Blueprint Genetics

Cardiomyopathy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		75	Male	12345

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

TEST RESULTS

***TTN* c.101113del, p.(Ser33705Leufs*4) and *DSG2* c.2315T>G, p.(Leu772*) are likely pathogenic.**

***MYH7* c.4030C>T, p.(Arg1344Trp) and *PRDM16* c.2455G>A, p.(Gly819Arg) are variants of uncertain significance (VUS).**

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
TTN	2:179400228	NM_001267550.1	c.101113del, p.(Ser33705Leufs*4)	frameshift	HET	**Likely pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	.	0/0	N/A	N/A	N/A	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Dilated cardiomyopathy (DCM), Hypertrophic cardiomyopathy (HCM)		AD	-	

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
DSG2	18:29122796	NM_001943.3	c.2315T>G, p.(Leu772*)	stop_gained	HET	**Likely pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs794728097	0/0	N/A	N/A	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Arrhythmogenic right ventricular dysplasia, Dilated cardiomyopathy (DCM)		AD	-	

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
MYH7	14:23887558	NM_000257.2	c.4030C>T, p.(Arg1344Trp)	missense	HET	**Variant of uncertain significance**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs727504352	2/120556	benign	deleterious	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Dilated cardiomyopathy (DCM), Hypertrophic cardiomyopathy (HCM), Myopathy, distal, Myopathy, myosin storage		AD&AR	-	

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
PRDM16	1:3329216	NM_022114.3	c.2455G>A, p.(Gly819Arg)	missense	HET	**Variant of uncertain significance**

ID	EXAC AC/AN	POLYPHEN	SIFT	MUTTASTER
rs755243204	0/28048	possibly damaging	tolerated	polymorphism

	PHENOTYPE	INHERITANCE	COMMENT
OMIM	Dilated cardiomyopathy (DCM), Left ventricular noncompaction	AD	-

Please see APPENDIX 2: Additional Findings

SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Cardiomyopathy Panel	134	2463	480171	479157	190	99.7

TARGET REGION AND GENE LIST

Blueprint Genetics Cardiomyopathy Panel (version 1, August 25, 2016) consists of sequence analysis of genes associated with arrhythmogenic right ventricular cardiomyopathy (ARVC), cardiomyopathy NAS, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), left ventricular non-compaction cardiomyopathy (LVNC), Noonan syndrome and RCM: A2ML1, AARS2, ABCC9, ACAD9, ACADVL, ACTA1, ACTC1, ACTN2, AGK*, AGL, ALPK3, ANKRD1, ANO5, APOA1, BAG3, BRAF*, CAPN3, CASQ2, CAV3, CBL, CHKB, COX15, CPT1A, CPT2, CRYAB, CSRP3, CTNNA3, DAG1, DBH, DES, DMD, DNAJC19, DSC2, DSG2, DSP, DYSF, EMD, ETFA, ETFB, ETFDH, EYA4, FBXO32, FHL1*, FKRP, FKTN, FLNC*, FOXRED1, FXN*, GAA, GATAD1, GBE1, GFM1, GLA, GLB1, GMPPB, GNE, GUSB*, HCN4, HFE, HRAS, ISPD, JPH2, JUP, KRAS*, LAMA2, LAMP2, LARGE, LDB3, LMNA, LZTR1, MAP2K1, MAP2K2, MTO1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOT, MYPN, NEXN, NF1*, NRAS, NSUN2, PKP2*, PLEC, PLEKHM2, PLN, PNPLA2, POMGNT1, POMT1, POMT2, PRDM16, PRKAG2, PTPN11, RAF1, RASA2, RBM20, RIT1, RRAS, RYR2, SCN5A, SCNN1B, SCNN1G, SCO2, SDHA*, SELENON, SGCA, SGCB, SGCD, SGCG, SHOC2, SLC22A5, SLC25A4, SLC25A20, SMCHD1, SOS1, SPRED1, TAZ, TCAP, TGFB3, TMEM43, TMEM70, TNNC1, TNNI3, TNNT2, TPM1, TRIM32, TSFM, TTN*, TTR, VCL, VCP and XK. 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 test covers the majority of arrhythmogenic right ventricular cardiomyopathy (ARVC), cardiomyopathy NAS, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), left ventricular non-compaction cardiomyopathy (LVNC), Noonan syndrome and RCM mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Cardiomyopathy Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

