

Supplementary data for Donato et al. “The ducky^{2J} mutation in Cacna2d2 results in reduced spontaneous Purkinje cell activity and altered gene expression”

Supplementary Table 1

List of primers used in qPCR

Gene	Accession number	Primer sequences (5'→3')
Actin	NM_007393	GACGTTGACATCCGTAAAGA; AATCTCCTTCTGCATCCTGT
RPL7a	BG075220	AAGGTGGAGACTGTATTGAC; AAGCAAGAGAAGAAGCAAAG
Cacnb1	NM_145121	TCTCAATCCAACACCTCAAT; CGCAGGCATCTCCAATT
Cacnb2	W41214	GGATCGCTACTGTGACAAGGA; GGACACGCACGGTCATTGG
Cacnb4	NM_146123	ACTCGTACCAGGACACTTAT; TACTATGGCAAATTGTCAACAG
Cacna2d1	NM_009784	CAAGCAGCCCAGATAACGAAA; ACACCACCACAGTCAGTATAATCC
Cav2.1 37a		AATCTCTTGTGCGTGTGTC; CATGTCCTTATAGTGAATGC
Cav2.1 37b		TGGGTCTGGGAAAGAAGTG; GAAGTGAACCTGTGTTGTATCTG
Cacna1b	NM_007579	TCTTTGACTGTGTGGTGAAC; GGCTTCTACTCTTCCTTCTC
Cacna1c	NM_009781	CCTCATCTCCTTGGCATCC; GCAGCAGAGTGGTGACAAT
Cacna1d	NM_028981	TGAAGCAAAGGGAAGGATAAA; ACTCGGTGTGGGCATAAT
Cacna1g	NM_009783	CCAGCAGTCAGGAAGAAC; GTGTCTCCTCTGTCGTC
Th	NM_009377	GCAGCCCTACCAAGATCAAAC; TACGGGTCAAACCTCACAGAG
Tnc	NM_011607	CCAAGCAGACCACACGGATCA; CCCAGAACCAAGAGAAGTAA
Slc4a1	BB448377	TAGGGAGCTGGGAGGATT; ACGATCCCCATTCTCTTGG
C1qr1	NM_010740	ACCTTCCTCCTTCCCATCTTG; AGACTGACCACACACAATT
Eraf	NM_133245	GAGCAGGAGGAGCAAGAC; ATGAGGAGGGCAGTGTATTG
Hba-a1	NM_008218	GATCCCGTCAACTTCAAG; GCAAGGAATTGTCCAGAGAG
BK	NM_010610	AGCACTCCGCAGACATTG; CAGAACAGATCACCATAACAAAC
BK beta4	NM_021452	ATCGGTTCCCAGCCATTG; GCAATCTCGTCGTGTGTG
SK2	AF357240	GATCGTCACCCCTGGAAAC; CTGCTGTCTGATGGTCTG
Nav1.1	XM_619757	GTGTTGGGTGAGAGTGGAGAG; AGTAGTGATGGCTGATAAGAGAC
Nav1.6	NM_011323	AGTGTCTTACGAGCCTATC; TGTTGGAAGTGTATCTTCTG
Scn1b	NM_011322	GTGCTCATTGTGGTGTG; TTGCTCTCGGATGTAATGG
Scn2b	NM_001014761	CTGGCTGTGGTCATCTG; CCTTCCTCTTCAGTCTTCAG
Scn4b	NM_001013390	TCTTGAGTGACCTGGAGTTC; GATGGTGGCAGAGTTGTTC

Supplementary Table 2

Affymetrix GeneChip results of comparison between WT and ducky cerebellar gene expression at age P21

