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Loss-of-function variants in DNM1 cause a specific form of developmental and epileptic encephalopathy only in bi-allelic state

Supplemental Material

Supplementary Table 1. *In silico* prediction and population allele frequencies of homozygous variants identified in individual III.1 (family 1).

Genomic location (GRCh38))	Gene	RefSeq	HGVS cDNA	HGVS protein	Allele frequency in gnomAD[a] [no of homozygous]	Prediction Scores			
						SIFT[b]	PolyPhen-2[c]	MutationTaster	CADD
Chr1:37540202	*SNIP1*	NM_024700.3	c.881T>C	p.(Ile294Thr)	0.00000399	T (0.25)	B 0.262	disease causing	22
Chr1:37613865	*RSPO1*	NM_001242908.1	c.464C>T	p.(Pro155Leu)	0.000242	T (0.18)	B 0.003	polymorphism	13
Chr1:111320412	*CHIA*	NM_201653.3	c.1377C>G	p.(Asn459Lys)	0.000404 [1]	T (0.5)	B 0.018	polymorphism	7
Chr1:113107745	*LRIG2*	NM_014813.2	c.1465G>T	p.(Asp489Tyr)	0	D (0.019)	B 0.264	disease causing	25
Chr1:119623184	*ZNF697*	NM_001080470.1	c.1159G>A	p.(Gly387Ser)	0	D (0.028)	PD 0.997	polymorphism	26
Chr1:155032708	*DCST2*	NM_144622.2	c.500G>A	p.(Arg167His)	0.0000239	D (0)	B 0.113	polymorphism	24
Chr1:160553480	*CD84*	NM_001184881.1	c.658C>T	p.(Arg220Cys)	0.0000992	T (0.07)	PoD 0.714	polymorphism	20
Chr3:113656782	*USF3*	NM_001009899.3	c.4900A>G	p.(Arg1634Gly)	0.000242	D (0)	PD 0.996	disease causing	26
Chr3:120185340	*GPR156*	NM_153002.2	c.655C>T	p.(Arg219Trp)	0.00035	D (0.01)	PoD 0.636	polymorphism	24
Chr3:130571276	*COL6A6*	NM_001102608.1	c.2860G>A	p.(Val954Met)	0.00005	T (0.09)	PoD 0.594	polymorphism	14
Chr3:134482945	*ANAPC13*	NM_015391.3	c.-27-14T>G	-	-	NA	NA	polymorphism	16
Chr3:142383406	*XRN1*	NM_019001	c.2510G>A	p.(Arg837Gln)	0.000143	T (0.27)	B 0.001	polymorphism	22

Chr3:151880910	*SUCNR1*	NM_033050.5	c.367A>G	p.(Ile123Val)	0.00000399	D (0.04)	B 0.003	polymorphism	15
Chr4:6575322	*MAN2B2*	NM_015274.2	c.112G>A	p.(Asp38Asn)	0.000269	D (0.01)	PD 1.000	disease causing	28
Chr4:69639085	*UGT2A2*	NM_001105677.2	c.556T>C	p.(Ser186Pro)	0	T (0.15)	PoD 0.750	polymorphism	23
Chr4:69639085	*UGT2A1*	NM_001252274.2	c.1159T>C	p.(Ser387Pro)	0	T (0.19)	B 0.358	polymorphism	23
Chr4:112618143	*ZGRF1*	NM_018392.4	c.1899A>C	p.(Lys633Asn)	0.000048	D (0.01)	B 0.368	polymorphism	18
Chr4:128209866	*LARP1B*	NM_018078.3	c.2558G>A	p.(Ser853Asn)	0	T (0.3)	B 0.136	disease-causing	17
Chr9:127928479	*PIP5KL1*	NM_001135219.1	c.233C>T	p.(Pro78Leu)	0.0000319	T (0.46)	B 0.001	polymorphism	7
Chr9:128203567	*DNM1*	NM_004408.3	c.97C>T	p.(Gln33*)	0	NA	NA	disease causing	26
Chr9:131504262	*POMT1*	NM_007171.3	c.44T>C	p.(Ile15Thr)	0	D (0)	B 0.126	disease causing	23
Chr15:43800640	*HYPK*	NM_016400.3	c.42T>G	p.(Asp14Glu)	0	T (0.11)	B 0.274	disease causing	18
Chr15:45253505	*SLC28A2*	NM_004212.3	c.155C>G	p.(Pro52Arg)	0.0000159	T (0.28)	B 0.035	polymorphism	0
Chr15:64153333	*SNX22*	NM_024798.2	c.353A>G	p.(Asn118Ser)	0.000114	T (1)	B 0.000	polymorphism	7
Chr18:8785987	*MTCL1*	NM_015210.3	c.1783G>A	p.(Glu595Lys)	0.0000823	T (0.17)	PoD 0.634	disease causing	23
Chr19:1109420	*SBNO2*	NM_014963.2	c.3220C>T	p.(Arg1074Cys)	0.000440 [2]	D (0)	PD 0.912	disease causing	24

