<table>
<thead>
<tr>
<th><strong>Allele</strong></th>
<th><strong>jinx</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutation Type</strong></td>
<td>splice donor site (57 bp from exon)</td>
</tr>
<tr>
<td><strong>Chromosome</strong></td>
<td>11</td>
</tr>
<tr>
<td><strong>Coordinate</strong></td>
<td>116,073,423 bp (GRCm38)</td>
</tr>
<tr>
<td><strong>Base Change (assembly)</strong></td>
<td>G T</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
<td><em>Unc13d</em></td>
</tr>
<tr>
<td><strong>Gene Name</strong></td>
<td>unc-13 homolog D (C. elegans)</td>
</tr>
<tr>
<td><strong>Synonym(s)</strong></td>
<td>2610108D09Rik, Munc13-4, Jinx</td>
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<td><strong>Chromosomal Location</strong></td>
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<td><strong>Accession Number</strong></td>
<td>NCBI RefSeq: NM_001009573; MGI: 1917700</td>
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<tr>
<td><strong>Mapped</strong></td>
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<tr>
<td><strong>Amino Acid Change</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Institutional Source</strong></td>
<td>Beutler Lab</td>
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<tr>
<td><strong>Phenotypic Category</strong></td>
<td>decrease in NK cell response, DSS: sensitive day 7, hematopoietic system, immune system, MCMV susceptibility</td>
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<tr>
<td><strong>Penetrance</strong></td>
<td>100%</td>
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<tr>
<td><strong>Alleles Listed at MGI</strong></td>
<td>All alleles(2) : Gene trapped(1) Chemically induced(1)</td>
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</table>
## Mutagenetix Phenotypic Mutation 'jinx'

### Lab Alleles

<table>
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<th>Lab Alleles</th>
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</table>

### Mode of Inheritance

- Autosomal Recessive

### Local Stock

- Live Mice, Embryos, gDNA

### Repository

- MMRRC: 016137-UCD

### Science Writers

- Alyson Mack, Eva Marie Y. Moresco

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### Illustrators

- Diantha La Vine

### Last Updated

- 10/11/2016 6:15 PM by Bruce Beutler

### Record Created

- unknown

### Record Posted

- 12/10/2007
Phenotypic Description

The *jinx* mutation was identified in a screen for susceptibility to mouse cytomegalovirus (MCMV) in which mice were challenged with $10^5$ plaque forming units (PFU) of MCMV, an inoculum that is normally harmless to wild type animals ([MCMV Susceptibility and Resistance Screen](#)) (1). Sickness was observed in the index mutant, and following expansion and fixation, the *jinx* mutation was found to be conditionally lethal following injection of $2.5 \times 10^5$ PFU of MCMV (2). The degree of susceptibility is similar to that of BALB/c mice, which lack the natural killer (NK) cell-activating receptor, Ly49H. *Jinx* homozygotes exhibit splenic viral titers that are four to five orders of magnitude higher than in wild type mice five days after inoculation with $10^5$ PFU of MCMV, as well as exaggerated production of cytokines interleukin (IL)-12, type I interferon (IFN-??), and IFN-?? (detected in serum), consistent with a post-sensing defect (Figure 1). Profound defects in degranulation of activated NK cells and cytolytic T lymphocytes (CTL) are observed, but IFN-?? secretion by these cells occurs at normal levels. *Jinx* mice have no associated coat color abnormality, and importantly, show robust health as long as they are not deliberately infected.
When inoculated with lymphocytic choriomeningitis virus (LCMV), *jinx* mice display clinical features of hemophagocytic lymphohistiocytosis (HLH) that include thrombocytopenia, neutrophilia, splenomegaly, and elevated serum IFN-γ. In addition, macrophages, dendritic cells and splenic CD8^+^ T cells all expressed markers indicative of increased activation relative to wild type cells. Infection with MCMV at a low level (2 x 10^4^ PFU) did not cause HLH, nor did infection with *Listeria monocytogenes*. 
The *jinx* mutation mapped to Chromosome 11 and corresponds to a C to A transversion located within intron 26 of the *Unc13d* gene (position 11328 in Genbank genomic region NC_000077 for linear DNA sequence of *Unc13d*). The lesion results in the creation of a consensus intron splice donor site (GTCAGT > GTAAGT), which is utilized to the complete exclusion of the normal donor splice site of intron 26 (beginning at position 11273 in the genomic sequence). Aberrant splicing using the new donor splice site causes the incorporation of 53 nucleotides from intron 26 into exon 26 of the *Unc13d* transcript. The predicted polypeptide contains 20 aberrant amino acids, followed by a premature stop codon (TGA) after residue 832 of the 1085 amino acid protein.

