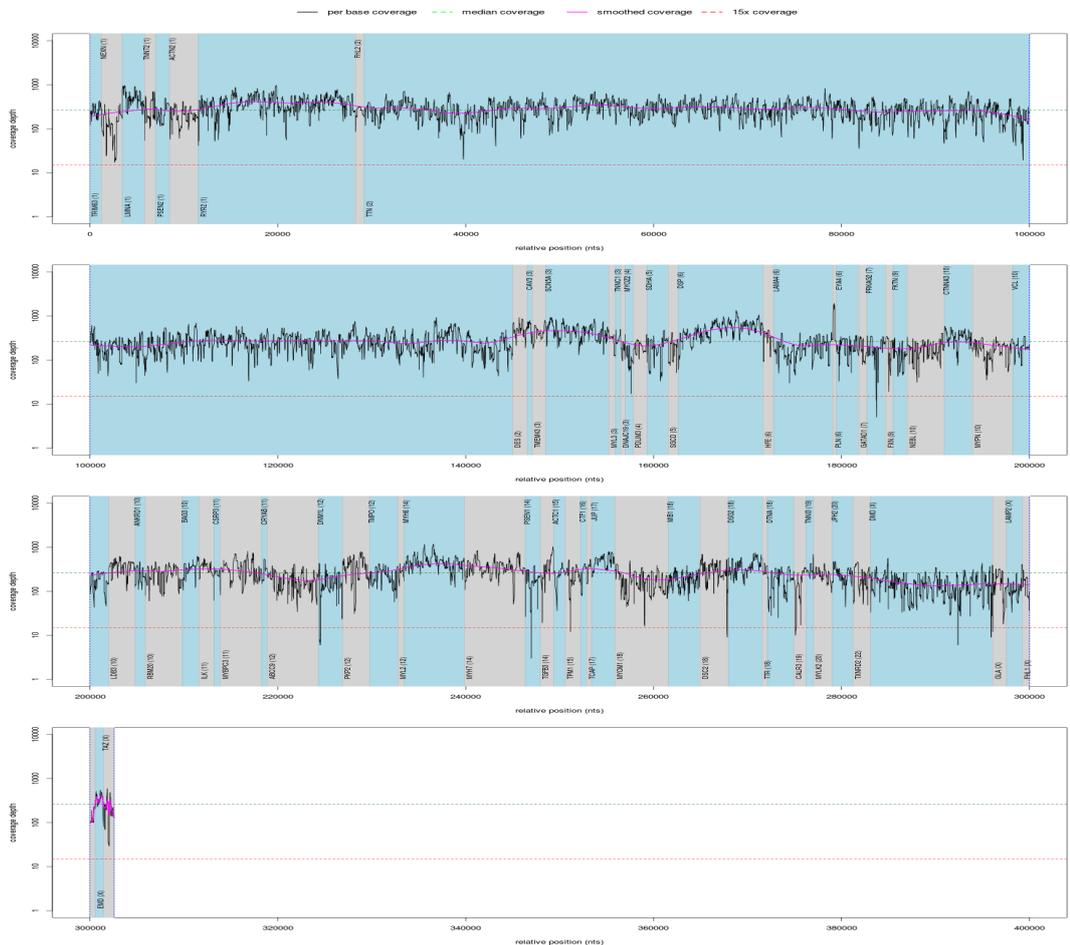
Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer

APPENDIX 3: SEQUENCING COVERAGE

COVERAGE PLOT

Coverage plot for sample 5239

Panel type: Core cardiomyopathy
Number genes: 72
Median coverage: 263
Mean coverage: 291
Percent above 15x coverage: 99.8



APPENDIX 4: SUMMARY OF METHODS

Total genomic DNA was extracted from the biological sample using a spin column method. DNA quality and quantity were assessed through gel electrophoresis and fluorometric analysis, respectively. The DNA was then enzymatically fragmented and adapters were then added using a ligation-based method. The sequencing library was amplified using PCR, after which quality and quantity were assessed through electrophoresis and fluorometric analysis, respectively. A proprietary OS-Seq method was used for targeted sequencing, which was performed using an Illumina sequencing device. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads of sufficient length were mapped to the human genome reference sequence (Hg19). Single nucleotide polymorphisms and short insertions / deletions were identified using a proprietary analysis pipeline. Variant findings were compared to our in-house curated and maintained mutation database to identify known pathogenic variants. The pathogenic potential of other variants was predicted by taking into account the biochemical properties of the codon change, the degree of evolutionary conservation as well as allelic frequencies from large population studies, including e.g. data from the 1000 Genomes project, the ExAC consortium and ClinVar archive. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data. Variant(s) classified as pathogenic or likely pathogenic were confirmed using Sanger sequencing. Reporting is done using HGNC-approved gene nomenclature and mutation nomenclature follows the HGVS guidelines. This test has been independently validated by Blueprint Genetics. This method has been accredited by FINAS Finnish Accreditation Service, accreditation requirement SFS-EN ISO/IEC 15189.