[a]Accessed in February 2021

[b]Score 1-0, *D* deleterious, *T* Tolerated

[c]HumVar prediction, Score 0-1, *B* benign, *PD* probably damaging, *PoD* possibly damaging

NA Not available

Supplementary Table 2. *In silico* prediction and population allele frequencies of homozygous variants identified in individual III.6 (family 2).

Genomic location (GRCh 38)	Gene	RefSeq	HGVS cDNA	HGVS protein	Allele frequency in gnomAD	Prediction Scores			
						SIFT[b]	PolyPhen-2[c]	MutationTaster	CADD
Chr9: 128222197	*DNM1*	NM_004408.4	c.850C>T	p.(Gln284*)	0	NA	NA	disease causing	43
Chr22:50448300	*SBF1*	NM_002972.4	c.5296C>T	p.(Arg1766Cys)	0,00008454	T (0.07)	PoD 0.540	disease causing	25

[a]Accessed in February 2021

[b]Score 1-0, *D* deleterious, *T* Tolerated

[c]HumVar prediction, Score 0-1, *B* benign, *PD* probably damaging, *PoD* possibly damaging

NA Not available

Supplementary Table 3. Genotypes and associated clinical phenotypes in DNM1 encephalopathy.

Reference	**EuroEPINOMICS-RES Consortium et al., 2014 [11]**					**Nakashima et al., 2016 [14]**		**Brereton et al., 2018 [16]**		**This report**	
DNM1 variant	c.529G>C; p.Ala177Pro	c.618G>C; p.Lys206Asn	c.1076G>C; p.Gly359Ala	c.709C>T; p.Arg237Trp	c.194C>A; p.Thr65Asn	c.127G>A; p.Gly43Ser	c.709C>T; p.Arg237Trp	c.1603A>G; p.Lys535Glu	c.1603A>G; p.Lys535Glu	c.97C>T; p.(Gln33*)	c.850C>T; p.(Gln284*)
Zygousity	het	het	het	het	het	het	het	het	het	hom	hom
Number of patients	1	1	1	1	1	1	1	1	1	1	1
Age at last follow-up	15 y	8 y	6 y	13 y	6 y	15 y	6 y	8 y, monozygotic twins		5 y	3 y 8 m
Somatic growth											
Weight at birth	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	>97th centile	50-90th cent.	+0.2 SD	-0.9 SD
Length at birth	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	>97th centile	>97th centile	+0.1 SD	-0.8 SD
HC at birth	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	80th centile	65th centile	+0.1 SD	-0.7 SD
Weight at follow-up	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	24th centile	30th centile	-2.1 SD	-3.7 SD
Length at follow-up	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	28th centile	22th centile	-2.4 SD	-3.1 SD
HC at follow-up	n. r.	n. r.	n. r.	microcephaly	n. r.	n. r.	n. r.	85th centile	95th centile	-4.4 SD	-4.5 SD
Motor and speech development											
Sitting	yes	yes	yes	no	no	yes	yes	yes	yes	no	no
Walking	yes	no	no	no	no	yes	yes	yes	yes	no	no
Functional hand use	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	yes	yes	no	no
Verbal expression	no	no	no	no	no	no	no	yes	yes	no	no
Neurological features											
Epilepsy (onset; type)	7 m; VS	6 m; VS	2 m; IS	12 m; VS	13 m; VS	11 m; LGS	10 m; West syn.	no	no	4 m; FS	6 m; FS
EEG	MFED, GSW, slow bg	Hyps, MFED, GSW, slow bg	GSW	Hyps, GSW, slow bg	Hyps, MFED, slow bg	partial hyps, MFED	MFED, GSW	normal	normal	Hyps, MFED	Hyps, MFED
Hypotonia	yes	yes	yes	yes	yes	n. r.	yes	yes	yes	yes	yes
Spasticity	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	yes	no
Dystonia	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	yes	n. r.	n. r.	yes	yes
Feeding problems	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	yes	yes
Visual impairment	n. r.	n. r.	n. r.	yes	yes	n. r.	n. r.	n. r.	n. r.	yes	yes
Miscellaneous			seizure free on ketogenic diet					mild-moderate ID, autism		scoliosis	mild bilat. optic atrophy
Neuroimaging	normal	normal	normal	cerebral atrophy	cerebral atrophy	normal	arachnoid cyst right temporal lobe	n. p.	normal	ACC	cerebral atrophy