The normal donor splice site of intron 26 is indicated in blue lettering. The new donor splice site, caused by the *jinx* mutation, is highlighted in gray; the mutated nucleotide is indicated in red lettering.

Cite this information as follows: Karine Crozat, Alyson Mack, Eva Marie Y. Moresco, Beutler B. Record for jinx, updated Oct 11, 2016. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu
Unc13d (also called Munc13-4) belongs to the Munc13 family of proteins, mammalian homologues of *Caenorhabditis elegans* Unc-13, which regulate vesicle priming in neurons and other cell types. Like other Munc13 protein family members, Unc13d contains two C$_2$ domains, Ca$^{2+}$/phospholipid-binding or protein interaction domains, separated by two Munc-homology domains (MHD) (Figure 2) (3). Unc13d is 37% identical to BAP3 [brain-specific angiogenesis inhibitor I (BAI1)-associated protein 3], which also has two C$_2$ and 2 MHD domains in the same arrangement (3). The function of BAP3, an interactor of the protein BAI1 which shows homology to the secretin receptor, is not understood (4). It appears that the known MHD-containing proteins in worms, flies, mice and humans are linked to secretory processes. However, although MHDs are present in organisms ranging from yeast to humans, and some mammalian MHD-containing proteins are ubiquitously expressed, the molecular function of this protein domain remains unknown.

The phorbol ester-binding C$_1$ domain found in the three other known Munc13 proteins (Munc13-1, Munc13-2, Munc13-3) is absent in Unc13d, but a domain of unknown function (pFAM DUF1041) is present between the N-terminal C$_2$ and MHD domains (2;3).

The predicted polypeptide encoded by *Unc13d*jinx contains 20 aberrant amino acids, followed by a premature stop codon after residue amino acid 832 of the 1085 amino acid protein. The truncated protein lacks part of the C-terminal MHD and all of the C-terminal C$_2$ domain (2).
Expression/Localization

Northern blot analysis reveals that *Unc13d* expression is widespread, with strong expression in lung, spleen and testis (3;5). Very low levels of transcript are detected in heart, kidney, liver, brain, and skeletal muscle (3;5). Expression of Unc13d in the lung and spleen is confirmed by Western blot analysis, but expression is below the level of detection in the other tissues (3). RT-PCR analysis detects *Unc13d* expression in all hematopoietic cells tested, including CD19+ B lymphocytes, CD4+ and CD8+ T lymphocytes, and mononuclear cells, as well as an NK cell line (5). Subcellularly, Unc13d localizes to secretory lysosomes in cytolytic lymphocytes (6); these vesicles are reported not to contain perforin or granzyme B (7).

Background
In mammals, the immediate members of the Munc13 family include Munc13-1 (Unc13a), Munc13-2 (Unc13b), and Munc13-3.
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(Unc13c). These, the closest relatives of Munc13-4 (Unc13d), are expressed in brain rather than in hematopoietic tissue, and are essential for the priming of synaptic vesicles to fusion competence in multiple types of synapses [reviewed in (8)] (Figure 3). Single knockout mutations of each mouse ortholog produce a CNS phenotype (9-11). While Munc13-1 knockouts are often born dead and show relatively severe neurological impairment (9), individual Munc13-2 and Munc13-3 knockout mice, and Munc13-2/3 double knockouts are viable (10), consistent with the conclusion that these paralogs serve at least partially redundant functions. Deletion of the more distantly related Munc18-1-encoding gene Stxbp1 (syntaxin binding protein-1) also causes perinatal lethality related to neurological dysfunction (12). In contrast, Unc13d is only very weakly expressed in the brain (3;5), and studies of jinx mice, and humans with mutations in the human homologue of Unc13d, indicate that Unc13d functions in priming lytic granule fusion in cytolytic T lymphocytes and NK cells (2;5).

