SUPPLEMENT

Disease relevance of rare *VPS13B* missense variants for neurodevelopmental Cohen syndrome

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Summary on literature reported disease-associated *VPS13B* missense variants

Six out of 29 disease-associated *VPS13B* missense variants were reported in compound heterozygosity or homozygosity with two further most likely truncating variants on both alleles (Lys1129Arg, Asp1210Tyr, Thr1289Ser, Ile2795Thr, Ser3303Arg, and Val3420Met. Thus, those rare missense variants can be suspected to be more likely benign [33, 34].

Five out of 29 disease-associated *VPS13B* missense variants were reported in compound heterozygosity with one most likely truncating variant on the second allele (Trp185Arg, Thr1068Ile, Leu2168, Tyr2316Cys, and Gly2704Arg).

Seven out of 29 disease-associated *VPS13B* missense variants were reported in different cases as homozygous variants (Ser824Ala, Ile1611Asn, Val2456Ile, Gly2620Asp, Ser2748, Asn2968Ser, and Arg3198Trp). Asn2968Ser was originally identified in a consanguine family in homozygosity [20]. However, the same mutation was more recently in compound heterozygosity with another missense variant Leu2821Ile [29]. For the missense variant Arg3198Trp in autism conflicting genetic data have been reported [15]. This variant is inherited by both male children in homozygosity from an unaffected homozygous father and an unaffected heterozygous mother in one family, and by another male patient in heterozygosity from an unaffected heterozygous father and an unaffected heterozygous mother in another family. For the latter family no further *VPS13B*-associated variation was identified.

Five plus one out of 29 disease-associated *VPS13B* missense variants were reported in compound heterozygosity with another missense variant (Gly567Glu, Pro1133Ser, Leu2821Ile, Thr3602Ile, and Ala3691Thr; plus Asn2968Ser).

Six out of 29 disease-associated *VPS13B* missense variants were reported in heterozygous inheritance where a second variant is still unknown (Phe274Val, Ala590Thr, Thr1289Ala, Lys1682Glu, Asn3088Tyr, and Pro3962Arg). Here, the three missense variants Phe274Val, Thr1289Ala, and Leu1682Glu) have been reported in targeted NGS for autism or primary immunodeficiency disease screens in occurrence with other heterozygous variants in further disease-causing genes [4, 21].The results from *in vitro* characterization of the hitherto cloned *VPS13B* missense variations and their update on ACMG classification are summarized in table 1.

Details on literature reported disease-associated *VPS13B* missense variants

The missense variant Trp185Arg was identified in one male with intellectual disability, microcephaly and joint hypermobility; combining typical features of Cohen syndrome [36]. At the age of 3 years clinical assessment did not reveal any ophthalmic or blood problems. The missense variant was detected as compound heterozygous with the fatal missense variant Cys733*.

The missense variant Phe274Val was identified in a male patient and was found in heterozygosity, lacking further variants in *VPS13B* [21]. The patient belonged to a study in which target gene enrichment of autism-associated genes occurred. However, a second heterozygous missense variant was identified in RAI1. The authors state that this missense variation is most unlikely disease causing.

The missense variant Gly567Glu was identified in compound heterozygosity with another Pro1133Ser missense variant during diagnostic family-based exome sequencing [11]. All patients were found to have a genetic etiology but lack a genetic diagnosis. Functional follow-up evidence of the hitherto identified missense variants remains elusive.

The missense variant Ala590Thr was identified as heterozygous in one female offspring from nonconsanguineous Italian parents [18]. A compound heterozygous second variant in *VPS13B* is missing so far. Age of clinical assessment was 24 years. The Cohen syndrome phenotype is incomplete without documented microcephaly, retinopathy and myopia. However, moderate intellectual disability and neutropenia indicate a Cohen syndrome.

The missense variant Ser824Ala was identified in a male patient with incomplete Cohen syndrome phenotype [37]. The analysis was performed in a screening of genetic causes for autism and was found

in homozygous state. The authors speculate an incomplete, hypomorph Cohen syndrome due to reduced protein availability.

The missense variant Thr1068Ile was identified in compound heterozygosity with a mild intragenic c.8016+7G > C, which has not been tested for its effect on the splicing event [36]. The major phenotype of the patient were repeated seizures and very mild intellectual disability.

The missense variant Lys1129Arg was identified in two siblings with Cohen syndrome [33]. However, both affected children carrier two compound heterozygous LoF variations in *VPS13B*, which segregate within the family and were therefore considered as solely responsible to cause Cohen syndrome. In a more recent study this missense variant reappeared in a genetic approach on patients with retinal disease [35]. The respective patient has a compound heterozygous pathogenic variant in *VPS13B* (c.10232delC) and was clinically assessed to have Cohen syndrome.

The missense variant Pro1133Ser was identified in compound heterozygosity with the above described Gly567Glu missense variant [11].

The heterozygous missense variant Asp1210Tyr was identified in a whole-genome sequencing approach on patients with retinal disease [3]. The respective patient presents with two other compound heterozygous pathogenic variants in *VPS13B* (Ser864* and Met2124Valfs*44), probably solely causative for the inherited retinal disease.

The heterozygous missense variant Thr1289Ala occurred in a targeted disease genes approach for primary immunodeficiency [4]. A second variant in *VPS13B* could not be identified; however, further heterozygous variants were identified in the respective patient in other primary immunodeficiency-associated disease genes: *SLC37A4, SCNN1G*, and *CXCR4*.

The missense variant Thr1289Ser in the second child of a nonconsanguineous Caucasian African couple was identified in combination with a paternal inherited 3 bp insertion (c. 11752_11753insATG) and a maternal inherited 315 kb deletion spanning from exon 4 of the *OSR2* gene to exon 17 of the *VPS13B* gene (chr8: 100015029…100347846del) [30]. Phenotypic assessment occurred at the age of 2 years with Cohen syndrome-like facial dysmorphism but without typical ophthalmological and/or hematological Cohen syndrome-associated findings.

The homozygous missense variant Ile16111Asn was recognized in Moroccan twins from consanguineous parents [9]. Both twins show typical Cohen syndrome-like features including intellectual disability, microcephaly, facial dysmorphism, and slender extremities.

The heterozygous missense variant Lys1682Glu occurred in a targeted disease genes approach for primary immunodeficiency [4]. A second variant in *VPS13B* could not be identified; however, further heterozygous variants were identified in the respective patient in other primary immunodeficiency-associated disease genes: *ADA, FERMT3*, and *CD79B*.