Supplementary Table 3. continued

Reference	**von Spiczak et al., 2017 [15]**													
DNM1 variant	c.127G>A; p.Gly43Ser	c.134G>A; p.Ser45Asn	c.194C>A; p.Thr65Asn	c.416G>T; p.Gly139Val	c.529G>C; p.Ala177Pro	c.616A>G; p.Lys206Glu	c.618G>C; p.Lys206Asn	c.709C>T; p.Arg237Trp	c.731 G>A; p.Ser238Ile	c.1037G>T; p.Gly346Val	c.1075G>A; p.Gly359Arg	c.1076G>C; p.Gly359Ala	c.1117G>A; p.Glu373Lys	c.1109G>A; p.Gly397Asp
Zygousity	het	het	het	het	het	het	het	het	het	het	het	het	het	het
Number of patients	1	1	1	1	1	1	1	7	1	1	2	1	1	1
Age at last follow-up	8 y	2 y	8 y	18 y	15 y	9 y	8 y	2 to 24 y	19 y	13 y	both 1 y	7 y	5 y	2 y
Somatic growth														
Weight at birth	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Length at birth	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
HC at birth	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Weight at follow-up	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Length at follow-up	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
HC at follow-up	microcephaly	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	microcephaly	microcephaly	n. r.	microcephaly
Motor and speech development														
Sitting	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Walking	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Functional hand use	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Verbal expression	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Neurological features														
Epilepsy (onset; type)	3 w; AS, MS	no	13 m; VS	4 m; VS	7 m; VS	2 m; VS	6 m; VS	3 to 12 m; VS	8 m; GTCS	6 m; VS	no / 1 m, VS	2 m; IS	4 y 6 m; VS	3 m; IS, MS
EEG	slow bg	normal	Hyps, MFED, slow bg	Hyps, MFED, slow bg	MFED, slow bg	MFED	Hyps, MFED, slow bg	Hyps, MFED, slow bg in most pat.	n. r.	Hyps, MFED, slow bg	MFED, slow bg	SSW	GSW, slow bg	Hyps, MFED, slow bg
Hypotonia	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	n. r.	yes
Spasticity	yes	n. r.	n. r.	yes	n. r.	n. r.	n. r.	yes	n. r.	n. r.	n. r. / yes	n. r.	n. r.	n. r.
Dystonia	yes	n. r.	n. r.	yes	n. r.	yes	n. r.	yes	n. r.	n. r.	n. r. / yes	n. r.	n. r.	n. r.
Feeding problems	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Visual impairment	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Miscellaneous											multifocal subcortical myoclonus		FIRES at 4.5 y	
Neuroimaging	normal	n. p.	cerebral atrophy	normal	normal	n. p.	normal	normal / cerebral atrophy	normal	hypoplasia of frontal lobes	thin CC / normal	cerebral atrophy	normal	n. p.

Abbreviations: ACC, agenesis of corpus callosum; AS, absence seizures; bg, background; CC, corpus callosum; FIRES, febrile infection-related epilepsy syndrome; FS, focal seizures; GSW, generalized spike-wave discharges; GTCS, generalized tonic-clonic seizures; ID, intellectual disability; HC, occipitofrontal head circumference; het., heterozygous; hom., homozygous; Hyps, hypsarrhythmia; IS, infantile spasms; LGS, Lennox-Gastaut syndrome; m, months; MFED, multifocal epileptiform discharges; MS, myoclonic seizures; n. p., not performed; n.r., not reported; SD, standard deviation; SSW, slow spike-wave discharges; VS, various seizure types; y, years; position referring to *DNM1* (ENST00000372923.8, NM_004408.3).

Supplementary Methods

Cytogenomic analysis

Array-comparative genomic hybridization (array-CGH) of individual III.1 (family 1) and her parents was performed on genomic DNA using the Human Genome 180 K CGH Microarray (Agilent Technologies) according to the manufacturer's recommendations. The results were scanned using the SureScan Dx Microarray scanner G5761A (Agilent Technologies), and image and data analysis was carried out using the Feature Extraction Software and the Cytogenomics software (Agilent Technologies). Chromosomal microarray (CMA) of individual III.6 (family 2) was performed using the Affymetrix Cytoscan 750K chip (Thermo Fisher Scientific Inc.), and analyzed using the Affymetrix Chromosome Analysis Suite 3.1 (Thermo Fisher Scientific Inc.), reporting deletions over 100kb and duplications over 200kb in cytoregions and deletions over 100Kb and duplications over 500Kb in the remaining genomic regions