Cytolytic T lymphocytes and NK cells kill virus-infected and tumor cells using a common cytolytic pathway that involves the localized exocytosis of secretory lysosomes containing perforin and granzymes (Figure 3). NK cells are particularly important for innate immune defense during infection with herpes viruses such as MCMV. The cytolytic process is a multi-step process beginning with cell activation. Cytolytic T cells are activated by specific antigen recognition, while engagement of activating receptors on NK cells leads to their activation. Upon interaction and activation by a target cell, lytic granules polarize towards the target cell contact site (the immunological synapse), become tethered to the inner surface of the plasma membrane (“docking”), gain competence for fusion (“priming”), and then fuse, releasing their contents at the immunological synapse. The molecular details of each of these steps have begun to be elucidated, and continue to be the subject of intense study.

Mutations affecting any of the various steps in the cytolytic process may cause immune disorders known as hemophagocytic syndromes (also called hemophagocytic lymphohistiocytosis, HLH), which are characterized by polyclonal CD8 T cell and macrophage activation and infiltration of multiple organs, as well as persistent release of cytokines including IFN-?, interleukin-1 (IL-1), IL-6, IL-18 and tumor necrosis factor (13). These events cause multisystem inflammation and can lead to death in untreated patients. Alteration of the Rab27a locus, which is required for vesicle docking, or the LYST locus, which is required for granule formation, causes Griscelli syndrome (14) or Chediak-Higashi syndrome (15;16), respectively. Both Rab27a and LYST are also involved in the intracellular transport of melanosomes, and partial albinism is associated with both Griscelli and Chediak-Higashi syndromes.

Familial forms of HLH (FHL) comprise the hemophagocytic phenotypes, but not the melanosome
transport phenotypes. Four types of FHL are known, designated by their association with distinct genetic loci: FHL1 is caused by mutations in an unknown gene that has been mapped to human chromosome 9 \((17)\); lesions in perforin \((Prf1)\), which polymerizes and serves to generate poly-perforin pores in the plasma membrane of the target cells, are responsible for FHL2 \((18)\); FHL3 is caused by defects in MUNC13-4 (the human homologue of Unc13d), a protein necessary for the priming of lytic granules \((5)\); and mutations in the syntaxin 11 gene, required for granule fusion, cause FHL4 \((19)\).

FHL3 is a fatal disease marked by massive hepatosplenomegaly, anemia, and thrombocytopenia, as well as high levels of cytokines and defective NK cell cytotoxicity \((5)\). MUNC13-4 is not essential for granule polarization and membrane docking, but is absolutely required for the regulated secretion of cytotoxic granules at the priming stage of the exocytic pathway \((5)\). Munc13-4 is believed to associate with Rab27a on the basis of immunoprecipitation studies performed with platelets and spleen cells \((6;20)\), and their association is thought to be important for the final steps in the exocytosis process. Recent work indicates that these final steps are more complicated than simply a direct association between Munc13-4 and Rab27a in cytolytic granules at the priming stage. Careful analysis of individual vesicles containing various Rab proteins, Rab27a and Munc13-4 suggests the existence of distinct endosomal/exocytic versus cytolytic vesicle populations \((7)\). Rab27a is associated with non-perforin-containing late endosomal vesicles \((Rab7^+)\), but not with Rab11^+ recycling endosomes \((7)\). Upon cell activation, Munc13-4 may promote fusion of these compartments, and subsequent colocalization (and probably fusion) of these endosomal/exocytic vesicles with cytotoxic vesicles, leading to the formation of mature cytolytic granules. Thus, Munc13-4 is postulated to bring together effectors of the exocytic machinery in a cytolytic-competent granule \((7)\).

**Putative Mechanism**

The \textit{jinx} phenotype, marked by normal health prior to infection with MCMV or LCMV, and the development of HLH in response to the latter pathogen, supports the view that human FLH3 is induced by an infectious trigger. Epstein-Barr virus (EBV) infection has been implicated as an agent that might possibly cause expression of the FHL3 phenotype, given that it is very common in human populations. In mice, MCMV, a herpesvirus, cannot induce HLH when inoculated at a low titer. Additionally, one study finds that neither EBV nor CMV is likely to be involved as the trigger for FHL3; only one of each case was found among ten Japanese FHL3 patients with confirmed MUNC13-4 mutations \((21)\). These observation contest (but do not refute) the hypothesis that EBV, also a herpesvirus, is the infectious trigger for human FLH3. The fact that LCMV (but neither \textit{Listeria}
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*monocytogenes* nor MCMV) can induce severe HLH indicates that the trigger is quite specific, at least in mice.

