

Supplemental Data: Case Reports

Detailed clinical description of individuals with PPCS-associated cardiomyopathy.

Family A

Individual 2 from family A (FA: II.2) is a female child born to healthy non consanguineous German parents, after uneventful full-term pregnancy. Birth weight was 3090 g (P 14). She showed minor dysmorphic signs such as cutis laxa, hands with abnormally placed thumbs without opposition, star-like palmar creases, high-arched feet, hypoplastic toe nails, hymen fimbrinatus and hypoplastic labia minora. After a good initial adaptation at birth (Apgar 8/9/10), she experienced an acute life-threatening event in the following hours with oxygen desaturation and bradycardia, followed by a tonic seizure that required intubation. Persistent pulmonary hypertension was treated with iNO and catecholamines. Initially lactic acidosis (maximal plasma lactate 132 mg/dl; normal value (N): <16 mg/dl), moderate hyperammonemia (150 μ mol/l; N: 12-53 μ mol/l), and elevation of creatine kinase activity (CK; 4,500 U/l; N: <286 U/l) were documented. In the following weeks the values normalized and the girl was discharged at the age of 5 weeks. She had severe muscular hypotonia requiring tube feeding and suffered frequent episodes of vomiting. After two weeks, she was re-admitted to the hospital with fever, tachypnea, and reduced consciousness. Echocardiogram showed dilated cardiomyopathy with severely impaired biventricular function. Laboratory examination showed clearly elevated CK (2,200 U/l), Troponin T (2,800 pg/ml; N: <50 pg/ml) and NT-ProBNP (max. 113,000 ng/l; N: <125 ng/l). Additionally, hyponatremia (118 mmol/l; N: 131-145 mmol/l), hypokalemia, and anemia (Hb 6.2 g/l; N: 9.5-13.5 g/l) as well as elevated aminotransferases were documented. Cardiac function deteriorated despite catecholamine therapy and mechanical ventilation. Morphine was administered as palliative therapy. However, the girl stabilized and recovered slowly. The clinical course was complicated by intestinal pseudo-obstruction which slowly improved. Urinary thymidine and deoxyuridine concentrations were normal. Because of suspected pituitary dysfunction, hydrocortisone was administered and subsequently tapered after normal cortisol and ACTH profile. Because of persistent hyponatremia, sodium chloride had to be supplemented. In clinically stable phase the girl had muscular hypotonia, but no abnormal or dystonic movements. At the age of three months the patient was re-admitted to the hospital for gastroparesis and decompensated dilated cardiomyopathy with severely compromised left ventricular function. She died shortly after from multiorgan failure. Cranial MRI showed an ectopic neurohypophysis but otherwise normal brain structures and normal myelination. The basal ganglia and substantia nigra were normal. Metabolic examinations revealed persistently elevated plasma and urine lactate. Initial acylcarnitine profiles in dry blood spots indicated clearly elevated concentrations of glutaryl carnitine (due to renal insufficiency after cardiogenic shock), C14:1 and long chain 3-OH-acylcarnitines, and later on low concentrations of long chain acylcarnitines (Table 2). Normal results were obtained for urinary organic acids (including 3-OH-glutaric acid), purines/pyrimidines, plasma amino acids, VLCFA, and sterols. Endocrinology examination showed normal thyroid function, normal cortisol and ACTH profile, reduced IGF1, IGF-BP3 and gonadotropines. Screening for cystic fibrosis showed elevated IRT/PAP concentrations, but sweat test was normal (done twice), therefore excluding cystic fibrosis.

Family B

Individual IV.1 from family B (FB:IV.1) is currently 21 years old and he is the elder male sibling. At the age of two years, he presented with asthma-like symptoms. The individual was considered generally healthy during his childhood and a diagnosis of dilated cardiomyopathy was made only at around 20 years of age with an echocardiogram showing dilated LV with decreased LV systolic function (LV Diameter (diastole/systole) 6cm/4.2cm, FS 22% (Normal, >28%), EF 44% (Normal, >55%)). Cardiac MRI/MRA showed a mildly dilated left ventricle with moderate to severe decrease in function of both ventricles (left ventricular end diastolic volume index of 117.07 ml/m², LV ejection fraction (EF) of 37.57% with no evidence of significant tricuspid regurgitation), consistent with non-ischemic cardiomyopathy (Figure 3). Abdominal ultrasound (performed to rule out hepatosplenomegaly, nephrocalcinosis and other abdominal visceral involvement), and brain MRI (to exclude cerebral involvement) were both considered normal. Metabolic screening tests including serum liver transaminases, ammonia, lactate, CPK, and lipid profile was unremarkable. Urine organic acid profile, urine reducing substances showed normal values. Endocrine investigations provided normal serum total testosterone, androstenedione, prolactin, cortisol, ACTH- within normal limits and low DHEAS (5.4 μ M; Normal values, 7.4-16.8). He is currently treated with a combination of diuretics, ACE inhibitors and beta blockers.

