<table>
<thead>
<tr>
<th><strong>Allele</strong></th>
<th><strong>drunk</strong></th>
</tr>
</thead>
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<tr>
<td><strong>Mutation Type</strong></td>
<td>critical splice donor site (2 bp from exon)</td>
</tr>
<tr>
<td><strong>Chromosome</strong></td>
<td>13</td>
</tr>
<tr>
<td><strong>Coordinate</strong></td>
<td>59,512,323 bp (GRCm38)</td>
</tr>
<tr>
<td><strong>Base Change (assembly)</strong></td>
<td>A ? T</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
<td><em>Agtpbp1</em></td>
</tr>
<tr>
<td><strong>Gene Name</strong></td>
<td>ATP/GTP binding protein 1</td>
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<tr>
<td><strong>Synonym(s)</strong></td>
<td>2310001G17Rik, Nna1, 1700020N17Rik, 4930445M19Rik, 2900054O13Rik, 5730402G09Rik</td>
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<td><strong>Chromosomal Location</strong></td>
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<tr>
<td><strong>Accession Number</strong></td>
<td>NCBI RefSeq: NM_023328 (isoform 1), NM_001048008 (isoform 2); MGI: 2159437</td>
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<tr>
<td><strong>Mapped</strong></td>
<td>Yes</td>
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<tr>
<td><strong>Institutional Source</strong></td>
<td>Beutler Lab</td>
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<td><strong>Phenotypic Category</strong></td>
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<td><strong>Penetrance</strong></td>
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<tr>
<td><strong>Alleles Listed at MGI</strong></td>
<td>All alleles(17) : Gene trapped(6) Transgenic(1) Spontaneous(6) Chemically induced(4)</td>
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### Mutagenetix Phenotypic Mutation 'drunk'

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<tr>
<th>Lab Alleles</th>
<th>APN: IGL00544 Agtpbp1, IGL00808 Agtpbp1, IGL01298 Agtpbp1, IGL01628 Agtpbp1, IGL01921 Agtpbp1, IGL02189 Agtpbp1, IGL02325 Agtpbp1, IGL02700 Agtpbp1, IGL02821 Agtpbp1, IGL03130 Agtpbp1, IGL03167 Agtpbp1, IGL03218 Agtpbp1</th>
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<td>UTSW: gru, rio, wobble, R0025 Agtpbp1, R0025 Agtpbp1, R0276 Agtpbp1, R0413 Agtpbp1, R0559 Agtpbp1, R0848 Agtpbp1, R0943 Agtpbp1, R1196 Agtpbp1, R1421 Agtpbp1, R1531 Agtpbp1, R1833 Agtpbp1, R1864 Agtpbp1, R1994 Agtpbp1, R1995 Agtpbp1, R2001 Agtpbp1, R2006 Agtpbp1, R2397 Agtpbp1, R2918 Agtpbp1, R3873 Agtpbp1, R3924 Agtpbp1, R4649 Agtpbp1, R4913 Agtpbp1, R4933 Agtpbp1, R4969 Agtpbp1, R5066 Agtpbp1, R5139 Agtpbp1, R5194 Agtpbp1, R5269 Agtpbp1, R5352 Agtpbp1, R5558 Agtpbp1, R5687 Agtpbp1, R5824 Agtpbp1, R5979 Agtpbp1, R6109 Agtpbp1, R6264 Agtpbp1, R6413 Agtpbp1, R6498 Agtpbp1, R6747 Agtpbp1</td>
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<th>Mode of Inheritance</th>
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<td>Local Stock</td>
<td>Embryos, gDNA</td>
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<tr>
<td>Repository</td>
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</tr>
<tr>
<td>Science Writers</td>
<td>Eva Marie Y. Moresco</td>
</tr>
<tr>
<td>Authors</td>
<td>Pia Viviani, Xin Du, Bruce Beutler</td>
</tr>
<tr>
<td>Illustrators</td>
<td>Diantha La Vine</td>
</tr>
<tr>
<td>Last Updated</td>
<td>05/22/2018 9:29 AM by Anne Murray</td>
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<td>Record Posted</td>
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Cite this information as follows: Pia Viviani, Xin Du, Eva Marie Y. Moresco, Beutler B. Record for drunk, updated May 22, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu
Phenotypic Description

The *drunk* phenotype was identified among ENU-induced G3 mutant mice; *drunk* mice display a severe ataxia of gait. Cerebellar Purkinje cells from homozygous *drunk* mice begin to degenerate beginning at approximately 3 weeks of age until virtually all cells are lost by 7 weeks of age. Degeneration of photoreceptor cells also begins at 6 weeks of age.