The compound heterozygous missense variant Leu2168Arg was found in combination with a 2 bp deletion (c.3348_3349delCT) in a finish patient with typical a finish phenotype of Cohen syndrome [19].

The missense variant Tyr2316Cys was identified as compound-heterozygous together with a nonsense variation (c.11240C>T) [13]. Clinical assessment of the 4-yearold female showed typical facial dysmorphism, intellectual disability and postnatal microcephaly. However, ophthalmological and hematological findings were unremarkable which is most likely due to the younger age of the patient at clinical assessment.

The homozygous missense variant Val2456Ile was identified in an exome sequencing approach in persons with severe intellectual disability [7]. The male patient was clinically assessed at the age of 4 years and presents with intellectual disability and microcephaly but normal motor development. MRI of the brain was normal, and at the age of around one year he lost his previously accomplished communication skills.

The missense variant Gly2620Asp was identified in two consanguine families from the Oman. All patients share a typical facial dysmorphism, intellectual disability, myopia and microcephaly in agreement with Cohen syndrome [13, 26]. Moreover, the oldest child (10 years at clinical assessment) showed initial ophthalmological abnormalities indicative of retinopathy and her MRT screening showed a comparable enlarged corpus callosum [26].

The missense variant Gly2704Arg was identified in a screening of genetic causes for autism [37]. However, the clinical reevaluation of the patient showed typical Cohen syndrome-like features. Moreover, the missense variant was identified to be compound heterozygous with a nonsense variation in *VPS13B* (c.8110G>A).

The missense variant Ser2748Leu was identified in two consanguineous families [34]. All three patients (two female, one male) showed typical Cohen syndrome-like facial appearance and intellectual disability. Two patients presented also with postnatal microcephaly. Due to their younger age (8, 5 and 2.5 years) the exclusion of later onset of ophthalmological problems could not be excluded.

The missense variant Ile2795Thr was recognized in 8 patients from an Amish community in US, Ohio [10]. However, all 8 patients carry an additional homozygous insertion (c.9183_9184insT) in *VPS13B*, which is most likely solely causative for the typical Cohen syndrome phenotype in all those patients. In another clinical study on bilateral angle closure glaucoma the missense variant Ile2795Thr was identified in heterozygosity in the described patient with Cohen syndrome. The patient has Amish background and was further genetically tested carrying the homozygous c.9258_9259insT [24].

The missense variant Leu2821Ile was detected in a whole-exome sequencing screen across clinical indicators with focus on family-based analysis and classified as very likely pathogenic [29]. However, from the supported material, one cannot clearly extract status of heterozygosity, inheritance or clinical presentation of the belonging patient.

The missense variant Asn2968Ser was identified in homozygous occurrence in Belgian twins [20]. Both patients presented with a typical Cohen syndrome including intellectual disability, microcephaly, neutropenia, retinopathy and myopia. However, the authors themselves speculated that this missense variant could represent a rare nonpathogenic change. This missense variant reoccurred in in a whole-exome sequencing screen across clinical indicators with focus on family-based analysis [29]. However, from the supported material, one cannot clearly extract status of heterozygosity, inheritance or clinical presentation of the belonging patient. In a large-scale genomic WES project on 267 genomes representing the healthy Spanish population the missense variant Asn2968Ser occurred one time on one allele [8].

The heterozygous missense variant Asn3088Tyr was identified in a genetic research study on short stature by target/whole exome sequencing [14]. A second variant in *VPS13B* was not identified. In addition to the short stature, the female patient was born with an atrial septal defect and mitral regurgitation and presents with intellectual disability as well as microcephaly. However, according to the ACMG guidelines, the authors categorized this missense variant as variance of uncertain significance.

The missense variant Arg3198Trp was identified in a sequenced-based study on identification of rare causal variants in Cohen syndrome and autism [15]. The variant appeared in 2 families, each with 2 affected siblings. The total allele count was 5 in affected individuals versus one in unaffected individuals. Reevaluation occurred by Sanger sequencing. However, the transmission showed also homozygosity in the unaffected father of one family.

The missense variant Val3445Met (c.10333G>A) was identified in heterozygosity with a homozygous Tyr413* (c.1239T>G) by whole-exome sequencing and was confirmed by Sanger sequencing. [23]. The patient presented with Cohen syndrome-agreeable features including normal head circumference at birth and postnatal microcephaly, intellectual disability and a typical facial appearance.

The missense variant Thr3602Ile (c.10805C>T) was identified on same allele in very close proximity to a deletion c.10808_10825delCGAGGCAGCTTGTGCACG [9]. Thus, it is more likely that both alterations occurred at the same DNA changing event. However, they were found to be compound heterozygous with nonsense variation (Arg692*). All patients with *VPS13B* variations had intellectual disability, a Cohen syndrome-typical facial dysmorphism, microcephaly and slender extremities with narrow hand/feet. Moreover, 11/12 patients had neutropenia.

The missense variants Ser3303Arg and Ala3691Thr were identified as compound heterozygous in one patient with an autisms-like phenotype [37]. However, reevaluation showed Cohen-syndrome-like facial dysmorphism but normocephalic brain development.

The heterozygous missense variant Pro3962Arg was identified in a male patient, lacking a second *VPS13B*-associated variant [21]. The patient belonged to a study in which target gene enrichment of autism-associated genes occurred. The authors state that this missense variation is most unlikely disease causing.

Genotype and phenotypical characteristics of these previously reported patients are summarized in supplementary table T1.

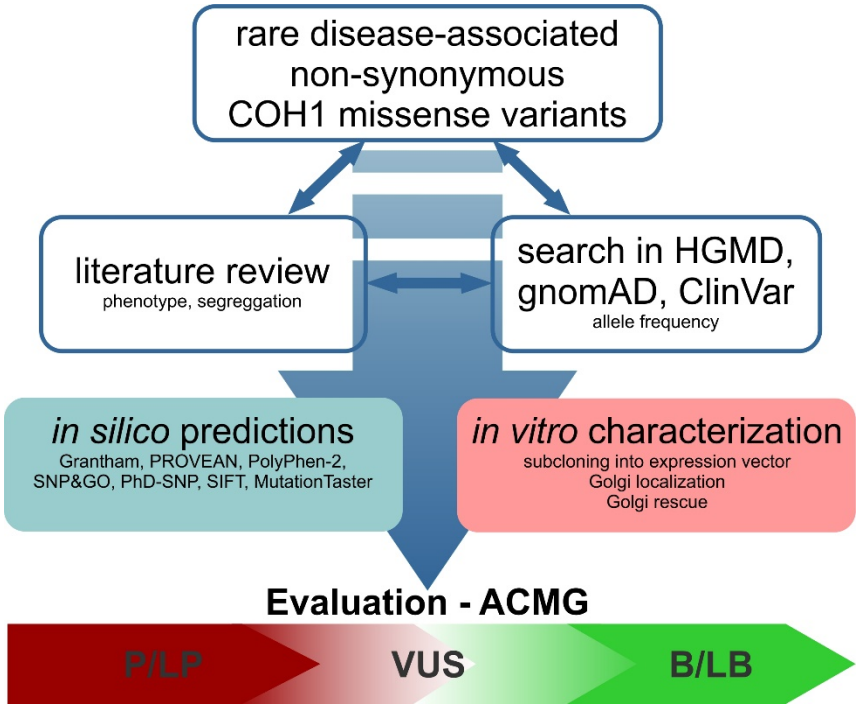
In silico analyses of disease-associated _VPS13B_ missense variants