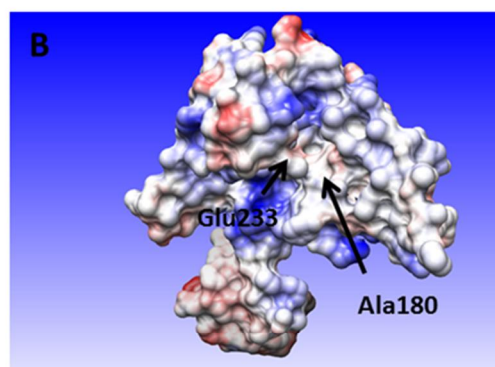
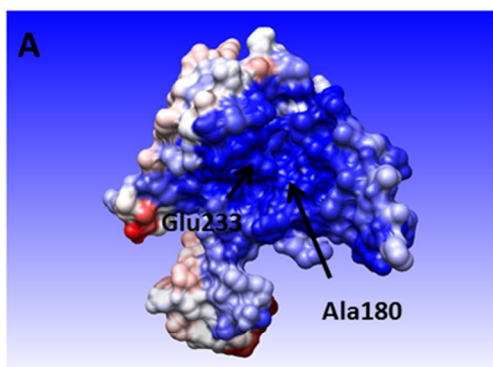
Individual IV.4 from family B (FB:IV.4) is currently 10 years old. This male sibling was considered healthy until he presented at four months of age with a hypovolemic shock and convulsions (following two days of recurrent episodes of vomiting), hypoglycemia (69mg/dl) and metabolic acidosis. Metabolic screening tests included: serum amino acids which were within normal limits; an abnormal acylcarnitine profile, with increased long chain acylcarnitines, especially C14:1, which initially led to the suspicion of raised the diagnostic possibility of Very Long-Chain Acyl-CoA-Dehydrogenase (VLCAD) deficiency (Table 2); abnormal urine organic acid profile (notable for excretion of ketones and lactate, and significantly elevated excretion of dicarboxylic acids, especially C12 and C14); and serum CPK and liver transaminases within normal limits. Subsequently, fatty acid oxidation was evaluated in lymphocytes, showing myristate oxidation and oleate oxidation of 73% and 68%, respectively. Echocardiogram performed thereafter showed LV contractility in the low-normal range (FS, 29%) and a small PDA, which was closed via cardiac catheterization at 16 months of age. He currently shows easy fatigability and limited exercise tolerance, and he is treated with diuretics, Digoxin, Enalapril and Coumadin (the latter due to an episode of deep vein thrombosis of the Right Common Iliac and Right Common Femoral veins, following central venous catheterization). Echocardiogram at 9 years and 10 months showed normal biventricular systolic function, LVD of 5.16/3.97 with FS of 23%, and EF of 40%. A recent cardiac MRI/MRA showed a dilated left ventricle with moderately decreased LV function (Left ventricular end diastolic volume index of 174.36 ml/m², LV ejection fraction (EF) of 37.01%) and no evidence of significant tricuspid regurgitation) (Figure 3, Movie S1). Abdominal ultrasound was considered normal. Of note, following the episode of DVT, coagulability investigations were pursued and revealed prolonged PTT, with decreased vitamin K-associated factors as well as factors XI and XII, and normal LAC, AT, protein C and S, APCR. He is concomitantly followed by a thrombophilia team. Currently, under Coumadin treatment, he shows INR of 2.8, PT 24%, PTT 64. Metabolic screening tests included: Serum liver transaminases, ammonia,

lactate, CPK, and lipid profile within normal limits; urine organic acid profile, urine reducing substances normal. Endocrine investigations show normal serum cortisol, ACTH, prolactin and DHEAS, and pre-pubertal testosterone levels.