STATEMENT

CLINICAL HISTORY

Patient is a 75-year-old male with DCM diagnosed in 2015. He has had atrial fibrillation. In Echo, LVEF was 26%. Coronary angiography did not identify coronary artery disease. After starting heart failure medical treatment, patient has felt better and LVEF increased to 40-45%. In latest echocardiography, LVEDD is 52 mm, LV-EF 45%, septum11 mm and LVPW 11 mm. There was also suspicion of non-compaction cardiomyopathy in cardiac MRI. Patient`s son has lightly enlarged left ventricle, systolic function normal, in MRI suspicion of non-compaction CMP, but diagnostic criteria was not fulfilled.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Cardiomyopathy Panel identified a heterozygous 1-bp deletion c.101113del, p.(Ser33705Leufs*4) in the A-band of *TTN*. It affects coding region of all long *TTN* trancsripts. This variant cause a frameshift leading to a premature stop codon at residue 4 in a new reading frame. Thus, it is predicted to cause loss of normal protein function either through protein truncation (33709 out of 35991 aa) or nonsense-mediated mRNA decay. It has not been observed in the Exome Aggregation Consortium (ExAC) data set, comprised in total of over 60,000 unrelated individuals or in larger reference population (Genome Aggregation Database, gnomAD, total of 126,216 exomes and 15,137 genomes). The *TTN* truncation variant classification software categorizes this variant into the group I (the most deleterious group of *TTN* truncations) in a scale where all *TTN* truncations are classified into groups I-VI. The group I has DCM disease odds ratio of 43 (95% CI 33-53) (PMID: 27625338; link to software: Deo 2016).

Mutations in the *TTN* have been previously reported to associate with cardiomyopathies such as dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC). *TTN* mutations also associate with congenital myopathy and limb-girdle muscular dystrophy. Few studies have applied next generation sequencing technologies to analyze the *TTN* variants in large cardiomyopathy patient cohorts and controls. Herman *et al.* sequenced the *TTN* gene in 312 subjects with DCM and 231 subjects with HCM. The frequency of truncating *TTN* mutations (nonsense, frame-shift and splice site mutations) was 27% in patients with DCM. Mutations in DCM patients (>84%) were overrepresented in the titin A-band and were absent from the Z-disc and M-band regions of titin. In families with DCM, *TTN* mutations co-segregated with DCM and showed over 95% penetrance within the family. Herman et al. concluded that *TTN* truncating mutations are a common cause of DCM, occurring in approximately 25% of familial DCM cases and in 18% of sporadic cases. Their results suggested that *TTN* truncations rarely, if ever, cause HCM (PMID: 22335739). Similarly, we have identified nonsense, frame-shift and consensus splice site *TTN* mutations in 21% and 15% of patients with familial and sporadic DCM, respectively (Finnish DCM study cohort: n=145, PMID: 26084686). We have performed meta-analysis of *TTN* truncations in all large DCM cohorts and ExAC reference population (PMID: 26701604 and 26777568). We found out that the enrichment of *TTN* truncations is most prevalent in A-band and the least significant in Z-disk (A-band: 12.25% vs. 0.19% (p=1.3x10-251), I-band: 2.52% vs. 0.07% (p=1.9x10-46), M-band: 0.62% vs. 0.05% (p=3.2x10-08) and Z-disk: 0.17% vs. 0.03% (p=2.4x10-02) (PMID: 26777568). We identified truncating *TTN* variants affecting five out of seven transcripts in unselected DCM patients and estimated the probability of pathogenicity of these variants to be 97.8% (likelihood ratio 42.2) (PMID: 26777568). By evaluating the ExAC control population of over 60 000 individuals, we have shown that truncating *TTN* variants affecting all six long transcripts occur in 0.36% of the general population (PMID: 26701604). Jansweijer et al. observed recently differences in responses to conventional heart failure medication among genetically different DCM patients: LVEF elevated at least 10% among 46.9% of DCM patients with a truncating *TTN* variant but only in 18% of idiopathic DCM patients or in 6.5% of cardiomyopathy patients with a *LMNA* variant (PMID: 27813223). European Society of Cardiology does not recommend competitive sports for cardiomyopathy patients and even family members who are genotype positive yet phenotype negative are advised to avoid competitive sports (PMID: 15923204).