To study missense variants in *VPS13B*, we started with a literature search on PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). 29 missense variants were retrieved as associated with Cohen syndrome, autism, intellectual disability, retinal disease, primary immunodeficiency disease, or short stature [3, 4, 7, 9-11, 13-15, 17, 19-21, 26, 29, 30, 33-37]. However, this search warrants no completeness. We reevaluated all 29 missense variants using several established pathogenicity prediction algorithms for *in silico* analysis, which can be broadly subdivided into three types: (1) sequence and evolutionary conservation-based method, (2) protein sequence and structure-based methods and (3) supervised machine learning methods.

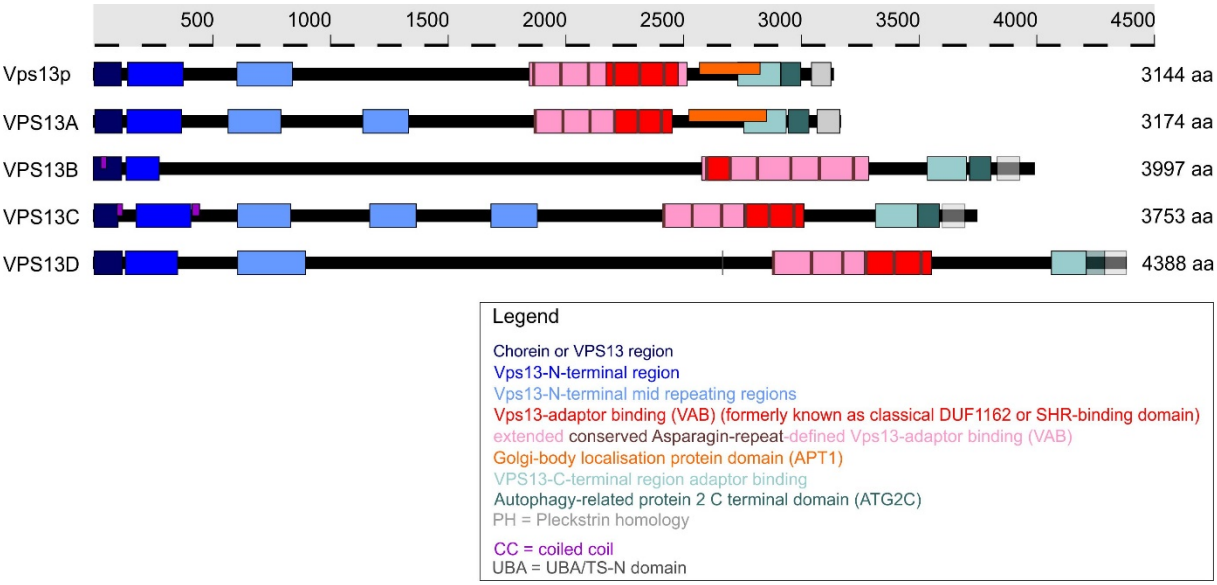
The Grantham substitution matrix is one of the earliest and simplest methods and provides the prediction of effects in substitutions between amino acids (aa) based in their chemical properties, including polarity and molecular volume. The Grantham score subdivides the substitutions by their increasing chemical dissimilarity in 4 different classes: conservative (0-50), moderately conservative (51-100), moderately radical (101-150), or radical (≥151) [12]. For the hitherto analyzed 29 missense variants, the Grantham substitution matrix identified three variants (Asp1210Tyr, Ile1611Asn and Tyr2316Cys) as radical, eight as moderately radical (Trp185Arg, Leu2168Arg, Gly2704Arg, Ser2748Leu, Asn3088Tyr, Arg3198Trp, Ser3303Arg, and Pro3962Arg), 12 as moderately conservative (Gly567Glu, Ala590Thr, Ser824Ala, Thr1068Ile Pro1133Ser, Thr1289Ala, Thr1289Ser, Lys1682Glu, Gly2620Asp, Ile2795Thr, Thr3602Ile, and Ala3691Thr), and six as conservative (Phe274Val, Lys1129Arg, Val2456Ile, Leu2821Ile, Asn2968Ser, and Val3420Met). However, the Grantham score exclusively assesses the chemical dissimilarity of the exchanged substitution without regard to protein conservation, structure, domains and/or motifs. PROVEAN (protein variation effect analyzer, http://provean.jcvi.org) and SIFT (http://sift.jcvi.org/, sorting intolerant from tolerant) algorithms are comparable analysis tools for filtering sequence variants based on sequence homology. PROVEAN includes the quality of sequence alignment of the flanking neighborhood sequences [5, 6]. Thus, the prediction accuracy is based on the supporting set of homologous sequences which is influenced by the choice of underlying protein databases. PROVEAN discriminates its score thresholds between deleterious (lower than default -2.500) and neutral (higher than -2.500). To increase specificity (>90%) a lower score threshold (-4.100) can be used, while to increase sensitivity (>90%) a higher threshold can be used (-1.300). For the hitherto analyzed 29 missense variants, the PROVEAN algorithm identified 13 variants as deleterious (Trp185Arg, Asp1210Tyr, Ile1611Asn, Leu2168Arg, Tyr2316Cys, Gly2620Asp, Gly2704Arg, Ser2748Leu, Asn2968Ser, Asn3088Tyr, Ser3303Arg, Thr3602Ile, and Ala3691Thr) and 16 variants as neutral. SIFT is a multi-step algorithm, which basically scans each position for evolutionary conservation and calculates all probabilities [22, 27]. SIFT uses a multiple sequence alignment (MSA) strategy to assess

