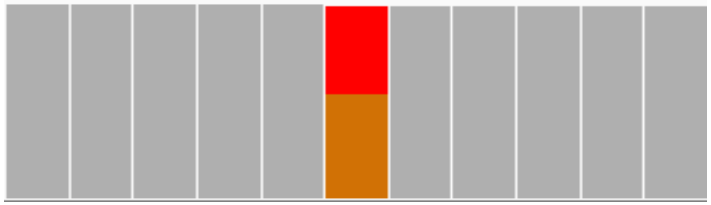
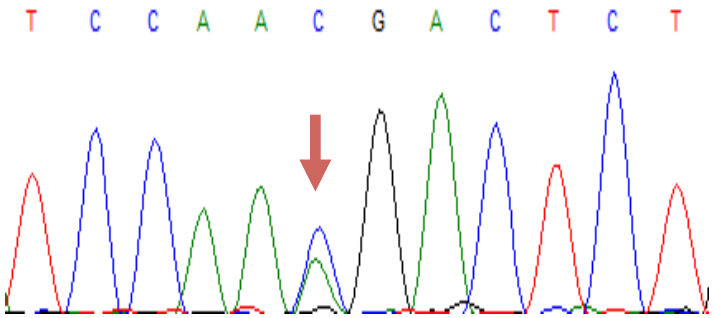
Confirmation of *TTN, MYL2* and *ALPK3* variants by Sanger sequencing