Individual IV.5 from family B (FB:IV.5) is a girl born via cesarean section, following a pregnancy notable for gestational diabetes. Considering the family history of two older siblings with cardiomyopathy, she underwent echocardiograms at 1.5 months and again at 12 months of age, which were both reportedly normal (LVEDD 1.9, FS 32%, and LVEDD 3, FS 32%, respectively). At 23 months, she presented with respiratory distress and obtundation during a week-long acute febrile respiratory illness, with oxygen saturation of 75% in room air. Upon physical examination at admission she showed tachypnea, dyspnea and tachycardia, with peripheral cyanosis, a systolic murmur, hepatomegaly and signs of cardiogenic shock. Echocardiogram revealed evidence of severe dilated cardiomyopathy, with an EF of 11%. She was intubated and mechanically ventilated, and required intensive care and full inotropic support, including Dopamine, Milrinone, Adrenaline and Nitroprusside, as well as diuretics, Zaxoxillin and Levosimendan. Metabolic screening tests were obtained, and included: serum ammonia which was within normal limits; elevated free and total carnitine (41 and 112 μ M, respectively); an abnormal however non-specific urine organic acid profile (mild ketonuria, moderate dicarboxylic aciduria, increased tyrosine metabolites attributed to hepatic dysfunction, and large excretion of HVA and DOPAC consistent with Dopamine administration); Acylcarnitine profile which was considered extremely abnormal (most carnitines were elevated, especially free carnitine, C2 and OH-C4, attributed to a combination of carnitine administration, ketosis and premorbid changes) (Table 2); and normal enzymatic activity of mitochondrial respiratory chain complexes in both liver and muscle tissue. Eight days after admission she further deteriorated, requiring prolonged cardiopulmonary resuscitation (CPR), and she succumbed.

Individual IV.8 from family B (FB: IV.8) is the youngest of the eight siblings, second of twins. He underwent an echocardiogram at 12 months of age due to the family history of dilated cardiomyopathy, which was considered normal. He was reportedly healthy until the age of three years, when following an adenovirus-associated acute febrile respiratory illness, he showed severe clinical deterioration and presented in a state of cardiogenic shock. He was intubated and mechanically ventilated, and transferred to the Pediatric Intensive Care Unit. Echocardiogram at admission showed dilated cardiomyopathy with extremely reduced LV function (EF, 6-10%), moderate mitral regurgitation and mild tricuspid regurgitation. Diuretics were initiated, and intravenous immunoglobulins (IVIG) was administered for the diagnostic suspicion of myocarditis. He required full inotropic support, and one week after the admission, the blood pressure dropped and he underwent a cardiac resuscitation and Extracorporeal Membrane Oxygenation (ECMO) support was initiated; pulmonary artery (PA) banding was performed. On the ninth day of ECMO support, a large pericardial effusion required pericardiocentesis. Of note, during ECMO support he was prepared for heart transplantation which was aborted due to the finding of over 50% antibodies against the potential donor erythrocytes. Following an echocardiogram demonstrating an improvement in systolic ventricular function, he was weaned from ECMO support after six weeks; he was subsequently extubated and weaned from inotropic support as well. Two weeks later, while still hospitalized at the PICU, a febrile episode was noted with evidence of acute tonsillitis. A quick and dramatic deterioration of his general condition, with decrease of oxygen

saturation and bradypnea ensued. Cardiopulmonary resuscitation was performed, however despite all the efforts, he succumbed. With consent of the parents, a skin biopsy was obtained.