the probability of a deleterious effect. A highly conserved residue is generally assumed to be intolerant to substitutions while low conserved residues tolerate most substitutions. SIFT discriminates in damaging scores (≤0.05) and tolerated (≥0.05) scores. For the hitherto analyzed 29 missense variants, the SIFT algorithm identified 27 variants as damaging and only two variants as tolerated (Val2456Ile and Ala3691Thr). The Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/, polymorphism phenotyping) provides an algorithm for filtering sequence variants based on structural homology [1]. Polyphen-2 discriminates in three different categories – probably damaging (<0.96), possibly damaging (<0.2-<0.96) and benign (>0.2). For the hitherto analyzed 29 missense variants, the Polyphen-2 algorithm identified 14 variants as probably damaging (Trp185Arg, Lys1129Arg, Asp1210Tyr, Thr1289Ala, Thr1289Ser, Ile1611Asn, Tyr2316Cys, Gly2620Asp, Gly2704Arg, Ser2748Leu, Asn2968Ser, Asn3088Tyr, Arg3198Trp, and Ala3691Thr), nine variants as possibly damaging (Phe274Val, Ala590Thr, Thr1068Ile, Lys1682Glu, Leu2168Arg, Ile2795Thr, Leu2821Ile, Ser3303Arg, and Thr3602Ile) and six variants as tolerated (Gly567Glu, Ser824Ala, Pro1133Ser, Val2456Ile, Val3420Met, and Pro3962Arg). Further MutPred2 (http://mutpred.mutdb.org/, random forest method), MutationTaster2 (http://mutationtaster.org/, Bayes classifier) and SNP&GOAnalyzer (http://snps.biofold.org/snps-and-go/snps-and-go.html) are machine learning-based algorithms, which combine genetic and molecular data to predict the probability of pathogenicity of amino acid substitutions. MutPred2 discriminates at a cut-off of 0.5 between pathogenic and non-pathogenic predictions [28]. Here, 17 out of 29 missense variants have been classified as pathogenic variants (Trp185Arg, Phe274Val, Thr1068Ile, Pro1133Ser, Asp1210Tyr, Ile1611Asn, Lys1682Glu, Leu2168Arg, Tyr2316Cys, Gly2620Asp, Gly2704Arg, Ser2748Leu, Asn2968Ser, Asn3088Tyr, Ser3303Arg, Thr3602Ile, and Ala3691Thr). With respect for missense variants, MutationTaster2 integrates information spanning evolutionary conservation, different biochemical databases and established analyses tools [31, 32]. The output distinguishes disease causing from polymorphism. Regarding the VPS13B-missense variants, except for 4 missense variants (Ser824Ala, Val2456Ile, Val3420Met, and Arg3198Trp) all other variants were predicted to be disease causing. SNPs&GO, a support vector machine (SVM) method, combines information on sequence (multiple sequence alignment, MSA), alleged protein function (Gene Ontology, GO) and Panther Evolutionary Analysis of Coding SNPs results [2, 25]. The SNPs&GO is similar to the PhD-SNP method. Both methods differentiate between neutral and disease. For the SNPs&GO algorithm 5 out of 29 have been classified as disease (Trp185Arg, Asp1210Tyr, Tyr2316Cys, Gly2704Arg, and Val3420Met). According to PhD-SNP, 12 out of 29 missense variants have been classified as neutral (Phe274Val, Gly567Glu, Ala590Thr, Ser824Ala, Thr1068Ile, Lys1129Arg, Thr1289Ala, Thr1289Ser, Val2456Ile, Leu2821Ile, Val3420Met, and Ala3691Thr). Further analytical tools implementing higher order protein structures (e.g. nsSNPAnalyzer, ERIS, Clustal, ConSurf, I-Tasser, SuperPose, ClusPro) could not be carried out due to the lack of further information on the protein structure of VPS13B. Allele frequency was extracted from GnomAD browser [16]. All prediction results are summarized in supplementary table T2.

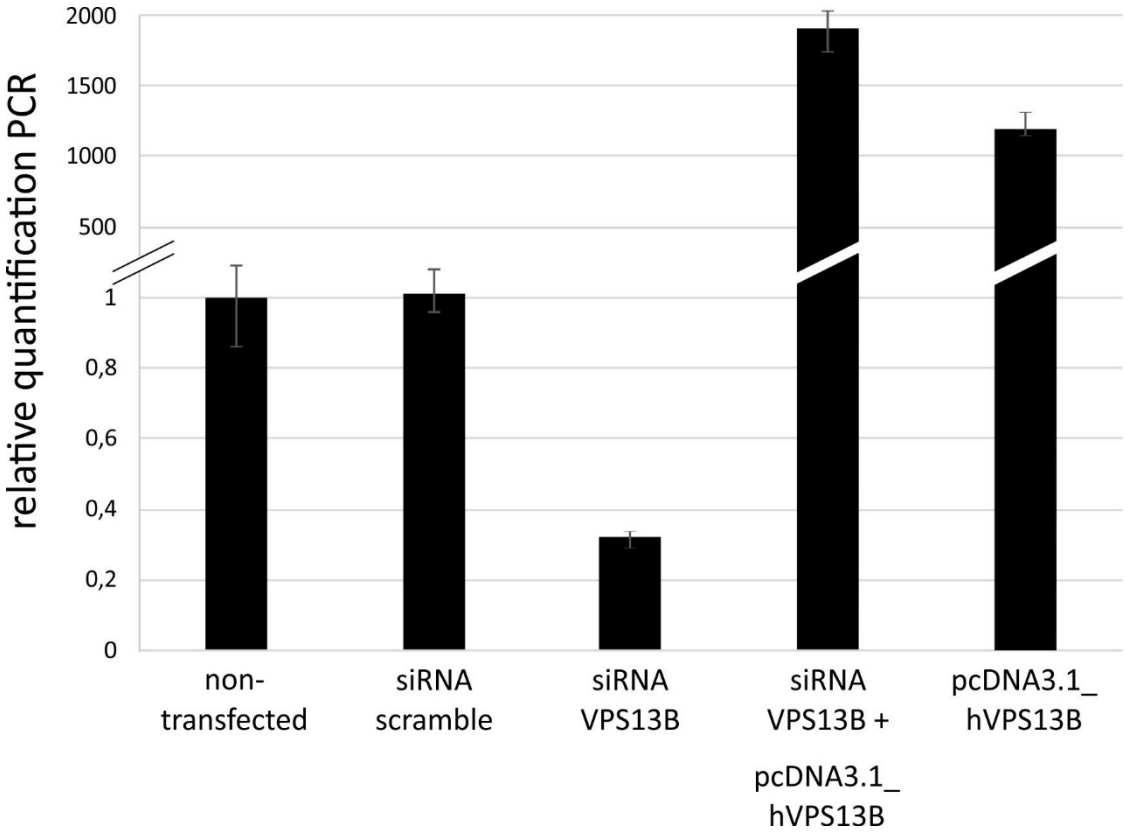
Supplementary figure S1: **Study design**. The study design involved *in silico* and *in vitro* analysis to predict and investigate the impact of *VPS13B* missense variants (P - pathogenic, LP - likely pathogenic, VUS - variant of unknown significance, LB - likely benign, B - benign).