Arscott et al. published two patients who had truncating *TTN* variants and the other had DCM+LVNC phenotype and the other isolated LVNC. In the family, where the proband had isolated LVNC, the detected Pro28985Glnfs*2 segregated either with LVNC or DCM phenotype in the family members (http://ondemand.hrsonline.org/common/presentation-detail.aspx/15/23/1391/10345). Recently, Waldmuller *et al*. sequenced a total of 25 cardiomyopathy samples including two patients with LVNC. One of the patients carried two relatively common (MAF 0.4%) missense variants in the *TTN* gene, which were kept as disease causing for recessive LVNC. This is based on the use of non-accepted variant classification scheme and thus the statement of pathogenicity is not clinically valid (PubMed 25979592). In most cases, when we found truncating *TTN* variants in patients with LVNC, the patient's phenotype actually fulfills DCM criteria and should be thus classified as DCM with LVNC features.

In addition, we identified a heterozygous nonsense variant *DSG2* c.2315T>G, p.(Leu772*). It generates a premature stop codon at position 772 out of 1118 amino acids. It is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. It is absent in ExAC and GnomAD. The p.(Leu772*) has been detected in some unrelated individuals at GeneDx, who also had a published missense mutation in *DSG2* (ClinVar ID_199830) We have previously detected the p.(Leu772*) in 40-year-old patient with ectopic atrial focus but no other features of cardiomyopathy was reported at referral.

DSG2 gene encodes a member of the desmoglein family and cadherin cell adhesion molecule superfamily of proteins. Desmoglein-2 is a calcium-binding transmembrane glycoprotein component of desmosomes. Together with the adherens and gap junctions, desmosomal proteins connect myocardial cells and maintain the mechanical and electrical integrity of the heart. The encoded preproprotein is proteolytically processed to generate the mature glycoprotein. It has only one transcript with RefSeq ID. Mutations in *DSG2* have been associated with arrhythmogenic right ventricular cardiomyopathy (ARVC, OMIM *610193) and dilated cardiomyopathy (DCM, OMIM *612877). Generally, *DGS2* associate with dominant disease but recessive mutations have been described. We have detected the *DSG2* p.(Thr335Ala) variant as homozygous state in two unrelated Finnish ARVC patients and one DCM patient. Subsequent family studies demonstrated recessive co-segregation of the variant with ARVC (5 affected homozygotes and 16 unaffected persons who were heterozygotes or wild type homozygote). Interestingly, Rasmussen et al. identified the same variant as homozygote in two brothers with severe ARVC and their heterozygote family members were healthy (PMID 23381804). Classically cardiac desmosomal proteins have been linked to ARVC but recent studies on DCM patients have revealed that mutations in cardiac desmosomal genes, such as *DSG2, DSC2, JUP, PKP2*, and *DSP*, associate also with DCM (PMID 21859740, 20716751). End-stage ARVC may be indistinguishable from DCM (PMID 20716751). Clinical evaluation of relatives may reveal classic isolated RV involvement in some cases to clarify differential diagnostic dilemma. There are 32 *DSG2* variants classified as pathogenic or likely pathogenic in ClinVar without conflicts: frameshifts (11), missense (10), nonsense (6), consensus splice site (4) and in-frame deletion (1). Truncating *DGS2* variants are rare in general population as 1 per 1,385 individuals in ExAC have such variant.