HLH probably occurs when antigen presenting cells (APCs; in all probability macrophages and myeloid dendritic cells) are either infected with LCMV or at least acquire and cross-present antigen from other infected cells. These cells drive the expansion of CD8⁺ T cells, which would normally eliminate the infection. However, because they cannot degranulate, they are unable to effect sterilizing immunity. Because the *jinx* mutation does not impair production of IFN-γ, the expanded CD8⁺ T cell pool probably drives proliferation of the APC compartment, and macrophage infiltration of many tissues follows, including the spleen and bone marrow, where hematopoiesis is suppressed as a result. Anemia due to impaired hematopoiesis, and occasional phagocytosis of erythrocytes that gives the syndrome its name, are both observed as consequences of activated macrophage proliferation. Although it is unclear whether IFN-γ has a non-redundant pathogenic role in *jinx* mice, administration of anti-IFN-γ has been reported to mitigate HLH in perforin-deficient (*Prf*) mice (22).

Most, but not all, of the hallmarks of the human disease are observed in *jinx* mutants (2). Hepatomegaly did not occur in mice, however, and neutrophilia was observed rather than neutropenia. The latter discrepancy may reflect a different role of the Unc-13d protein in mouse, as compared to human, neutrophils. Alternatively it may reflect the need for a different microbial trigger in humans, affecting different aspects of the innate immune response. Of interest in this regard, neutropenia was observed in *Prf*⁻/⁻ mice infected with LCMV (22). No evidence of platelet dysfunction is observed in *jinx* homozygotes, despite work suggesting that MUNC13-4 is required for dense core granule secretion in human platelets (20). Alternative mechanisms for dense granule priming, independent of Unc-13d, may exist in mice.

The strong NK cell phenotype of *jinx* mice suggests non-redundancy of function, and while it may not apply to all classes of NK cell granules, it is clear that the LAMP1 (CD107a) compartment does not undergo exocytosis in *jinx* homozygotes. Fas ligand, granzymes, and perforin co-localize within the same class of vesicles in NK cells (23-25) and it is therefore possible that in *jinx* mice, a defect of Fas ligand release also exists. This may contribute to the proliferative syndrome that is observed, to the extent that Fas ligand initiates apoptosis in the target cell population. However, the absence of spontaneous lymphoproliferative disease in *jinx* mice (as compared to *Gld* mice, for example) indicates that the NK cells and CTLs do not act as the essential source of Fas ligand required for homeostatic control of T cells *in vivo*.
Genotyping

*Jinx* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.

**Primers for PCR amplification**
jinx(F): 5’-CTACATGAACACCAACCTGGTCCAGGAG-3’
jinx(R): 5’-GGATCATGAAGAGGAAGGAGATGCAGTTAGG-3’

**PCR program**
1) 94°C 2:00
2) 94°C 0:30
3) 58°C 0:30
4) 72°C 0:30
5) repeat steps (2-4) 39X
6) 72°C 7:00
7) 4°C ∞

**Primers for sequencing**
jinx_seq(F): 5’-TACATGAACACCAACCTGGTCC-3’
jinx_seq(R): 5’-AGAGGAAGGAGATGCAGTTAGG-3’

The following sequence of 308 nucleotides (from Genbank genomic region NC_000077 for linear DNA sequence of *Unc13d*) is amplified:

```
11234  ctacatq aacaccaacc tggtccagga gaacttcagc 
aggtacccag
11281  cagccccccga tctgcctctcg gccgccatgt ctccaaaggct cacgagteag 
tggcatgttg
11341  ggattccccct ttggtgggat tcccttttgt gacctctcttg gaggatgtaa 
c wagtcggg
11401  ggacagggag ctcactgctt cccttaaagg tccttttttc ttctgcctcg 
ctctaaagcac
11461  ggcaagccttt cccctgtatc cctccaggcc tgggcccatc attaatggcc 
cctcttcctgc
11521  tctctcttctttctcatgatc c
```

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated C is shown in red text.
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References


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(Generated on Apr 07, 2017)
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