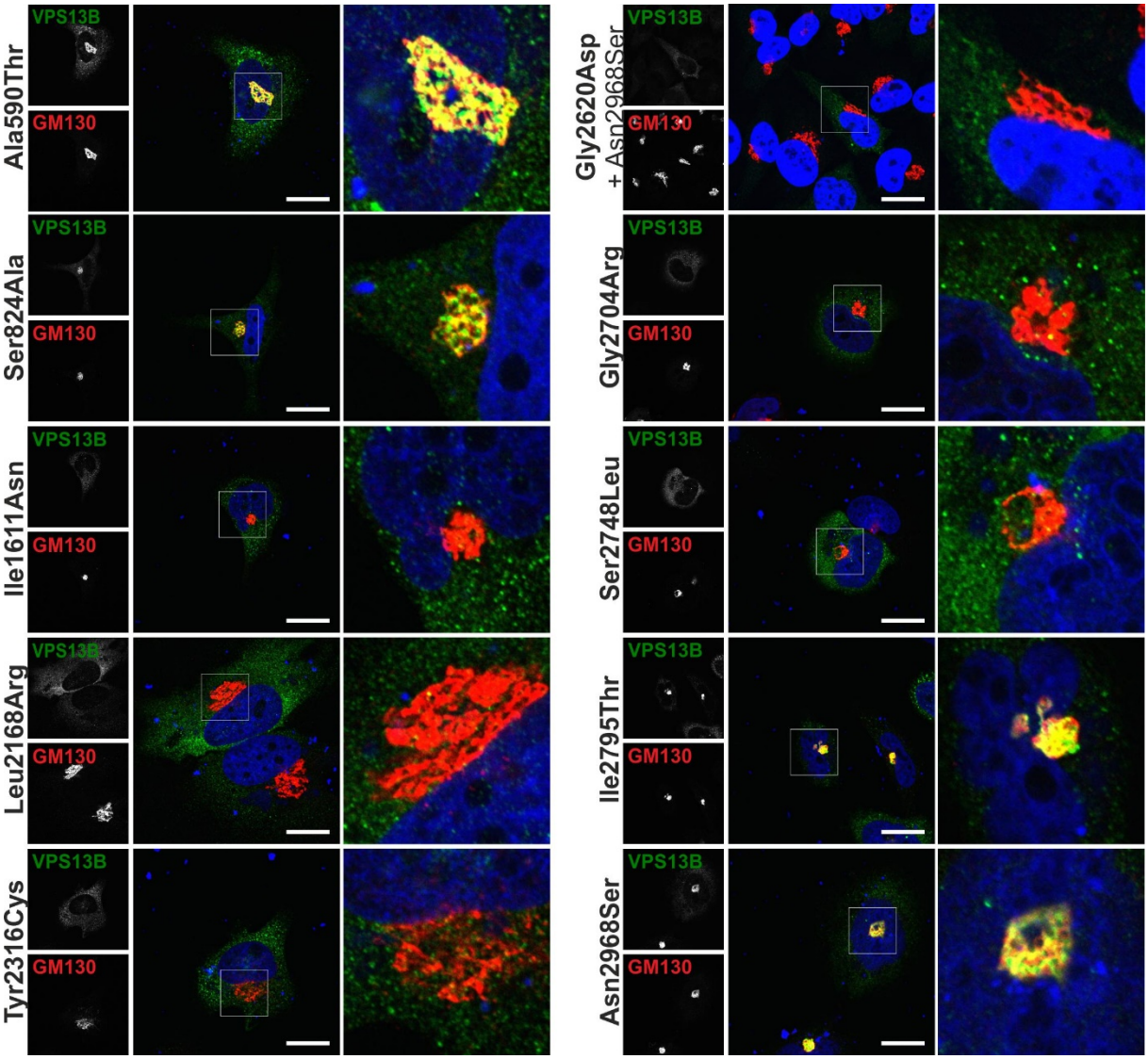
Supplementary Figure S2: **VPS13 family.** The figure summarizes the protein length and domain organization of yeast Vps13p and human VPS13 family members VPS13A (CHAC), VPS13B (COH1), VPS13C, and VPS13D.



Supplementary Figure S3: Expression analysis with qPCR assessing relative *VPS13B* transcript levels during siRNA treatment and transient overexpression in HeLa cells.



Supplementary Figure S4: **Localization of mutant VPS13B in HeLa cells.** HeLa cells were transfected with the respective pcDNA3.1_VPS13B-mut constructs. 24h later, cells were processed for immunostaining of VPS13B (green) and GM130 (red). Imaging occurred with confocal microscopy. Nuclei were stained using DAPI (blue), scale bars 10 µm.



Supplementary Table T1: Summary of clinical symptoms of patients with missense variants in *VPS13B*.