***TTN*:c.39909A>C (p.Lys22368Asn)**

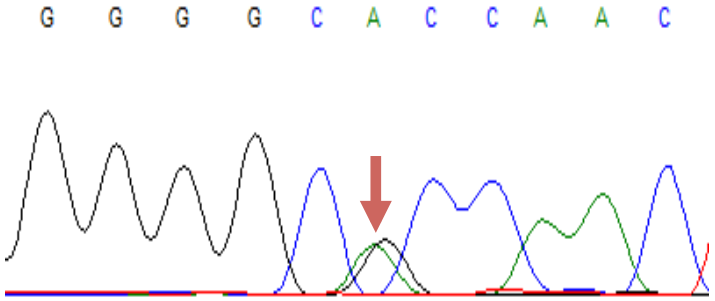
81 reads, 37A, 44C





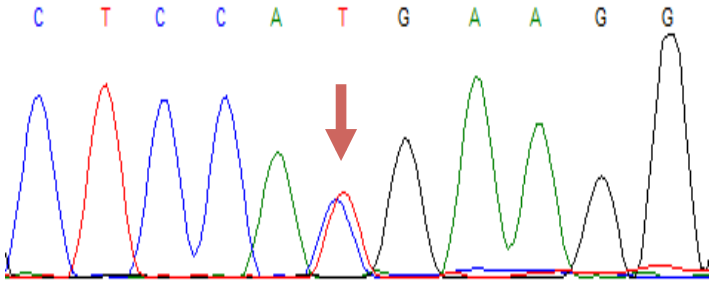
***MYL2*:c.37G>A (p.Ala13Thr)**

74 reads, 41G, 33A



***ALPK3*:c.242C>T (p.Thr81Met)**

83 reads, 41C, 42T



Online Table I

	II-2			III-1			III-4			Average		
GENE	**1X**	**10X**	**20X**	**1X**	**10X**	**20X**	**1X**	**10X**	**20X**	**1X**	**10X**	**20X**
MYH7	100.0	95.9	88.7	100.0	97.3	91.8	99.5	95.8	88.6	99.8	96.3	89.7
MYBPC3	100.0	99.2	96.2	100.0	100.0	96.6	100.0	99.4	96.6	100.0	99.5	96.5
TNNT2	100.0	99.9	96.5	100.0	100.0	100.0	100.0	98.5	95.3	100.0	99.5	97.3
TNNI3	100.0	100.0	95.5	100.0	100.0	94.5	100.0	100.0	90.5	100.0	100.0	93.5
TPM1	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	98.4	100.0	100.0	99.4
ACTC1	100.0	98.2	91.1	100.0	98.3	93.5	100.0	100.0	92.1	100.0	98.8	92.2
MYL2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
MYL3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
TTN	98.5	97.6	96.5	98.8	97.9	96.9	98.8	97.9	96.8	98.7	97.8	96.7
ALPK3	100.0	95.4	92.4	100.0	96.5	94.0	100.0	95.5	93.2	100.0	95.8	93.2
FHL1	100.0	98.5	91.0	100.0	91.5	80.0	100.0	100.0	95.6	100.0	96.7	88.9
PLN	100.0	100.0	88.6	100.0	99.0	92.9	100.0	100.0	98.0	100.0	99.7	93.2
GLA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
LAMP2	100.0	99.7	91.9	100.0	93.0	83.6	100.0	98.6	96.1	100.0	97.1	90.5
PRKAG2	100.0	97.7	97.7	100.0	99.9	97.7	100.0	98.3	97.7	100.0	98.7	97.7
NEXN	100.0	100.0	97.7	100.0	100.0	99.7	100.0	100.0	97.1	100.0	100.0	98.2
Average	99.9	98.9	95.2	99.9	98.3	95.1	99.9	99.0	96.0			
SD	0.3775	1.53495	4.0687	0.293	2.655	5.96	0.3048	1.508	3.382			

Online Table II

Gene	Protein Change	Mutation Type	EXAC Non-Finnish European	GTEx (100XFPKM)$^{1/2}$
ADAM28	p.Tyr107Asn	Nonsynonymous	0.009% ± 0.01%	4.00
ADRB1	p.Asp382Gly	Nonsynonymous	0.078% ± 0.025%	32.00
ALPK3	p.Thr81Met	Nonsynonymous	0.148% ± 0.053%	54.00
ALS2CR12	p.Gln168GlufsTer3	Deletion (frameshift)	0.03% ± 0.016%	10.00
ANK3	c.1690-4delT	Splice region		25.00
ANKRD11	p.Ala618Thr	Nonsynonymous	0.071% ± 0.023%	26.00
ANKRD13C	p.Gly138Val	Cryptic splicing	0.215% ± 0.044%	17.00
ANO9	p.ArgGlnAla49GlnGlnVal	Cryptic splicing		5.00
ARG1	p.Gly169Ala	Nonsynonymous	0.016% ± 0.013%	5.00
ARSB	p.Asn458Ser	Nonsynonymous	0.11% ± 0.028%	14.00
ASXL3	p.Leu2067Arg	Nonsynonymous	0.966% ± 0.077%	6.00
ATXN1	p.Ile730Val	Cryptic splicing	0.03% ± 0.016%	15.00
BCOR	p.Ala161Val	Nonsynonymous	0.114% ± 0.028%	21.00
BRCA1	c.2077_2082delGACAGCinsGACAGT(p.%3D)	Cryptic splicing		10.00
BRICD5	p.Thr69Ile	Nonsynonymous	0.259% ± 0.11%	22.00
C1orf106	p.Arg469Cys	Cryptic splicing	0.253% ± 0.068%	1.00
C9orf47	p.Arg66Gly	Nonsynonymous	0.026% ± 0.028%	4.00
CCDC180	p.Ile1679Val	Cryptic splicing	0.049% ± 0.02%	9.00
CDC14A	p.Val260Met	Nonsynonymous	0.303% ± 0.044%	10.00
CDH4	p.Arg677His	Nonsynonymous	0.044% ± 0.019%	2.00
CEP131	p.Ser642Gly	Nonsynonymous	0.231% ± 0.04%	17.00
CEP131	p.His238Arg	Nonsynonymous	0.21% ± 0.037%	17.00
CEP250	p.His2129Arg	Nonsynonymous	0.256% ± 0.041%	14.00
CFAP43	p.Val197Met	Nonsynonymous	0.576% ± 0.061%	2.00
CFHR1	c.435T>G(p.%3D)	Cryptic splicing	0.137% ± 0.033%	13.00
CLDN23	p.Ala90Thr	Nonsynonymous	0.609% ± 0.069%	6.00
CLTCL1	p.Gly169Asp	Nonsynonymous	0.021% ± 0.015%	19.00
CLUAP1	p.Ala107Val	Nonsynonymous		13.00
COL17A1	p.Val991Met	Nonsynonymous	0.152% ± 0.032%	3.00
COL24A1	p.Asp844Ala	Nonsynonymous	0.004% ± 0.009%	7.00
COL4A2	p.Pro650Ser	Nonsynonymous	0.209% ± 0.038%	66.00
COL6A5	p.Tyr2438Ser	Nonsynonymous	0.264% ± 0.041%	0.43
COL6A5	p.Glu2272Ter	Stopgain	1.15% ± 0.098%	0.43
COPB1	c.813C>G(p.%3D)	Cryptic splicing	0.099% ± 0.027%	38.00
CPTP	c.504C>T(p.%3D)	Cryptic splicing	0.045% ± 0.02%	48.00
CRYBB1	p.Arg110Cys	Nonsynonymous	0.365% ± 0.049%	4.00
CRYBG3	p.Lys978Gln	Nonsynonymous	0.049% ± 0.13%	21.00
DAP	p.Arg65Gln	Nonsynonymous	0.264% ± 0.042%	45.00
DDX20	c.1210+3delA	Splice region		14.00
DDX25	p.Tyr113Cys	Cryptic splicing	0.01% ± 0.014%	4.00
DENND2A	p.Arg542Gln	Nonsynonymous	0.052% ± 0.022%	20.00
DENND4C	c.*153C>T	Cryptic splicing		21.00
DMRTA1	p.Ser279Cys	Nonsynonymous	0.053% ± 0.021%	5.00