non conserved conserved

negative positive

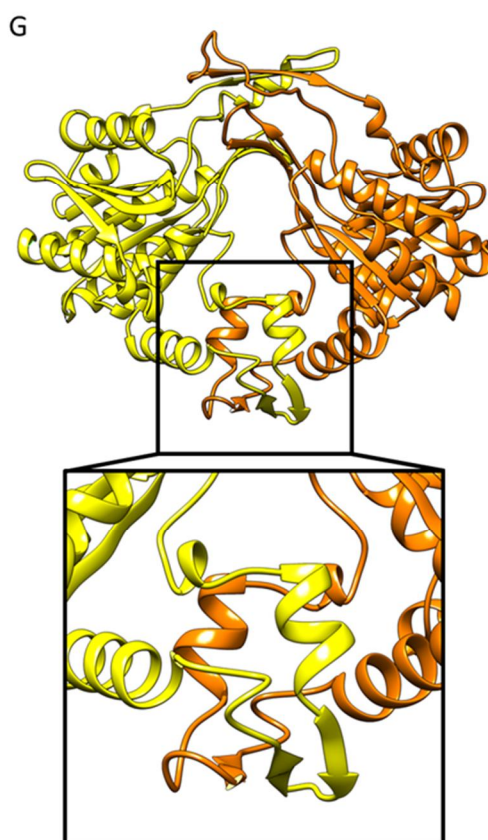
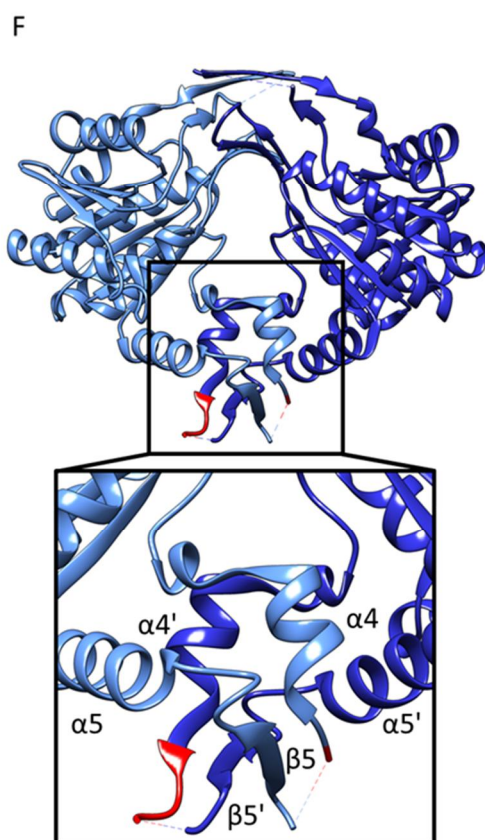
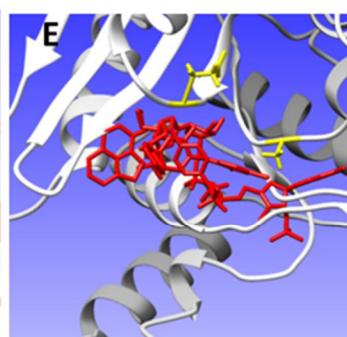
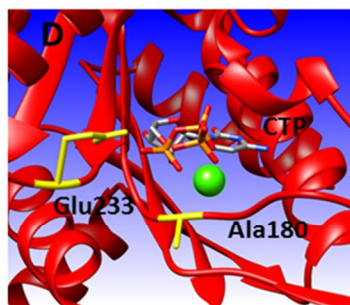
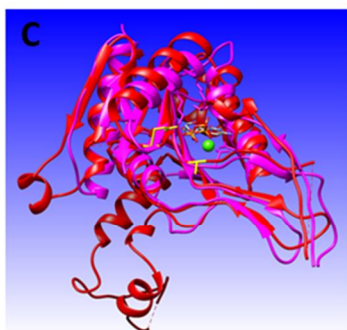


Figure S1. Structural analysis of the detected variants. A) Molecular surface representation of protein colored by evolutionary sequence conservation. Positions 180 and 233 are located in a conserved region (based on ConSurf server) and are partially exposed to the solvent. B) Molecular surface representation of protein colored by electrostatic potential. C) Structural alignment of the human protein (red) and the *E.coli* protein (pink) which include also the CTP cofactor (CPK colors wireframe view) and the calcium atom (green ball) which were solved in the later structure are also shown. Residues Glu233 and Ala180 are shown in yellow. D) Zoom in of the CTP binding region which includes only the human protein and the superimposed CTP with the mutated residues indicated in yellow. E) Cluster of ligands (in red) suggested independently by the COACH algorithm which suggests that the cavity near the detected variants is indeed a binding site. Residues Glu233 and Ala180 are shown in yellow. F) Ribbon representation of human Phosphopantothencysteine synthetase (PDB code 1P9O). Zoom in of the second dimerization domain with residues 107-111 in red. Residues for which no electron density was observed are represented by a broken line. G) Ribbon representation of the deletion mutant 107-111 PPCS SWISS-MODEL homology model (3-6). The zoom-in region corresponds to the second dimerization region where the deletion is observed.