Protein change (NP_689777.3)	cDNA Change (NM_152564.4)	Protein change (NP_060360.3)	cDNA Change (NM_017890.4)	Exon	Inheritance	Sex	Age at diagnosis (a)	I. Facial Dys-morphism	II. Micro-cephaly	III. Intellectual Disability	IV. Myopia/ Retino-pathy	V. Neutro-penia	VI. Other CS features	Initial Diagnosis	Score for CS	Detection method	Reference
Trp185Arg	c.553T>C	Trp185Arg	c.553T>C	5	compound heterozygous with Cys733*	M	3	+	++	++	-	-	++[1]	CS	3.5/6	WGS (NextSeq500, illumina)	Yang et al., 2018
Phe274Val	c.820T>G	Phe274Val	c.820T>G	7	heterozygous; second variant unknown; another missense variant het in RAI1	M	11	n/p	n/p	++	n/p	n/p	-[4]	Autism	1/2	target gene enrichment (PGM, Thermo Fisher Scientific versus MiSeq, illumina)	Koshimizu et al. 2013
Gly567Glu	c.1700G>A	Gly567Glu	c.1700G>A	13	compound heterozygous with Pro1133Ser	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	undiagnosed genetic condition	n.d.	diagnostic WES (SureSelect Target Enrichment System, Agilent Technologies or SeqCap EZ VCRome 2.0, Roche NimbleGen)	Farwell et al., 2015
Ala590Thr	c.1768G>A	Ala590Thr	c.1768G>A	13	heterozygous; second variant unknown	F	24	- (yes/no)	-	++	-	+	+[2]	CS	2/6	Sanger sequencing, DHPLC, TaqMan	Katzaki et al. 2007
Ser824Ala	c.2470T>G	Ser824Ala	c.2470T>G	17	homozygous, consanguine	M?	n/p	+	n/p	n/p	n/p	n/p	+[1]	Autism	1/2	WGS (HiSeq, illumina), confirmed by Sanger sequencing	Yu et al. 2013
Thr1068Ile	c.3203C>T	Thr1068Ile	c.23278C>T	22	compound heterozygous with c.8016+7G>C	M	5	-	-	+	-	-	-[4]	Epilepsy	0.5/6	WGS (NextSeq500, illumina)	Yang et al., 2018
Lys1129Arg	c.3386A>G	Lys1129Arg	c.3386A>G	23	compound heterozygous with c.10232delC	n/p	n/p	n/p	n/p	n/p	++	n/p	n/p	Retinal disease/CS	n.d.	target specific sequencing, SNP calling and chromosome analysis (Sanger sequencing; EP1 system, Fluidigm ; T-ARMS PCR, chromosomal microarray)	Stone et al., 2017
					compound heterozygous with 2 other CS causing variants (Gln2229Hisfs*10/ Trp3649*)	F,F	8,5	++	++	++	++	++	+	CS	5.5/6	Sanger sequencing	Seifert et al. 2009
Pro1133Ser	c.3397C>T	Pro1133Ser	c.3397C>T	23	compound heterozygous with Gly567Glu	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	undiagnosed genetic condition	n.d.	diagnostic WES (SureSelect Target Enrichment System, Agilent Technologies or SeqCap EZ VCRome 2.0, Roche NimbleGen)	Farwell et al., 2015
Asp1210Tyr	c.3628G>T	Asp1210Tyr	c.3628G>T	24	compound heterozygous with 2 other CS causing variants (Ser864*/ Met2124Valfs*44)	F	n/p	n/p	n/p	n/p	++	n/p	n/p	Retinal disease, CS	1/1	WES and WGS	Carss et al., 2017
Thr1289Ala	c.3865A>G	Thr1289Ala	c.3865A>G	27	heterozygous, second variant unknown; other het variants in SLC37A4, SCNN1G, CXCR4	M	2	n/p	n/p	n/p	n/p	++	-[6]	Primary immunodefi-ciency disease	1/1	targeted HTS (Agilent SureDesign with NextSeq500, illlumina)	Chi et al., 2018
Thr1289Ser	c.3866C>G	Thr1289Ser	c.3866C>G	27	compound heterozygous with 2 other CS causing variants (ex01-17del/ Asp3942_Gly3943insAsp)	F	2	++	-	++	-	-	n/p	CS	2.5/5	Genome-wide screen for CNVs (SNP array 6.0, affymetrix))	Rivera-Brugués et al. 2011
Ile1611Asn	c.4832T>A	Ile1636Asn	c.4907T>A	31	homozygous, consanguine	n/p;n/p	n/p; n/p	++	++	++	n/p	n/p	+[3]	CS	3.5/4	Sanger sequencing	El Chehadeh et al. 2010
Lys1682Glu	c.5044A>G	Lys1707Glu	c.5119A>G	32	Heterozygous; second variant unknown; other	M	1	n/p	n/p	n/p	n/p	++	-[5]	Primary immunodefi-ciency disease	1/1	targeted HTS (Agilent SureDesign with NextSeq500, illlumina)	Chi et al., 2018

					het variants in ADA, FERMT3, CD79B												
Leu2168Arg	c.6503T>G	Leu2193Arg	c.6578T>G	37	compound heterozygous with Cys1092fs*7	n/p	n/p	++	++	++	++	++	+[1]	CS	5.5/6	Sanger sequencing	Kolehmainen et al. 2003
Tyr2316Cys	c.6947A>G	Tyr2341Cys	c.7022A>G	39	compound heterozygous with Gln3747*	F	4	++	++	++	+	-	++[1,2,3]	CS	4.5/6	Sanger sequencing	Hennies et al. 2004
Val2456Ile	c.7366G>A	Val2481Ile	c.7441G>A	41	homozygous	M	4	++	++	++	n/p	n/p	n/p[9]	Intellectual disability	3/3	diagnostic WES	de Ligt et al., 2012
Gly2620Asp	c.7859G>A	Gly2645Asp	c.7934G>A	43	homozygous, consanguine	F;M;M	7,5,2	++	++	++	++	n/p	++[1,2,3]	CS	5/5	Sanger sequencing	Hennies et al. 2004
Gly2704Arg	c.8110G>A	Gly2795Arg	c.8185G>A	45	compound heterozygous with Trp963*	M	n/p	++	n/p	n/p	n/p	n/p	n/p	Autism/CS	1/1	NGS (HiSeq, illumina), confirmed by Sanger sequencing	Yu et al. 2013
Ser2748Leu	c.8243C>T	Ser2773Leu	c.8318C>T	45	homozygous, consanguine	F,F M	8,5 2	++ ++	++ ++	++ ++	++ n/p	n/p n/p	+[2] -	CS	4.5/5 3/4	Sanger sequencing	Seifert et al.2006
Ile2795Thr	c.8384T>C	Ile2820Thr	c.8459T>C	46	homozygous, but consanguine amish people with second homozygous Leu3062fs*19 compound heterozygous amish with homozygous c.9258_9259insT	M,F,F,M,F F,F,F F	15,9,4,9,7 6,14,13 28	++ ++ ++	++ ++ n/p	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++	CS CS CS	6/6 6/6 5/5	Sanger sequencing n/p	Falk et al.2004 Li et al 2018
Leu2821Ile	c.8461C>A	Leu2846Ile	c.8536C>A	47	compound heterozygous with Asn2968Ser	n/p[10]	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n.d.	WES	Retterer et al., 2016
Asn2968Ser	c.8903A>G	Asn2993Ser	c.8978A>G	49	homozygous, consanguine compound heterozygous with Leu2821Ile heterozygous	n/p;n/p n/p[10] n/p	n/p;n/p n/p n/p	++ n/p -	++ n/p -	++ n/p -	++ n/p -	++ n/p -	++ n/p -	CS n/p healthy	6/6 n.d. 0/6	Sanger sequencing WES WES	Kolehmainen et al. 2004 Retterer et al., 2016 Dopazo et al. 2016
Asn3088Tyr	c.9262A>T	Asn3113Tyr	c.9337A>T	51	heterozygous, second variant is missing	F	1	-	++	++	n/p	n/p	+[7,8]	Short stature	2.5/3	target sequencing (ClearSeq Inherited Disease panel) and WES (SureSelect All Exon V5, Agilent with HiSeq4000, illumina)	Huang et al., 2018
Arg3198Trp	c.9592C>T	Arg3223Trp	c.9667C>T	52	4 patients: 1 homozygous 3 heterozygous cases with unknown second variant 1 control: heterozygous	 n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 n/p n/p n/p n/p n/p	 Autism Autism Autism Autism healthy	 n.d. n.d. n.d. n.d. n.d.	WES	Ionita-Laza et al., 2014
Ser3303Arg	c.9907A>C	Ser3328Arg	c.9907A>C	56	compound heterozygous with 2 other CS causing variants c.10808_10825delCGAGGCAGCTTGTGCACG/ Arg692*	n/p	n/p	++	++	++	n/p	n/p	+[3]	CS	3.5/4	Sanger sequencing	El Chehadeh et al. 2010
Val3420Met	c.10258G>A	Val3445Met	c.10333G>A	56	compound heterozygous with homozygous stop CS causing variant	F	1	++	++	++	n/p	n/p	+[3,11, 12]	CS	4/4	WES (Agilent SureSelect XT Human All Exon v5 kit) confirmed by Sanger sequencing	Lee et al. 2020
Thr3602Ile Ala3691Thr	c.10805C>T c.11071G>A	Thr3627Ile Ala3716Thr	c.10880C>T c.11146G>A	5? 58	compound heterozygous	M	n/p	+	-	n/p	n/p	n/p	+	Autism	1/3	NGS (HiSeq, illumina), confirmed by Sanger sequencing	Yu et al. 2013