DNTT	p.Pro140Leu	Nonsynonymous	0.086% ± 0.025%	0.46
DPP6	p.Leu252Cys	Nonsynonymous		2.00
EPB41L5	p.Arg28Cys	Nonsynonymous	0.492% ± 0.055%	14.00
ERVV-2	c.*151_*152insGG	Cryptic splicing		0.06
FAM162A	c.263+1G>A	Splicing	0.094% ± 0.026%	63.00
FANCM	p.Leu57Phe	Nonsynonymous	0.252% ± 0.04%	7.00
FRMPD1	c.738+3A>G	Splice region	0.273% ± 0.043%	4.00
FUCA2	p.Leu20_Leu21dup	Insertion (nonframeshift)		37.00
GCGR	c.660G>A(p.%3D)	Splice region	0.152% ± 0.119%	0.99
GCNT1	p.Pro368Leu	Nonsynonymous	0.918% ± 0.074%	7.00
GCSAML	p.Asn73His	Nonsynonymous	0.665% ± 0.064%	2.00
GDI2	c.45+1253delT	Splice region		49.00
HES3	p.Gly108Arg	Nonsynonymous	0.152% ± 0.293%	0.10
HMCN1	p.Thr1232Met	Nonsynonymous	0.698% ± 0.065%	11.00
IGHV3-53	p.Ile31Val	Cryptic splicing		1.00
IGSF9	p.Arg195Gln	Nonsynonymous	0.08% ± 0.024%	6.00
IL2RG	p.Glu109Lys	Nonsynonymous	0.109% ± 0.028%	10.00
IPMK	p.Thr28Ala	Nonsynonymous	0.071% ± 0.026%	9.00
KBTBD13	p.Gly265Asp	Cryptic splicing		13.00
KCNK15	p.Thr260Pro	Nonsynonymous		10.00
KLF14	p.His47Pro	Nonsynonymous		2.00
LAMTOR5	p.Pro45Leu	Nonsynonymous	0.068% ± 0.023%	65.00
LAP3	p.Asn271Ser	Cryptic splicing	0.053% ± 0.021%	40.00
LRIG2	p.Thr765Ile	Nonsynonymous	0.129% ± 0.03%	13.00
LRRC3	c.*1C>T	Cryptic splicing	0.283% ± 0.046%	9.00
LRRC37B	p.Thr183Ile	Nonsynonymous	0.25% ± 0.04%	9.00
MAOA	p.Lys520Arg	Nonsynonymous	0.072% ± 0.024%	52.00
MC1R	p.Tyr152Ter	Stopgain	0.108% ± 0.028%	10.00
MDH2	p.Lys328Gln	Nonsynonymous	0.342% ± 0.047%	110.00
MIB2	p.Gln646Arg	Nonsynonymous	0.216% ± 0.044%	35.00
MIEF1	p.Gly90Asp	Nonsynonymous	0.022% ± 0.015%	20.00
MIR4477B		Cryptic splicing		2.00
MUC12	p.Val20Leu	Nonsynonymous		1.00
NEB	c.20467-4dupT	Splice region		14.00
NECAB3	p.Arg203Gln	Nonsynonymous	0.034% ± 0.09%	42.00
NIPAL2	c.*344C>G	Cryptic splicing		20.00
NOTCH2	p.Arg1786Gln	Nonsynonymous	0.003% ± 0.008%	21.00
NUDT16	p.Ala4Ser	Nonsynonymous	0.815% ± 0.211%	34.00
OR1J4	p.Glu4Lys	Nonsynonymous	0.002% ± 0.007%	0.87
OR2T4	p.PheTyrVal135LeuTyrLeu	Nonsynonymous		0.37
OR4C3	p.ThrLeuPro289IleLeuSer	Nonsynonymous		0.49
OR4C3	p.ValHis284MetLeu	Nonsynonymous		0.49
OR56A3	p.Gly314Glu	Nonsynonymous	0.013% ± 0.012%	0.29
OR6B1	p.Met256Leu	Nonsynonymous	0.015% ± 0.012%	0.25
OR8B8	p.Asn42Lys	Nonsynonymous	0.105% ± 0.027%	1.00
OR8U1	p.Phe200Leu	Nonsynonymous		0.12
PABPC3	p.Met326GlyfsTer21	Deletion (frameshift)		28.00