ARVC is cardiac disease associated with ventricular arrhythmias, heart failure and sudden cardiac death. *DSG2* mutations are the second most common genetic

defect in ARVC (*PKP2* 22.6% (10-52%), *DSG2* 3-19%, *DSP* 1-16%, *DSC2* 1-13% whereas all other genes are rarely involved) (PMID 22527912, ARVC - GeneReviews). ARVC is generally autosomal dominant disease but recessive inheritance is rarely found in association with homozygous or compound heterozygous mutations in *DSP, JUP, DSC2* and *DSG2* (ARVC - GeneReviews, PMID 18957847, 24793512, 23381804). Variability in clinical manifestations, reduced penetrance and digenic inheritance are commonly associated with ARVC and increase the complexity of genetic diagnostics in this disease. Life style issues such as exercise activity can play significant role in determining the age related penetrance in people carrying mutations associated with ARVC (PMID 23871885). Multiple desmosomal gene mutations and male sex are independent predictors of lifetime arrhythmic events with a HR of 3.71 and 2.76 (PMID 24070718).

In addition, we identified a heterozygous missense variant *MYH7* c.4030C>T, p.(Arg1344Trp). There are two heterozygotes in the ExAC and four heterozygotes in the GnomAD. *In silico* tools SIFT and MutationTaster predict it deleterious whereas as PolyPhen as benign. The p.(Arg1344Trp) was reported by Ladakwala et al in one individual with DCM (PMID 22464770) and in one HCM patient (PMID 27532257) but no co-segregation has been evaluated. It has been also identified in clinical testing in patients with no phenotype information available (ClinVar ID_177839).

MYH7, encoding the protein myosin heavy chain beta, is one of the most commonly mutated genes in patients with HCM (OMIM #192600). It is also associated with dilated cardiomyopathy (DCM), left ventricular non-compaction cardiomyopathy (LVNC; OMIM #613426) and restrictive cardiomyopathy (RCM). Mutations in *MYH7* can cause also pure skeletal myopathies and a combination of myopathy and cardiomyopathy. A significant proportion of mutations have been associated with more than one phenotype (PMID: 25935763). This has been observed among and within families, and multiple patients have shown overlapping phenotypes (HCM, DCM, LVNC and RCM). In patients with HCM, mutations in *MYH7* have been associated with an earlier age of onset than mutations in *MYBPC3* (PMID: 19666645).Van Driest et al. analyzed *MYH7* variants in 389 patients with HCM (PMID 15358028). They observed altogether 40 different mutations in 57 (15%) patients. They concluded that HCM patients with *MYH7* mutations were younger at diagnosis, had more hypertrophy, underwent more frequently myectomy and had more often a family history of HCM when compared to HCM patients without a *MYH7* mutation. However, no difference in the family history of sudden death was observed between the groups although in other study some of the converter domain *MYH7* mutations have been associated with a particularly adverse outcome (PMID: 25935763). Overall, over 500 *MYH7* mutations have been described and they are predominantly missense mutations (PMID: 25125180). Mutations causing premature truncation have been submitted to ClinVar but rarely there is clear evidence for pathogenicity. There are 413 *MYH7* variants classified as disease causing listed in HGMD, of which 389 (94.4%) are missense, 10 (2.4%) small deletions, 4 (1.0%) nonsense, 4 (1.0%) small indels, 3 (0.7%) splicing, 1 (0.2%) each of gross deletion and complex variant.

Moreover, a heterozygous missense variant *PRDM16* c.2455G>A, p.(Gly819Arg) was identified. It is absent in the ExAC but there are five heterozygotes in the GnomAD. It is predicted deleterious by PolyPhen but benign by SIFT and MutationTaster. It has not been described in literature or relevant mutation databases.