Homozygous *drunk* and wild type mice recover at the same rate after sciatic nerve crush operation (please see Background).

Male *drunk* mice are sterile due to a reduced number of abnormally shaped sperm cells. The *drunk* stock was maintained by breeding homozygous females with heterozygous males.
The *drunk* mutation mapped to Chromosome 13, and corresponds to a T to A transversion in the donor splice site of intron 11 (GTAGGG -> GAAGGG) in the *Agtpbp1* (hereafter *Nna1*) gene (position 44998 in Genbank genomic region NC_000079 for linear genomic DNA sequence of *Agtpbp1*). Sequencing of cDNA from *drunk* mice demonstrates that the mutation results in skipping of exon 11, thus destroying the reading frame after codon 302 (encoding glutamine) and creating a premature stop codon at codon 318.

```
<--exon 10   <--exon 11 intron 11-->  exon 12-->
43219 ACTTCTCAA…….CCGCTGAAG GAGGGTG…………ACGACATTGATTTAG 49311
300   -T--S--Q--….P--P--E-- …..T--T--L--I--* 318
```  
correct  deleted  aberrant

The donor splice site of intron 11, which is destroyed by the *drunk* mutation, is indicated in blue lettering; the mutated nucleotide is indicated in red lettering.

**Protein Prediction**

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Figure 1. Domain structure of NNA1. The drunk mutation corresponds to a T to A transversion in the donor splice site of intron 11 in the Agtpbp1 gene. A premature stop codon is predicted to truncate the protein after amino acid 318. Click on each mutation in red for more specific information.

Nna1 (nervous system nuclear protein induced by axotomy) is a 1218 amino acid-containing protein, translated from 25 exons with no alternative splicing (1,2). Nna1 is conserved in the bacterium Zymomonas mobilis, flies, worms, mice and humans (J). Nna1 has a number of predicted protein domains and motifs (J). The most prominent feature of Nna1 is the presence of a zinc carboxypeptidase-like sequence located within a highly conserved 300-amino acid region towards the C-terminus. This 300-amino acid region is 96% identical in mice and humans. The carboxypeptidase domain of Nna1 contains a zinc-binding motif, which is characterized by two conserved histidines and a catalytic glutamate residue, at positions H912, E915 and H1009 in Nna1. In this region, there are also consensus sequences for a tyrosine phosphorylation site, nuclear localization signal, and an ATP/GTP binding motif of the P-loop type. In the rest of the protein, there are several more predicted tyrosine phosphorylation sites, one nuclear localization signal, and consensus phosphorylation sites for protein kinase C, casein kinase II and cGMP/cAMP-dependent kinases. The N-terminal 450 amino acids of Nna1 are leucine-rich and possess weak homology to armadillo repeat proteins.

The drunk mutation causes the skipping of exon 11, creating a predicted premature stop codon after the insertion of fifteen aberrant amino acids. The mutation likely results in nonsense mediated decay of the aberrant transcript.

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Nna1 mRNA is expressed throughout the brain, with prominent expression in cerebellar Purkinje and granule cells, mitral cells of the olfactory bulb, thalamic neurons, dorsal root ganglia, and hippocampal CA3 neurons (1;3). In the retina, Nna1 is expressed in photoreceptor cells (3). Nna1 is highly expressed in testes, in developing and mature sperm (3), and in heart, skeletal muscle and kidney (1). No Nna1 expression was found in ovary, liver, stomach, small intestine, lung, adrenal gland, spleen and thymus (1). During development, Nna1 is expression is found in the embryonic central and peripheral nervous systems (1).

Nna1 has two nuclear localization signals, and GFP-fusions with Nna1 localize to both the nucleus and the cytoplasm in primary cortical neurons (1). However, no zinc carboxypeptidases except AEBP1 have yet been found in the nucleus [discussed in (4)]. Definitive localization of the native Nna1 is unknown.