PABPC3	p.Phe335LeufsTer19	Deletion (frameshift)		28.00
PABPC3	p.Thr319Ile	Nonsynonymous	0% ± 0.006%	28.00
PCDHA8	p.Ser150Tyr	Nonsynonymous	0.041% ± 0.019%	1.00
PEX6	p.Glu592Lys	Nonsynonymous	0.044% ± 0.023%	31.00
PHTF1	p.Gln237Lys	Nonsynonymous	1.116% ± 0.081%	11.00
PKD1	c.11388C>G(p.%3D)	Cryptic splicing	0.026% ± 0.016%	32.00
PLCB2	p.Glu435Lys	Nonsynonymous	0.191% ± 0.036%	16.00
PLXDC1	p.Arg282Cys	Nonsynonymous	0.101% ± 0.027%	21.00
PPRC1	p.Leu1228Ile	Nonsynonymous	0.117% ± 0.029%	24.00
PRPF39	p.Asn399Ser	Nonsynonymous	0.247% ± 0.04%	25.00
PRSS3	p.Met166Leu	Nonsynonymous	0.01% ± 0.012%	3.00
PYGM	p.Val624Ile	Nonsynonymous	0.078% ± 0.024%	50.00
QRICH2	p.Arg1403Cys	Cryptic splicing	0.238% ± 0.04%	9.00
RAMP3	p.Asp116Glu	Nonsynonymous	0.049% ± 0.02%	48.00
RC3H1	p.Val146Ala	Nonsynonymous	0.482% ± 0.055%	16.00
RECQL5	c.2806-2A>G	Splicing	0.1% ± 0.038%	13.00
RFWD2	p.Ser477Ile	Nonsynonymous	0.024% ± 0.014%	24.00
ROBO1	c.4283-6_4284delCCTTAGGCinsTCTTAGGT	exonic_splicing		15.00
RPL3L	p.Asp149Asn	Nonsynonymous	0.028% ± 0.016%	62.00
RRP8	p.Glu9Ter	Stopgain	0.027% ± 0.027%	16.00
SBF2	p.Lys1672del	Deletion (nonframeshift)	0.061% ± 0.021%	20.00
SEPP1	p.Arg368Cys	Nonsynonymous	0.432% ± 0.052%	46.00
SEPP1	p.Met263Ile	Nonsynonymous	0.434% ± 0.052%	46.00
SIGIRR	p.Ala236Gly	Nonsynonymous	0.264% ± 0.066%	27.00
SLC12A2	p.Ala99_Ala101del	Deletion (nonframeshift)		12.00
SLC22A8	p.Ala310Val	Cryptic splicing	0.064% ± 0.022%	0.47
SLC25A32	p.Ala39Ser	Nonsynonymous	0.021% ± 0.015%	22.00
SLC30A9	p.Met50Val	Nonsynonymous		43.00
SLC5A1	p.Ile34Thr	Nonsynonymous	0.027% ± 0.016%	55.00
SLCO1B1	p.Arg57Gln	Nonsynonymous	0.037% ± 0.018%	0.39
SLIT1	p.Asn700Lys	Nonsynonymous	0.144% ± 0.031%	0.41
SMTNL2	p.Glu461Lys	Nonsynonymous	0.57% ± 0.059%	23.00
SORBS3	p.Ile556Thr	Nonsynonymous		45.00
SPACA3	p.His100Arg	Cryptic splicing	0.123% ± 0.029%	0.55
SPATA31E1	p.Met65Arg	Nonsynonymous	0.375% ± 0.055%	0.31
SRRM3	c.566C>T(p.%3D)	Cryptic splicing	1.364% ± 0.271%	16.00
TACC3	p.Gln4Arg	Nonsynonymous	0.122% ± 0.029%	15.00
TAS2R1	p.Ala193Asp	Nonsynonymous	0.024% ± 0.014%	0.39
TICRR	p.Gln1399Glu	Nonsynonymous	0.107% ± 0.027%	3.00
TMC3	c.1273-7_1273-6dupTC	Splice region		2.00
TMEM175	p.Pro14Leu	Nonsynonymous	0.256% ± 0.042%	29.00
TRBV11-2		Cryptic splicing		1.00
TRIOBP	p.Ala322Val	Cryptic splicing	0.069% ± 0.023%	29.00
TSPYL2	p.Ile472Val	Cryptic splicing	0.067% ± 0.022%	65.00