Experiment 1

	Control		12 mM Vitamin B5		8 mM pantethine	
total	homo	hetero	homo	hetero	homo	hetero
	124	438	156	497	181	346
% homo	22%		23,8%		34,3%	

Experiment 2

	Control		12 mM Vitamin B5		8 mM pantethine	
total	homo	hetero	homo	hetero	homo	hetero
	54	549	76	585	107	345
% homo	9%		11,5%		23,7%	

Figure S2. Viability in *dPPCS¹ Drosophila melanogaster*. Viability of homozygous and heterozygous *dPPCS¹ Drosophila melanogaster* in presence or not of 8mM pantethine. Absolute numbers differ between experiments due to variable parameters such as humidity. However, the rescue with pantethine is consistently observed. Results are from two independent experiments.

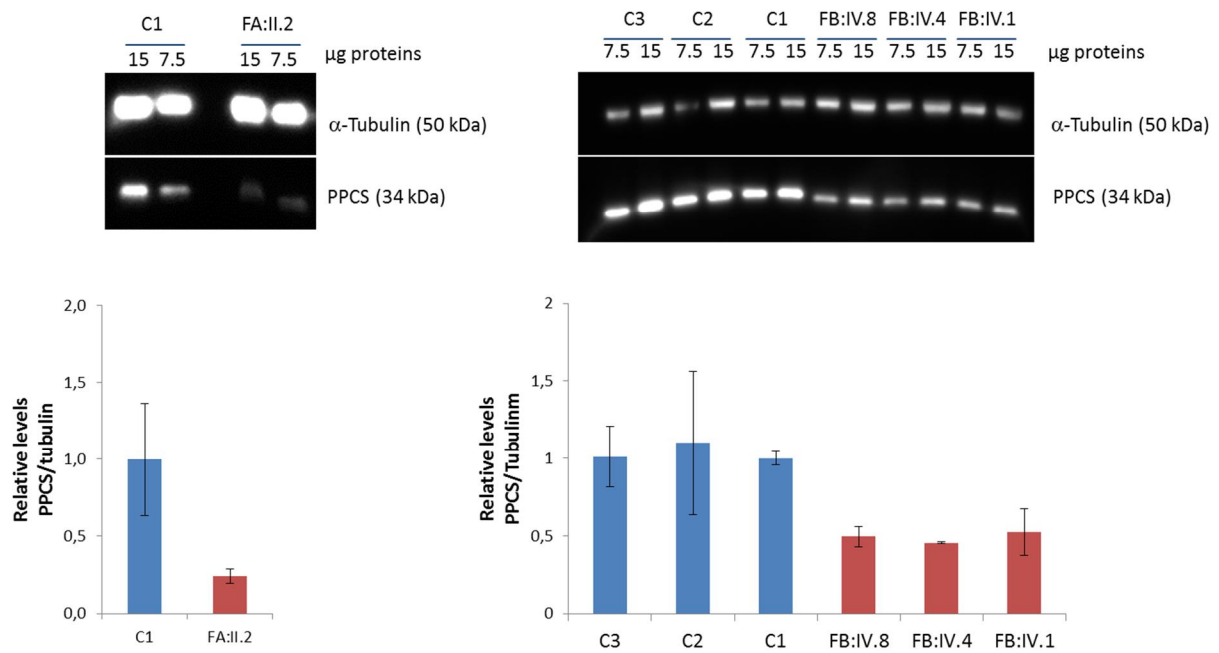


Figure S3. Western Blot analysis of PPCS protein in *PPCS*-mutant fibroblasts. Analysis of PPCS protein level was performed on whole cellular lysate of *PPCS*-affected individuals (FA:II.2, FB:IV.8, FB:IV.4, FB:IV.1) and of three unrelated controls (C1, C2, C3).

Program name	Ala180Pro	Glu233Val	URL	Full reference
SNAP	Functional effect (score=85, accuracy=91%)	Functional effect (score=49, accuracy=71%)	http://rostlab.org/services/snap/	Hecht M, Bromberg Y & Rost B. Better prediction of functional effects for sequence variant. BMC Genomics. 2015; 16(Suppl 8):S1
Polyphen2	Probably damaging (score=1.0, rankscore=0.97)	Probably damaging (score=1.0, rankscore=0.91)	http://genetics.bwh.harvard.edu/pph2/index.shtml	Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 7(4):248-249 (2010).
Sift	Damaging (score=0, rankscore=0.91)	Damaging (score=0, rankscore=0.91)	http://sift.jcvi.org/	Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073-81.
LRT	Deleterious (score=0.0, rankscore=0.84)	Deleterious (score=0.0, rankscore=0.84)	http://www.genetics.wustl.edu/jflab/lrt_query.html	Chun S and Fay JC. Identification of deleterious mutations within three human genomes. Genome Research (2009) 19:1553-1561.
MutationTaster	disease_causing (score=1, rankscore=0.81)	disease_causing (score=1, rankscore=0.81)	http://www.mutationtaster.org/	Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing

				age. Nat Methods. 2014 Apr;11(4):361-2.
MutationAssessor	High impact (score= 3.97, rankscore=0.972)	High impact (score= 3.96, rankscore=0.971)	http://mutationassessor.org/r3/	Reva B, Antipin Y, Sander C. Nucleic Acids Research (2011) "Predicting the Functional Impact of Protein Mutations: Application to Cancer Genomics"
Provean	Damaging (score= -4.83, rankscore= 0.82	Damaging (score= -6.75, rankscore= 0.92	http://provean.jcvi.org/seq_submit.php	Choi Y, Sims GE, Murphy S, Miller JR, Chan AP (2012) Predicting the Functional Effect of Amino Acid Substitutions and Indels. PLoS ONE 7(10): e46688.
Mupro	Mixed predictions	Mixed predictions	http://www.ics.uci.edu/~baldig/mutation.html	J. Cheng A. Randall, P. Baldi. Prediction of Protein Stability Changes for Single-Site Mutations Using Support Vector Machines. Proteins, vol. 62, no. 4, pp. 1125-1132, 2005.
I-mutant 2.0	Neutral (confidence 0)	Stabilize (confidence 2)	http://folding.biofold.org/cgi-bin/i-mutant2.0.cgi	Nucleic Acids Res. 2005 Jul 1;33(Web Server issue):W306-10. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Capriotti E1, Fariselli P, Casadio R.

Maestro	destabilizing ($\Delta\Delta G=0,62$ kcal/mol, confidence=0.88)	Destabilizing($\Delta\Delta$ $G=0,69$ kcal/mol, confidence=0.86)	https://biwww.che.sbg.ac.at/maestro/web	MAESTRO - multi agent stability prediction upon point mutations. Laimer J, Hofer H, Fritz M, Wegenkittl S and Lackner P. BMC Bioinformatics. 2015 Apr 16;16(1):116.
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Table S1. Computational predictions for the consequence of the Ala180Pro and Glu233Val variants.

Most tools listed here predict the functional consequence of the mutations. The last three programs predict change in thermostability.

No	Chr	Position	Gene	RefAlt	KggSeq annotation	Mutation	Near Splice Site	Mutation Refseq
1	1	42925359	PPCS	A/T	PPCS:NM_001287511:C.612+2338 A>T:(3EXONS):INTRON2;PPCS:NM_024664:C.698A>T:P.E233V:(3EXONS):EXON3:MISSENSE;PPCS:NM_001077447:C.179A>T:P.E60V:(3EXONS):EXON3:MISSENSE;PPCS:NM_001287508:C.179A>T:P.E60V:(3EXONS):EXON3:MISSENSE;PPCS:NM_001287510:C.179A>T:P.E60V:(2EXONS):EXON2:MISSENSE;PPCS:NM_001287509:C.179A>T:P.E60V:(3EXONS):EXON3:MISSENSE;PPCS:NM_001287506:C.179A>T:P.E60V:(3EXONS):EXON3:MISSENSE;PPCS:NM_001287507:C.80A>T:P.E27V:(3EXONS):EXON3:MISSENSE	MISSENSE	NO	INTRON2,MISSENSE
2	4	159630907	PPID	T/C	PPID:NM_005038:C.1094A>G:P.Y365C:(10EXONS):EXON10:MISSENSE	MISSENSE	NO	MISSENSE
3	8	80567102	STMN2	C/T	STMN2:NM_007029:C.289-4C>T:(5EXONS):INTRON3;STMN2:NM_001199214:C.289-4C>T:(6EXONS):INTRON3	INTRONIC	YES;3	INTRON3
4	15	59974718	BNIP2	G/C	BNIP2:NM_004330:C.307-5C>G:(10EXONS):INTRON1	INTRONIC	YES;-5	INTRON1
5	18	33282964	GALNT1	G/+TATC	GALNT1:NM_020474:C.1398+5G>+TATC:(11EXONS):INTRON9	INTRONIC	YES;-5	INTRON9
6	8	18658821	PSD3	T/C	PSD3:NM_015310:C.1982A>G:P.N661S:(16EXONS):EXON7:MISSENSE;PSD3:NM_206909:C.380A>G:P.N127S:(13EXONS):EXON4:MISSENSE	MISSENSE	NO	MISSENSE
7	4	997190	IDUA	C/G	IDUA:NR_110313:(14EXONS):NCRNA;IDUA:NM_000203:C.1582C>G:P.P528A:(14EXONS):EXON11:MISSENSE	MISSENSE	NO	NCRNA,MISSENSE
8	9	38610336	ANKRD18A	C/T	ANKRD18A:NM_147195:C.674G>A:P.R225H:(16EXONS):EXON5:MISSENSE	MISSENSE	NO	MISSENSE
9	11	93554357	VSTM5	G/A	VSTM5:NM_001144871:C.224C>T:P.T75M:(4EXONS):EXON2:MISSENSE	MISSENSE	NO	MISSENSE