**Background**

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(Generated on Nov 12, 2018)
Figure 2. Overview of cell types in the cerebellum. The cerebellum functions in fine motor control. Cellular components of the cerebellum include Purkinje cells, granule cells, mossy fibers, and deep cerebellar nuclei. Mossy fibers enter the granule cell layer and synapse onto granule cells. Granule cells, which receive excitatory input from mossy fibers, send axons into the molecular layer where they split to form parallel fibers. Purkinje cell bodies are oriented in a single layer of the cerebellum (Purkinje cell layer), with their characteristic, extensive dendritic arbors occupying the molecular layer within which they receive synaptic input from the parallel fibers. Climbing fibers originating from the inferior olive split into multiple terminal branches that also innervate Purkinje cell dendrites in the molecular layer. The deep cerebellar nuclei receive inhibitory input from Purkinje cells and excitatory input from mossy fibers and climbing fibers. The deep cerebellar nuclear cells...
Zinc carboxypeptidases in the nervous system generally serve regulatory roles, removing a C-terminal amino acid (usually arginine) from secreted proteins and peptides in order to activate or inhibit them. For example, carboxypeptidase E/H (CPE/H) proteolyses several neuropeptides including proenkephalin, proinsulin, proneurotensin, promelanin-concentrating hormone [discussed in (4)], and procholecystokinin [discussed in (5)]. A spontaneous mutation eliminating enzyme activity in CPE/H exists in the Cpe\textit{fat/fat} mouse, which develops obesity (6). Other carboxypeptidases also function in the nervous system (4).

\textit{Nna1} was first identified in a screen for inducible genes in a sciatic nerve transection paradigm and it is expressed in spinal motor neurons undergoing axon regeneration. Its role in these events is currently unknown (1). \textit{Nna1} is also mutated in the spontaneously occurring \textit{pcd} (Purkinje cell degeneration) mutants. There are currently eight known phenotypic alleles of \textit{pcd}, out of which four have identified genetic lesions. In each of these four cases, protein levels are dramatically reduced by the genetic lesions (2;3). \textit{drunk} is a new addition to the \textit{pcd} allelic series.

The hallmark feature of \textit{pcd} mice, as for \textit{drunk} mice, is development of an ataxic gait between three and four weeks of age, which correlates with the onset of cerebellar Purkinje cell degeneration (7). Purkinje cells (Figure 2) proceed to deteriorate rapidly and die over the subsequent two week period. Distinct areas of the cerebellum display different rates of Purkinje cell degeneration, but all eventually die. Experiments with wild type-\textit{pcd} chimeras demonstrated that this phenotype is cell autonomous (8). In addition to Purkinje cells, cerebellar granule cells (Figure 2) also display progressive death, with near normal numbers at three months declining to 5% by 20 months of age (7). Selected thalamic neurons also degenerate between postnatal days 50 and 60, and in addition, degeneration of retinal photoreceptors and olfactory bulb mitral cells progresses slowly over a year (3). Male \textit{pcd} homozygous mice are sterile due to a reduction in the number of spermatozoa, which are sometimes abnormally shaped and immotile as well (3).

**Putative Mechanism**

The molecular mechanisms underlying the \textit{pcd} phenotypes have been under investigation for decades, yet much is still unknown. Several groups published findings supporting a role for abnormally increased apoptosis in \textit{pcd} mice. A 5-fold increase in the mRNA levels of \textit{c-fos, junB} and \textit{krox-24}, which are associated with neuronal apoptosis, has been detected specifically in cerebellar Purkinje cells of \textit{pcd} mice at postnatal day 22, at the onset of cell death (9). These transcription factors may dictate cellular outcome by coordinating the expression of various anti- and pro-apoptotic proteins. In
fact, mRNA levels of the anti-apoptotic protein Bcl-2 are reduced by 35% while those of the pro-apoptotic Bax remain unchanged in pcd mice at 22 days of age (9). Because Bcl-2 prevents apoptosis by binding and physically inhibiting Bax, a decrease in Bcl-2 may favor apoptosis by de-repressing Bax activity. Consistent with the hypothesis that pcd Purkinje cells undergo increased apoptosis, nuclear DNA fragmentation (10) and activated caspase-3 (11) have been observed in these cells.