Pro3962Arg	c.11885C>G	Pro3987Arg	c.11960C>G	62	heterozygous, second variant is unknown	M	12	n/p	n/p	++	n/p	n/p	+[2]	Autism	1.5/2	target gene enrichment (PGM, Thermo Fisher Scientific versus MiSeq, illumina)	Koshimizu et al.,2013

whole genome sequencing (WGS), whole exome sequencing (WES), ++ clear phenotype, + incomplete phenotype, - no phenotype, n/p not provided by literature; [1]joint hyperextensibility, [2]truncal obesity, [3]narrow hand/feet, [4]epilepsy, [5]recurrent respiratory tract infections, [6]recurrent cough and fever, [7]atrial septal defect; mitral regurgitation, [8]developmental delay of bilateral frontotemporal lobe gyrus, [9]normal motor development, lost communication skills at the age of 1 year, [10]abnormalities of the nervous system, [11] hearing loss, [12] cleft palate, micrognathia, glossoptosis

Supplementary Table T2: Computational predictions of pathogenicity and frequency (gnomAD, release August 2019) of the hitherto summarized missense variants in VPS13B.

Protein change (NP_689777.3)	Grantham Score	PROVEAN Score	Polyphen-2 (HumVar)	MutPred 2 Probability	SNPs&GO – PhD-SNP	SNPs&GO – SNPs&GO	SIFT Score	MutationTaster (Probability)	gnomAD Allele Count TOTAL	gnomAD Allele Number TOTAL	gnomAD Number of Homozygotes TOTAL	gnomAD Allele Frequency TOTAL	gnomAD Other variants at same position (Allele Number TOTAL)	ClinVar	Potentially affected PROSITE and ELM Motifs	Vps13 protein domain
Trp185Arg	101	-10.298	0.998	0.918	0.941	0.734	0.0	disease causing 0.999999867501632	no data	no data	no data	no data	-	-	LIG_PEX14_1, DOC_MAPK_GEN_1, LIG_ACTIN_WH2_2	-
Phe274Val	50	-1.732	0.844	0.752	0.412	0.134	0.0	disease causing 0.999987094277913	no data	no data	no data	no data	-	-	-	-
Gly567Glu	98	-1.501	0.015	0.263	0.264	0.070	0.0	disease causing 0.999724479670851	114	282342	0	0.0004442	Gly567Gly (2)	VUS	-	-
Ala590Thr	**58**	**-0.692**	**0.651**	**0.312**	**0.220**	**0.050**	**0.0**	**disease causing 0.99934312757362**	**236**	**282742**	**2**	**0.0008347**	**Ala590Glu (3) Ala590Val (14) Ala590Ala (31)**	**VUS**	**-**	**-**
Ser824Ala	**99**	**-0.520**	**0.079**	**0.159**	**0.244**	**0.062**	**0.0**	**polymorphism 0.999999999726318**	**45**	**282612**	**1**	**0.0001592**	**Ser824Phe (42) Ser824Ser (19)**	**VUS**	**-**	**-**
Thr1068Ile	89	-0.173	0.675	0.711	0.449	0.115	0.0	disease causing 0.999973855141115	2836	281306	35	0.01056	Thr1068Thr (6)	Benign	LIG_FHA_1, DOC_CYCLIN_1, (CLV_PCSK_SKI1_1), LIG_SUMO_SIM_PAR_1	-
Lys1129Arg	26	-0.948	0.937	0.162	0.450	0.101	0.0	disease causing 0.990735321628732	1303	282622	5	0.004610	Lys1129Glu (1)	Benign	-	-
Pro1133Ser	74	-1.692	0.374	0.586	0.539	0.134	0.0	disease causing 0.999999315450176	1	251222	0	0.000003981	Pro1133Thr (1)	LP	MOD_CK2_1, LIG_TRAF2_1, CK2_PHOSPHO_SITE	-
Asp1210Tyr	160	-6.983	0.999	0.922	0.928	0.525	0.0	disease causing 0.99999909362335	no data	no data	no data	no data	-	VUS	LIG_SH2_SRC, LIG_SH2_STAT5	-
Thr1289Ala	58	-1.544	0.991	0.343	0.215	0.048	0.0	disease causing 0.999926322700007	26	282234	0	0.00009212	Thr1289Ser (150)	VUS	-	-
Thr1289Ser	58	-0.827	0.991	0.216	0.162	0.030	0.0	disease causing 0.999995577038185	150	282188	1	0.0003788	Thr1289Ala (26)	VUS	-	-
Ile1611Asn	**194**	**-4.377**	**0.992**	**0.898**	**0.839**	**0.377**	**0.0**	**disease causing 0.999993993014875**	**no data**	**no data**	**no data**	**no data**	**Ile1611Ile (2)**	**P**	**(MOD_N-GLC_1), ASN_GLYCOSYLATION**	**-**
Lys1682Glu	56	-1.550	0.475	0.608	0.691	0.290	0.0	disease causing 0.999387572062778	1	282516	0	0.000003540	Lys1682Lys (1)	-	LIG_FHA_1, MOD_GSK_1, DOC_WW_Pin1_4, LIG_SH3_3, MOD_ProDKin_1, ELME000173	-
Leu2168Arg	**102**	**-3.350**	**0.814**	**0.789**	**0.793**	**0.307**	**0.0**	**disease causing 0.966354918683841**	**no data**	**no data**	**no data**	**no data**	**-**	**P**	**MOD_PKA_1, MOD_PKA_2, MOD_CK2_1, (CLV_NRD_NRD_1), LIG_ACTIN_WH2_2, MOD_NEK2_1, CAMP_PHOSPHO_SITE**	**-**