PRDM16 gene is a zinc finger transcription factor and contains an N-terminal PR domain. The translocation results in the overexpression of a truncated version of this protein that lacks the PR domain, which may play an important role in the pathogenesis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In 2013, Arndt et al. published a study where they linked *PRDM16* to non-syndromic non-compaction and dilated cardiomyopathies (PMID 23768516). Two out of seven *PRDM16* variants claimed to be disease causing mutations had some features suggesting true pathogenicity as being *de novo* truncations. However, their study included several methodological failures, especially when accounting role of missense variants in cardiomyopathy as all but one had several carriers in ExAC; e.g. Val1101Met as found as heterozygote in 428 individuals and homozygous in seven in EXAC and no segregation of any of *PRDM16* was demonstrated. In 2014 Nicole de Leeuw published a study where they question the role of *PRDM16* in cardiomyopathies (PMID 24387995). In 2016, Hertz et al investigated 47 victims of sudden unexpected death in infancy using a large NGS panel and identified two missense variants in *PRDM16* that have carrier frequencies of 0.8% and 1 per 3,000 that question their role as being pathogenic (PMID 26350513).All *PRDM16* variants classified as pathogenic or likely pathogenic in ClinVar are those identified by Arndt et al. Truncating variants affecting canonical *PRDM16* transcript are extremely rare (carrier frequency is 1 per 40,000 in ExAC; Nov 2016), thus likely poorly tolerated. We consider *PRDM16* as gene of uncertain significance.

Mutation nomenclature is based on GenBank accession NM_001943.3 (*DSG2*), NM_001267550.1 (*TTN*), NM_000257.2 (*MYH7*), NM_022114.3 (*PRDM16*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

We classify the identified *DSG2* c.2315T>G, p.(Leu772*) and *TTN* c.101113del, p.(Ser33705Leufs*4) as likely pathogenic and probable cause for patient's disease, considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations, mutation type (frameshift (*TTN*), nonsense (*DSG2*))). However, additional information is still needed to confirm the pathogenicity of the variants, which could allow independent risk stratification based on these mutations. Genetic counseling and family member testing is recommended. Disease caused by *DSG2 and TTN* mutations is inherited in an autosomal dominant manner, thus each child of an affected individual has a 50% chance of inheriting each mutation. A proband with autosomal dominant cardiomyopathy may have the disorder as a result of a *de novo* mutation. BpG offers mutation testing for the family if requested.

As there is not enough data to support or rule out pathogenicity, we classify the identified *MYH7* c.4030C>T, p.(Arg1344Trp) and *PRDM16* c.2455G>A, p.(Gly819Arg) as variants of uncertain significance (VUS). Additional information is needed to assess the clinical significance. Screening of the variants should not be used for risk evaluation within family members.

CONFIRMATION

DSG2 c.2315T>G, p.(Leu772*), *TTN* c.101113del, p.(Ser33705Leufs*4) and *MYH7* c.4030C>T, p.(Arg1344Trp) were confirmed by bidirectional Sanger sequencing.

STEP	DATE
Order date	Mar 02, 2017
Sample received	Mar 03, 2017
Reported	Apr 07, 2017

On Apr 07, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Sari Tuupanen, Ph.D.

Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

APPENDIX 2: ADDITIONAL FINDINGS

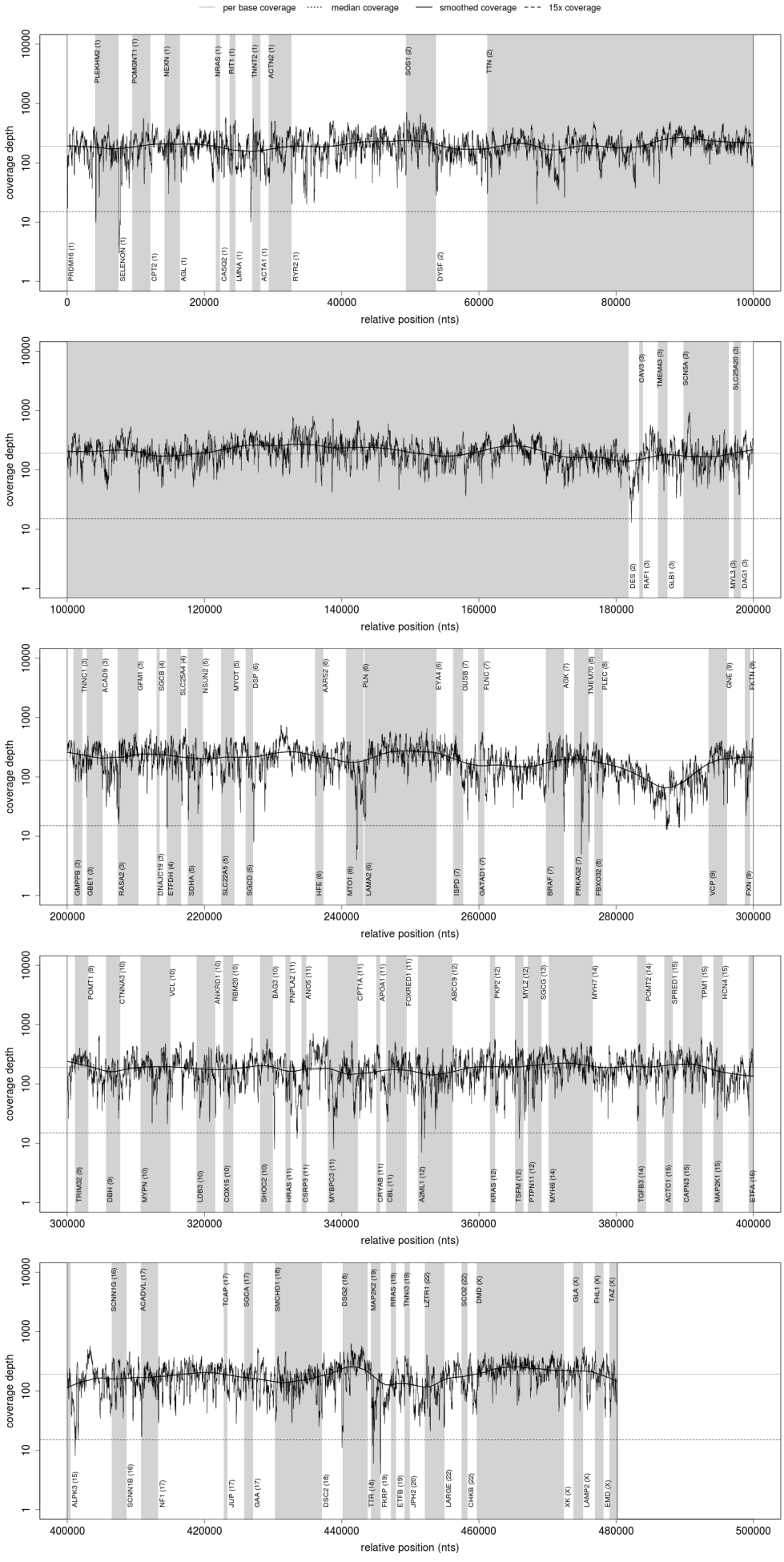
This table includes variants that either are not thought to be the likely cause for patient's phenotype (carrier status of variants of uncertain significance for recessive/X-linked disorders or heterozygous VUS variants for autosomal dominant disorders not likely related to the patient's phenotype), are secondary findings potentially relevant to patient's medical care (risk variants, heterozygous pathogenic or likely pathogenic variants for autosomal dominant disorders not related to patient's current phenotype) or carrier status for pathogenic or likely pathogenic variants for autosomal recessive or X-linked disorder not suspected in the patient.

VARIANT TABLE: ADDITIONAL GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
LAMA2	6:129573388	NM_000426.3	c.2049_2050del, p.(Arg683Serfs*21)	frameshift	HET	**Likely pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs751627052	11/121244	N/A	N/A	N/A	
	OMIM	**PHENOTYPE** Muscular dystrophy, congenital merosin-deficient, Schizophrenia		**INHERITANCE** AD&AR	**COMMENT** -	

NOTES REGARDING ADDITIONAL FINDINGS

The *LAMA2* c.2049_2050del, p.(Arg683Serfs*21) has been described in patients with autosomal recessive congenital muscular dystrophy (PMID: 9541105, 25544356). This variant is not sufficient to cause disease without another disease-causing variant in the same gene.