Change wt→du ^{2J}	Fold change		Accession number	Affymetrix Gene Title	Unigene cluster
	Set 1	Set 2			
↓	5.7	6.5	AF247139	calcium channel, voltage-dependent, alpha 2 delta subunit 2	Mm.100236
↓	2.1	1.4	NM_011607	tenascin-C	Mm.980
↓	2.6	1.4	BB483357	angiotensin receptor-like 1	Mm.29368
↑	2.5	2.3	NM_009377	tyrosine hydroxylase	Mm.1292
↑	2.8	1.5	NM_011361	serum/glucocorticoid regulated kinase	Mm.28405
↑	2.6	1.9	U16959	FK506 binding protein 5	Mm.154390
↑	2.5	1.4	BC017637	glioma tumour suppressor candidate region gene 2	Mm.277634
↓	3.5	3.2	AA266723	EST	Mm.29940
↓	2.0	2.5	NM008218	haemoglobin alpha, adult chain 1	Mm.196110
↓	2	3.2	AK004987	McKusick-Kaufman syndrome protein	Mm.26763
↓	2.8	1.9	NM_133245	erythroid associated factor	Mm.218857
↓	2.3	1.9	BB448377	solute carrier family 4 (anion exchanger), member 1	Mm.7248
↓	2.3	1.9	BB039247	complement component 1, q subcomponent, receptor 1	Mm.681
↓	2.1	1.5	AV301324	Ribonucleotide reductase M2	Mm.99
↓	2	1.5	NM_021272	fatty acid binding protein 7, brain	Mm.3644
↓	2	1.6	NM_026331	RIKEN cDNA 1700020E22 gene	Mm.36710
↑	3	8	NM_007398	adenosine deaminase	Mm.388
↑	2.8	1.4	AI467657	similar to mPLZF(B)=promyelocytic leukemia zinc finger protein	Mm.34106
↑	2.3	1.6	AA419994	similar to mPLZF(B)=promyelocytic leukemia zinc finger protein	Mm.34106
↑	2.1	1.9	AK018202	EST	Mm.183116
↑	2	1.5	NM_010098	opsin (encephalopsin)	Mm.32744
↑	2	1.7	NM_007705	cold inducible RNA binding protein	Mm.17898
↑	2.1	1.5	AF173681	thioredoxin interacting protein	Mm.77432

Supplementary Table 3

Affymetrix GeneChip results of comparison between WT and ducky cerebellar gene expression at age P10

Change wt→du ^{2J}	Fold change		Accession number	Affymetrix Gene Title	Unigene cluster
	Set 1	Set 2			
↓	6.5	7.0	AF247139	calcium channel, voltage-dependent, alpha 2 delta subunit-2	Mm.100236

Supplementary Table 4**Affymetrix GeneChip results of comparison between WT and ducky cerebellar gene expression at age P0**

Change wt→du ^{2J}	Fold change		Accession number	Affymetrix Gene Title	Unigene cluster
	Set 1	Set 2			
↓	4.9	3.2	AF247139	calcium channel alpha-2-delta-2 subunit (Cacna2d2)	Mm.100236
↓	4.3	1.9	AA516748	EST	Mm.172523
↓	4.3	1.9	BB558275	EST	Mm.134041
↓	3.5	2.1	C88202	EST	Mm.42451
↓	3.5	9.8	BB485470	EST	Mm.209495
↓	3.5	1.4	BE952603	EST	Mm.151179
↓	3.5	2.1	BB085124	EST	Mm.120256
↓	3.2	1.9	BB638160	EST, Weakly similar to ELM1	Mm.133830
↓	2.8	5.7	AK006578	RIKEN cDNA 1700031F05	Mm.158984
↓	2.8	2.5	BF607489	Eno1 enolase 1, alpha non-neuron	Mm.90587
↓	2.6	1.5	NM_008990	Mus musc. poliovirus sensitivity (Pvs)	Mm.4341
↓	2.6	1.9	NM_007921	Mus musc. E74-like factor 3 (Elf3), expressed	Mm.3963
↓	2.6	1.4	BG092516	sequence AI447904	Mm.447
↓	2.6	1.5	AV045081	ESTs, Weakly similar to Chain A, Fv Fragment	Mm.46118
↓	2.5	1.4	U49865	5E6 (5E6Ly-49C) mRNA	Mm.196054
↓	2.5	1.6	AV364767	EST	Mm.56633
↓	2.5	1.4	BM237812	EST	Mm.103545
↓	2.5	1.6	C77489	D4Ert41e DNA segment, Chr 4, ERATO Doi 41, expressed	Mm.195245
↓	2.3	1.4	BB710064	EST	Mm.212146
↓	2.3	1.4	BG069821	EST	Mm.26241
↓	2.3	1.7	AK003253	RIKEN cDNA 1110001M24 gene	Mm.28359
↓	2.3	1.6	BM247957	EST	Mm.216189
↓	2.3	1.6	AI645293	expressed sequence AI448571	Mm.89570
↓	2.3	1.7	AB000500	Kcnq2 Mus musculus mRNA for alternative splicing: see accession between AB000494 and AB000504,	Mm.40615
↓	2.3	1.9	NM_028625	small proline rich-like 2 (Sprrl2)	Mm.89230
↓	2.3	2	AV314845	RIKEN cDNA 5830426C09 gene	Mm.61414
↓	2.1	2.8	BM934740	EST	Mm.150943
↓	2.1	1.5	BG066263	expressed sequence C78651	Mm.24996
↓	2.1	1.9	NM_023784	RIKEN cDNA 2310016N21	Mm.154776
↓	2.1	3	BB209605	EST	Mm.34482
↓	2.1	1.5	BB767243	EST	Mm.188372
↓	2.1	1.9	AW554852	EST	Mm.103115
↓	2.1	1.6	AV352659	EST	Mm.180845
↓	2	1.5	NM_008358	interleukin 15 receptor, α chain (Il15ra)	Mm.200196
↓	2	2.3	BB105328	EST	Mm.157190
↓	2	1.4	AV369255	EST	Mm.96903
↓	2	1.6	BB152070	EST, Weakly similar to A54595 transcription factor E2F-2	Mm.207587
↓	2	1.9	BB277142	EST	Mm.133826
↓	2	1.6	BE955693	EST	Mm.122885
↓	1.9	2.5	AK019674	RIKEN cDNA 4930511E03 gene	Mm.67065
↓	1.7	2	NM_025806	RIKEN cDNA 1100001H23	Mm.3311
↓	1.7	2	U96702	Spi16 serine proteinase inhibitor mBM17 mRNA	Mm.206796