10	X	101910520	GPRAS P1	T/C	SE ARMCX5- GPRASP2:NM_001199818:C.- 109216+49939T>C:(7EXONS):INTR ON4;GPRASP1:NM_001184727:C.1 679T>C:P.V560A:(6EXONS):EXON 6:MISSENSE;GPRASP1:NM_01471 0:C.1679T>C:P.V560A:(5EXONS):E XON5:MISSENSE;GPRASP1:NM_0 01099410:C.1679T>C:P.V560A:(4E XONS):EXON4:MISSENSE;GPRAS P1:NM_001099411:C.1679T>C:P.V 560A:(3EXONS):EXON3:MISSENS E	MISSENSE	NO	INTRON4,M ISSENSE
11	12	2760940	CACNA 1C	C/T	CACNA1C:NM_199460:C.4218+6C >T:(50EXONS):INTRON34;CACNA1 C:NM_001167624:C.4074+6C>T:(4 8EXONS):INTRON32;CACNA1C:N M_001129830:C.4074+6C>T:(48EX ONS):INTRON32;CACNA1C:NM_00 1129844:C.4065+6C>T:(47EXONS): INTRON32;CACNA1C:NM_0011298 37:C.4041+6C>T:(46EXONS):INTR ON31;CACNA1C:NM_001129834:C. 4074+6C>T:(47EXONS):INTRON32; CACNA1C:NM_001129836:C.4125+ 6C>T:(47EXONS):INTRON32;CACN A1C:NM_001129838:C.4041+6C>T: (46EXONS):INTRON31;CACNA1C: NM_001129829:C.4140+6C>T:(47E XONS):INTRON32;CACNA1C:NM_ 001129827:C.4218+6C>T:(49EXON S):INTRON34;CACNA1C:NM_0011 29843:C.4074+6C>T:(47EXONS):IN TRON32;CACNA1C:NM_00112983 5:C.4074+6C>T:(47EXONS):INTRO N32;CACNA1C:NM_001129832:C.4 134+6C>T:(48EXONS):INTRON33; CACNA1C:NM_001129842:C.4074+ 6C>T:(47EXONS):INTRON32;CACN A1C:NM_001129841:C.4074+6C>T: (47EXONS):INTRON32;CACNA1C: NM_001167625:C.4041+6C>T:(47E XONS):INTRON31;CACNA1C:NM_ 001129839:C.4035+6C>T:(46EXON S):INTRON31;CACNA1C:NM_0011 29833:C.4074+6C>T:(47EXONS):IN TRON32;CACNA1C:NM_00112984 6:C.4041+6C>T:(46EXONS):INTRO N31;CACNA1C:NM_001129831:C.4 158+6C>T:(48EXONS):INTRON33; CACNA1C:NM_000719:C.4074+6C >T:(47EXONS):INTRON32;CACNA1 C:NM_001167623:C.4074+6C>T:(4 7EXONS):INTRON32;CACNA1C:N M_001129840:C.4074+6C>T:(47EX ONS):INTRON32	INTRONIC	YES;-6	INTRON34,I NTRON32,I NTRON31,I NTRON33
12	9	71629038	PRKAC G	C/+G CGGC G	PRKACG:NM_002732:C.- 30G>CGCCGC+:(1EXONS):5UTR	5UTR	YES;1	5UTR
13	X	153137622	L1CAM	G/A	L1CAM:NM_001278116:C.385C>T: P.R129W:(29EXONS):EXON5:MISS ENSE;L1CAM:NM_000425:C.385C> T:P.R129W:(28EXONS):EXON4:MI SSENSE;L1CAM:NM_001143963:C. 370C>T:P.R124W:(26EXONS):EXO N3:MISSENSE;L1CAM:NM_024003: C.385C>T:P.R129W:(27EXONS):EX ON4:MISSENSE	MISSENSE	NO	MISSENSE
14	10	61956386	ANK3	G/+AA	ANK3:NM_001204403:C.1672-	INTRONIC	YES;-3	INTRON15,I