per base coverage
median coverage
smoothed coverage
15x coverage
coverage depth
relative position (nts)
PRDM16 (1)
PLEKHM2 (1)
SELENON (1)
POMGNT1 (1)
CPT2 (1)
NEXN (1)
AGL (1)
NRAS (1)
CASQ2 (1)
RIT1 (1)
LMNA (1)
TNNT2 (1)
ACTA1 (1)
ACTN2 (1)
RYR2 (1)
SOS1 (2)
DYSF (2)
TTN (2)
DES (2)
CAV3 (3)
RAF1 (3)
TMEM43 (3)
GLB1 (3)
SCN5A (3)
SLC25A20 (3)
MYL3 (3)
DAG1 (3)
GMPPB (3)
TNNC1 (3)
GBE1 (3)
ACAD9 (3)
RASA2 (3)
GFM1 (3)
DNAJC19 (3)
SGCB (4)
ETFDH (4)
SLC25A4 (4)
SDHA (5)
NSUN2 (5)
SLC22A5 (5)
MYOT (5)
SGCD (5)
DSP (6)
HFE (6)
AARS2 (6)
MTOT (6)
PLN (6)
LAMA2 (6)
EYA4 (6)
ISPD (7)
GUSB (7)
GATAD1 (7)
FLNC (7)
BRAF (7)
AGK (7)
PRKAG2 (7)
TMEM70 (8)
FBXO32 (8)
PLEC (8)
VCP (9)
GNE (9)
PXN (9)
EKTN (9)
TRIM32 (9)
POMT1 (9)
DBH (9)
CTNNA3 (10)
MYPN (10)
VCL (10)
LDB3 (10)
ANKRD1 (10)
COX15 (10)
RBM20 (10)
SHOC2 (10)
BAG3 (10)
HRAS (11)
PNPLA2 (11)
CSRP3 (11)
ANO5 (11)
MYBPC3 (11)
CPT1A (11)
CRYAB (11)
APOA1 (11)
CBL (11)
FOXRED1 (11)
A2ML1 (12)
ABCC9 (12)
KRAS (12)
PKP2 (12)
TSPAN (12)
MYL2 (12)
PTPN11 (12)
SGCG (13)
MYH6 (14)
MYH7 (14)
TGFB3 (14)
POMT2 (14)
ACTC1 (15)
SPRED1 (15)
CAPN3 (15)
TPM1 (15)
MAP2K1 (15)
HCN4 (15)
ETFA (16)
ALPK3 (16)
SCNN1G (16)
SCNN1B (16)
NF1 (17)
ACADVL (17)
JUP (17)
TCAP (17)
GAA (17)
SGCA (17)
DSC2 (18)
SMCHD1 (18)
TTR (18)
MAP2K2 (19)
PKRP (19)
ETFB (19)
DSG2 (18)
RRAS (19)
JPH2 (20)
TNNI3 (19)
LZTR1 (22)
LARGE (22)
CHKB (22)
SCO2 (22)
DMD (X)
XK (X)
GLA (X)
LAMP2 (X)
FHL1 (X)
EMD (X)
TAZ (X)

APPENDIX 5: SUMMARY OF METHODS

OS-SEQ (SEQUENCE ANALYSIS)

Sequencing. Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.
Data analysis. 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 were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.
Confirmation. SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons.
Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
Accreditation. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. Expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.
Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).
Reporting. Reporting was done using an HGNC-approved gene nomenclature.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
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GLOSSARY OF USED ABBREVIATIONS:

POS = genomic position of the variant in the format of chromosome:position
ID = rsID in dbSNP
Transcript = GenBank accession for reference sequence used for variant nomenclature
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
ExAC AC/AN = allele count/allele number in the Exome Aggregation Consortium Database
AD = autosomal dominant
AR = autosomal recessive
OMIM = Online Mendelian Inheritance in Man®
ExAC = Exome Aggregation Consortium Database (>60,000 unrelated individuals)
het = heterozygous
hom = homozygous
Del/Dup = Deletion and Duplication
CNV = copy number variation
PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.