Supplementary Table 5**Comparison of gene expression in *du^{2J}/du^{2J}* and +/+ cerebellum at P21 by qPCR**

Expressed gene	n	Expression of <i>du^{2J}/du^{2J}</i> relative to WT	Expressed gene	n	Expression of <i>du^{2J}/du^{2J}</i> relative to WT
Tenascin-C	10	0.44 ± 0.06***	Ca _v 2.1	5	1.13 ± 0.10
Tenascin-R	10	0.91 ± 0.06	Ca _v 2.1 37a	10	1.00 ± 0.07
			Ca _v 2.1 37b	10	1.33 ± 0.10**
			Ca _v 1.2	5	1.12 ± 0.20
			Ca _v 1.3	5	0.99 ± 0.11
Tyr hydroxylase	10	16.29 ± 0.79***	Ca _v 2.2	5	1.16 ± 0.16
<i>du^{2J}/du^{2J}</i>			Ca _v 3.1	5	0.96 ± 0.14
<i>du^{2J}/+</i>	8	0.96 ± 0.17	Ca _v $\alpha_2\delta$ -1	5	1.11 ± 0.10
			Ca _v β1	5	1.07 ± 0.17
			Ca _v β2	5	1.13 ± 0.14
			Ca _v β4	5	1.11 ± 0.12
Eraf	10	0.60 ± 0.13	Na _v 1.1	5	0.97 ± 0.11
C1qr1	10	0.55 ± 0.03**	Na _v 1.6	5	0.92 ± 0.05
Slc4a1	10	0.64 ± 0.10	Na _v β1	5	1.20 ± 0.24
			Na _v β2	10	0.82 ± 0.04*
			Na _v β4	5	0.98 ± 0.08
			BK	5	0.96 ± 0.10
			BK β4	5	1.16 ± 0.10
			SK2	5	1.05 ± 0.10

RNA was isolated from the cerebellum of mice at developmental stage P21 and qPCR was carried out as described in Experimental procedures. Expression was corrected for the mean expression of the housekeeping genes actin and RPL7a, neither of which was found altered in *du^{2J}/du^{2J}* mice, and normalized to expression in WT cerebellum. The statistical significances of the changes observed are given by *** $P<0.0001$, ** $P<0.01$, * $P<0.02$, Student's t test.