				A	3C>TTT+:(44EXONS):INTRON15;ANK3:NM_001204404:C.1639-3C>TTT+:(44EXONS):INTRON14;ANK3:NM_020987:C.1690-3C>TTT+:(44EXONS):INTRON14			NTRON14
15	8	77763128	ZFH4	G/A	ZFH4:NM_024721:C.3971G>A:P.S1324N:(11EXONS):EXON10:MISSENSE	MISSENSE	YES;-7	MISSENSE
16	10	85962835	CDHR1	G/T	CDHR1:NM_001171971:C.739G>T:P.V247L:(17EXONS):EXON8:MISSENSE;CDHR1:NM_033100:C.739G>T:P.V247L:(17EXONS):EXON8:MISSENSE	MISSENSE	NO	MISSENSE
17	1	62940938	DOCK7	C/T	DOCK7:NM_001272001:C.5920G>A:P.A1974T:(48EXONS):EXON45:MISSENSE;DOCK7:NM_033407:C.5953G>A:P.A1985T:(49EXONS):EXON46:MISSENSE;DOCK7:NM_001271999:C.6013G>A:P.A2005T:(49EXONS):EXON46:MISSENSE;DOCK7:NM_001272000:C.5926G>A:P.A1976T:(49EXONS):EXON46:MISSENSE	MISSENSE	NO	MISSENSE
18	X	48213439	SSX3	T/A	SSX3:NM_021014:C.275A>T:P.N92I:(8EXONS):EXON4:MISSENSE	MISSENSE	YES;-6	MISSENSE
19	X	19983405	CXORF23	G/A	CXORF23:NM_198279:C.1031C>T:P.P344L:(11EXONS):EXON3:MISSENSE	MISSENSE	NO	MISSENSE
20	X	152939414	PNCK	G/A	PNCK:NM_001135740:C.-671C>T:(12EXONS):UPSTREAM;PNCK:NM_001039582:C.217C>T:P.P73S:(12EXONS):EXON1:MISSENSE	MISSENSE	NO	UPSTREAM,MISSENSE
21	X	50378384	SHROOM4	C/T	SHROOM4:NR_027121:(10EXONS):NCRNA;SHROOM4:NM_020717:C.689G>A:P.R230Q:(9EXONS):EXON4:MISSENSE	MISSENSE	NO	NCRNA,MISSENSE
22	4	187628341	FAT1	G/A	FAT1:NM_005245:C.2641C>T:P.R81C:(27EXONS):EXON2:MISSENSE	MISSENSE	NO	MISSENSE
23	18	43248391	SLC14A2	G/A	SLC14A2:NM_007163:C.1985G>A:P.G662D:(20EXONS):EXON15:MISSENSE;SLC14A2:NM_001242692:C.1985G>A:P.G662D:(21EXONS):EXON16:MISSENSE	MISSENSE	NO	MISSENSE
24	X	152939483	PNCK	C/G	PNCK:NM_001135740:C.-740G>C:(12EXONS):UPSTREAM;PNCK:NM_001039582:C.148G>C:P.G50R:(12EXONS):EXON1:MISSENSE	MISSENSE	NO	UPSTREAM,MISSENSE
25	X	50213063	DGKK	GGGC GAT/ G-----	DGKK:NM_001013742:C.A612DELATCGCC-:P.P205DEL?R?:(28EXONS):EXON1:NONFRAMESHIFT	NONFRAME SHIFT	NO	NONFRAME SHIFT
26	11	124790575	HEPACAM	A/G	HEPN1:NM_001037558:C.*2A>G:(1EXONS):DOWNSTREAM;HEPACAM:NM_152722:C.*459T>C:(7EXONS):3UTR	3UTR	YES;-2	DOWNSTREAM,3UTR

Table S2. Variant calling for individual IV.8 family B and his mother. Detailed are the final 26 variants filtered for analysis.