Tyr2316Cys	**194**	**-7.706**	**0.999**	**0.891**	**0.850**	**0.511**	**0.0**	**disease causing 0.999999164283618**	**2**	**282672**	**0**	**0.000007075**	**Tyr2316Tyr (2)**	**VUS**	**-**	**-**
Val2456Ile	29	-0.123	0.002	0.027	0.265	0.071	0.20	polymorphism 0.998023919296257	50	282710	0	0.0001768	-	Confl.	-	-
Gly2620Asp#	**94**	**-6.089**	**0.999**	**0.937**	**0.851**	**0.450**	**0.0**	**disease causing 0.999999996618357**	**no data**	**no data**	**no data**	**no data**	**-**	**Confl**	**MYRISTYL**	**SHR_BD (2607-2687)**
Gly2704Arg	**125**	**-6.956**	**1.000**	**0.877**	**0.931**	**0.764**	**0.0**	**disease causing 0.999999985985493**	**2**	**250782**	**0**	**0.000007975**	**-**	**VUS**	**(CLV_NRD_NRD_1), CLV_PCSK_KEX2_1**	**-**
Ser2748Leu	**145**	**-3.667**	**0.979**	**0.609**	**0.698**	**0.136**	**0.0**	**disease causing 0.999999504479773**	**1**	**250930**	**0**	**0.000003985**	**Ser2748Trp (1) Ser2748Ser (59)**	**VUS**	**ELME000173, DOC_MAPK_GEN_1, LIG_SUMO_SIM_PAR_1**	**-**
Ile2795Thr	**89**	**-1.678**	**0.801**	**0.344**	**0.608**	**0.163**	**0.0**	**disease causing 0.991215747760016**	**no data**	**no data**	**no data**	**no data**	**-**	**VUS**	**-**	**-**
Leu2821Ile	5	-1.311	0.892	0.297	0.364	0.101	0.0	disease causing 0.999758934712428	no data	no data	no data	no data	-	-	-	-
Asn2968Ser	**46**	**-4.611**	**0.994**	**0.871**	**0.635**	**0.215**	**0.0**	**disease causing 0.999998306414467**	**875**	**2282666**	**1**	**0.003096**	**-**	**Confl**	**(MOD_N-GLC_1), LIG_SUMO_SIM_PAR_1, ASN_GLYCOSYLATION**	**-**
Asn3088Tyr	143	-5.311	0.997	0.918	0.845	0.462	0.0	disease causing 0.999987671556758	no data	no data	no data	no data	Asn3088Asp (1)	-	LIG_FHA_1, MOD_N-GLC_1, LIG_PTB_Phospho_1, LIG_PTB_Apo_2, DOC_WW_Pin1-4, MOD_ProDKin_1, DOC_CKS1_1	-
Arg3198Trp	101	-1.729	0.409	0.139	0.656	0.267	0.0	polymorphism 0.999999999884398	1066	282148	4	0.003778	Arg3198Arg (1) Arg3198Gln (8)	Benign /LB	-	-
Ser3303Arg	110	-3.178	0.903	0.643	0.718	0.174	0.0	disease causing 0.999998165511474	no data	no data	no data	no data	-	-	MOD_GSK3_1, CLV_C14_CASPASE3-7	-
Val3420Met	21	-0.94	0.013	0.128	0.428	0.129	0.01	polymorphism 0.996794772703239	57	282858	0	0.000202	Val3445Val (1)	Confl	-	-
Thr3602Ile	89	-3.133	0.720	0.834	0.772	0.375	0.0	disease causing 0.999998165511474	2	251264	0	0.000007960	-	-	PKC_PHOSPHO_SITE	VPS13_C (3560-3706)
Ala3691Thr	58	-3.289	0.999	0.761	0.087	0.008	0.11	disease causing 0.999999998949835	643	253378	5	0.002538	Ala3691Asp (1)	Confl	LIG_FHA_1, MOD_GSK3_1, (MOD_N-GLC_1), MOD_NEK2_1, ASN_GLYCOSYLATION	VPS13_C (3560-3706)
Pro3962Arg	103	-2.366	0.101	0.101	0.719	0.213	0.0	disease causing 0.938688179688374	no data	no data	no data	no data	Pro3962Ala (247)	-	-	-

all cloned missense variants are in bold, #in combination with Asn2968Ser cloned, green indicates no negative impact, yellow indicates uncertain negative impact, red indicates potential negative impact

Supplementary Table T3: P value results of Dunnett's multiple comparisons test on Golgi ROI enrichment of overexpressed cloned VPS13B missense variants.

Dunnett's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value	Number of cells
wt						**24**
wt vs. Ala590Thr	4,409	-0,6013 to 9,420	No	ns	0,1159	**28**
wt vs. Ser824Ala	1,617	-4,312 to 7,546	No	ns	0,9904	**15**
wt vs. Ile1611Asn	15,55	9,733 to 21,36	Yes	****	<0,0001	**16**
wt vs. Leu2168Arg	14,84	9,791 to 19,90	Yes	****	<0,0001	**27**
wt vs. Tyr2316Cys	15,18	8,395 to 21,95	Yes	****	<0,0001	**10**
wt vs. Gly2620Asp	16,07	10,75 to 21,39	Yes	****	<0,0001	**22**
wt vs. Gly2704Arg	15,01	9,755 to 20,27	Yes	****	<0,0001	**23**
wt vs. Ser2748Leu	15,50	9,446 to 21,56	Yes	****	<0,0001	**14**
wt vs. Ile2795Thr	2,076	-3,634 to 7,786	No	ns	0,9379	**17**
wt vs. Asn2968Ser	8,234	3,135 to 13,33	Yes	***	0,0001	**26**

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