TTN	p.Lys22368Asn	Nonsynonymous	0.007% ± 0.01%	72.00
TVP23C-CDRT4	p.SerValPheTrp199ThrValPheArg	Nonsynonymous		
TVP23C				
UBAP2	p.Phe869Cys	Nonsynonymous	0.024% ± 0.014%	16.00
USP36	p.Val271Ile	Nonsynonymous		21.00
UTRN	p.Ile2227Val	Cryptic splicing	0.391% ± 0.05%	21.00
WBSCR27	c.389-3_389-2dupCA	Splicing		6.00
ZBTB10	p.Gly850Ala	Nonsynonymous	0.031% ± 0.018%	15.00
ZDHHC19	p.ThrProGly305SerProArg	Cryptic splicing		4.00
ZNF438	p.Ala187LeufsTer6	Deletion (frameshift)		21.00
ZP3	p.Ser315Pro	Nonsynonymous		9.00
ZSCAN10	p.Arg69Pro	Nonsynonymous	0.2% ± 0.112%	0.74

Online Table III

Prediction of Pathogenicity of the Variants

	MYL2: p.Ala13Thr	*TTN*: p.Lys22368Asn	*ALPK3*: p.Thr81Met
PolyPhen2_HVAR	B	D	B
SIFT	T	D	D
LRT	D	Unknown	Unknown
MutationTaster	D	D	N
MutationAssessor	L	M	N
ClinVar	P	Unknown	Unknown
CADD_phred	16.11	10.46	13.54

PolyPhen2_HVAR: Polymorphism Phenotyping v2 by Harvard, B: benign; D: Probably damaging

SIFT: Sorting Intolerant from Tolerant, D: Deleterious; T: tolerated

LRT: Likelihood Ratio Test, D: deleterious; N: neutral

MutationTaster, D: disease_causing; N: polymorphism

MutationAssessor, M: predicted functional; L, N: predicted non-functional

ClinVar, P: pathogenic

CADD_phred: Combined Annotation Dependent Depletion phred score