However, genetic studies investigating the potential contribution of several cell death pathways demonstrated that Bax is not involved in Purkinje cell death of pcd mice, as pcd3j/bax-/- mice still develop ataxia and lose Purkinje cells (4). The p53 pathway was also tested by generating homozygous ATM-null or Puma-null pcd mice. ATM serves to phosphorylate and activate p53 upon DNA damage, resulting in either DNA repair or apoptosis. Puma is a pro-apoptotic protein and a transcriptional target of p53. Neither combining ATM nor Puma mutations with the Nna1 mutation led to rescue of the pcd phenotype, strongly suggesting that the p53 pathway is not involved in pcd Purkinje cell death (4).

A recent report supports a role for ER stress in the pcd phenotype. The levels of endoplasmic reticulum (ER)-specific chaperone BiP, and the ER-stress related transcription factor CHOP are increased pcd mice at 23 and 26 days of age (11). Moreover, an unusual configuration of the ER with associated electron-dense particles was observed during the early characterization of pcd mice (12).

Finally, the putative carboxypeptidase substrate-binding site of Nna1 was demonstrated to be required for rescue of the pcd phenotype (13). Purkinje cell-specific transgenic mice expressing wild type Nna1 were generated and crossed with pcd3j homozygous mice. pcd3j/Nna1-transgenic mice showed no Purkinje cell degeneration or ataxia. Sequence comparisons of Nna1 with other carboxypeptidases and structural modeling predicted R962 as part of the catalytic site, while N970 and R971 constituted the substrate-binding site of Nna1. Significantly, Purkinje cell-specific pcd transgenic mice expressing a putative substrate-binding site mutant (Nna1N970A R971A) still displayed ataxia and Purkinje cell degeneration. These results indicate that N970 and R971 are essential for Nna1 to support Purkinje cell survival, and suggest that Nna1 is a true carboxypeptidase.
Drunk genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition. The same primers are used for PCR amplification and for sequencing.

**Primers**
Drunk(F): 5'-TTCCAGTACCGCAGTGCTGAGCTGTG -3'
Drunk(R): 5'-CATGGCTCAGTGGGACTGTGGCAAG -3'

**PCR program**
1) 94°C 2:00
2) 94°C 0:30
3) 62°C 0:30
4) 72°C 1:00
5) repeat steps (2-4) 35X
6) 72°C 5:00
7) 4°C ∞

The following sequence of 689 nucleotides (from Genbank genomic region NC_000079 for linear genomic sequence of Agtpbp1, sense strand) is amplified:

44764  ttccagt accgcagtgc tgagctgtg  tctaatcttc atcatcttgtg ttgaacagga
44821  gtgcttgccg gtcaggacct ttagctctct tgtcaacaca tccagtctga taatgagaaa
44881  atgtctcccc aaaaaccgcc ttccgctccc caccattaat agttcttccc accttcaatt
44941  gccaattatc cctgtgacct gcacgctctac agcttgccgc ctgaagg tag
45001  ggggtgggca tgggtgtgct tgggagtccg tgactctgtt gtcgaaatat gttatctggt
tggtgggcca tgggctgctg tgggagtccg tgactctgtt gtcgaaatat gttatctggt
tgtttaatgt ggatctgagg tgtgaaata cacaaaatt tgtcagcaat caatgtaaat
45521  attaaggaga taaggtggag acattatata agttgatgtc atacaagata gtagacattac
45581  attaaagaga taagagctgct gctgtgagc ggatctggtc gtttatatat tttttaaaaa
45541  gaattttaat taacatctgg tagtgaatct attattgtca ctatgtgggc atccacatat
45591  gttttgttgg tcaatttggt gcacatcttg gcctctgtgg gtttgagcag gtagagctca
gttttgttgg tcaatttggt gcacatcttg gcctctgtgg gtttgagcag gtagagctca
ggaccaagcc ttccatgttt gtttctttcc tttcagagtt gttgcaagg atcaatgtgaattt
45421  tttcagcttt gccacagtcc cactgagcca tg

Primer binding sites are underlined; the mutated T is highlighted in red.

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