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Short Communication

# The open-eyelid mutation, lidgap-Gates, is an eight-exon deletion in the mouse *Map3k1* gene

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**Abstract**

The BALB/cGa mouse strain and its descendants, now called the SELH/Bc strain, have produced two waves of high frequency of spontaneous heritable mutations. One of these, the recessive lidgap-Gates ($lg^{Ga}$) mutation, causes the same open-eyelids-at-birth phenotype as the gene knockout mutations of *Map3k1* and co-maps to distal Chr 13. The $lg^{Ga}$ mutation is demonstrated to be a 27.5-kb deletion of exons 2–9 in the *Map3k1* gene, the first spontaneous mutant allele described at this locus. The $lg^{Ga}$ mutation is consistent with a pattern suggesting that the waves of mutation in BALB/cGa and its descendants tend to be large deletions or ETn insertions, whose elevated rate of occurrence is due to an unknown mechanism.


*Keywords:* Deletion; Mutation; Eyelid; Mouse; MEKK1; Map3k1; MAPKKK; Lidgap; Instability

Lidgap-Gates, $lg^{Ga}$, is a recessive mutation in mice that causes a failure of fetal eyelid development, resulting in the defect "open eyelids at birth"; it was mapped to distal Chr 13 [1]. The eyelid defect is highly penetrant and usually bilateral. Affected eyes develop corneal opacity by adulthood. There are no other known defects in the mutant.

Lidgap-Gates is one of seven spontaneous heritable mutations—asebia ($Scd1^{ab}$), shaker-3 (Sh-3), clasper-underweight (*cu*), pigtail-rotator (pr), ear malformed (Em), ophthalmatrophy (oa; now $lg^{Ga}$), and first arch (*Far*)—that were recovered from the BALB/cGa strain or its outcross by Dr. Allen Gates at Stanford University in the period 1962–1969 [2,3]. The cause of this wave of mutations is unknown. One of these seven mutations, *Far,* still exists and is mapped to Chr 2 [4]. Another mutation occurred in our BALB/cGa colony in 1982, cartilage matrix deficiency ($Agc1^{cmd\text{-}Bc}$) [5], but no further burst of mutations has recurred within the strain.

A second wave of mutations, however, occurred in mice descended in part from BALB/cGa. The $lg^{Ga}$ mutation was imported to our mouse colony by Dr. James R. Miller in 1968 and maintained on mixed genetic backgrounds from BALB/cGa, 129/RrGa, CBA/−, and ICR random-bred mice. During early generations of inbreeding of the $lg^{Ga}$ stock on a largely ICR-derived background, a new wave of mutations occurred, beginning with $Spna1^{sph\text{-}2Bc}$ on Chr 1 in 1978 [6]; the exencephaly and cleft cerebellum-causing mutations *Exen1, Exen2,* and *Exen3* on Chrs 13, 5, and possibly 11, in 1981–1982 [7]; $Foxn1^{nu\text{-}Bc}$ on Chr 11 in 1983 [8]; $Tyr^{c\text{-}Bc}$, $Tyr^{c\text{-}Bc2}$, and $Tyr^{c\text{-}Bc3}$ on Chr 7 in 1989–1990 [8,9]; "lens rupture" (unmapped) in 1990; *wam* on Chr 11 in 1993 [10]; and an allele of $Kit^{W}$ on Chr 5 in 1994. The mutations that occurred after $Spna1^{sph\text{-}2Bc}$ were in a subline that was inbred to become the SELH/Bc inbred strain, used for the study of cranial neural tube closure defects [7], and all mutations except the *Exen* genes were purged from the SELH/Bc strain into sublines when they occurred. We have estimated, based on colony size of the SELH stock compared with the other stocks in our colony, that the mutation rate in the SELH/Bc lineage was approximately 10 times higher than normal [8,9].

We hypothesize that the cause of the high mutation rate in the $lg^{Ga}$-derived lineage, SELH/Bc, was inherited from

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the BALB/cGa ancestor, along with the $lg^{Ga}$ mutation, possibly by linkage on Chr 13. One explanation is that the "instability" reflects a high level of retrotransposition of a transposable element, activated by an unknown mechanism. In support of this hypothesis is our recent finding that the SELH/Bc strain has a highly transcribed variant of the ETnII retrotransposon, whose transposition may be enabled by a variant MusD element [11].

As one approach to the ongoing question of the mechanism behind the waves of elevated mutation rate in BALB/cGa and its descendants, we have identified the molecular nature of the mutations when possible, and they are summarized in Table 1. Of the six mutations previously analyzed, one is a point mutation, two are due to insertion of the ETnII element, and three are large deletions of at least several exons. A high level of retrotransposon insertion can lead to large deletions [12]. From this pattern, we hypothesized that the $lg^{Ga}$ mutation would be either an ETn insertion or a large deletion.

$lg^{Ga}$ had been mapped to a chromosomal region of approximately 1 cM relative to "Mit" SSLP markers [1] and, with the availability of the mouse sequenced genome [13,14], identification of the mutated gene became feasible. We noted that the homozygous knockout mutants of the *Map3k1* (MEKK1) gene have a highly penetrant open-eyelid defect, very similar to the $lg^{Ga}$ mutant [15,16]. Although the MGD map [17] placed *Map3k1* approximately 10 cM proximal to the SSLPs flanking $lg^{Ga}$, the mapping data for the exact location were not strong. We therefore mapped the *Map3k1* locus relative to the SSLP markers that flank $lg^{Ga}$. The four crucial recombinant individuals from our archived recombinant mapping panel used to map $lg^{Ga}$ precisely [1] were typed for an informative SSLP derived from intron 18 of the *Map3k1*

Table 1
The nature of the identified heritable spontaneous mutations that occurred in the lineage from BALB/cGa through the $lg^{Ga}$ stock to the SELH/Bc strain

| Mutation[a] | Name | Background | Year occurred | Chromosome | Molecular lesion | Ref. |
|---|---|---|---|---|---|---|
| *Scd1*[ab] | asebia | BALB/cGa | 1962 | 19 | Not known | [3] |
| "Sh-3" | shaker 3 | BALB/cGa × 129/RrGa stock | 1962 | — | Not known | [3] |
| *cu* | clasper-underweight | BALB/cGa | 1963 | — | Not known | [3] |
| "pr" | pigtail-rotator | BALB/cGa | 1964 | — | Not known | [3] |
| "Em" | ear malformed | BALB/cGa | 1966 | — | Not known | [3] |
| $lg^{Ga}$ ("oa") | lidgap-Gates (formerly ophthalmatrophy) | BALB/cGa | 1966 | 13 | Deletion of exons 2–9[b] | [1,3]; this report |
| *Far* | first arch | BALB/cGa | 1969 | 2 | Not known | [4,23] |
| $Agc1^{cmd\text{-}Bc}$ | cartilage matrix deficiency | BALB/cGaBc | 1982 | 7 | Deletion of exons 2–19 | [5,20] |
| $Spna1^{sph\text{-}2Bc}$ | spherocytosis | $lg^{Ga}$ stock (from BALB/cGa) | 1978 | 1 | G→T transversion in first nucleotide of intron 41, causing skipping of exon 41 in mRNA | [6,24] |
| *Exen1* | exencephaly (oligogenic) | $lg^{Ga}$ stock via *sph-2Bc* line | 1981 | 13 | Not known | [7] |
| *Exen2* | | | | 5 | | |
| *Exen3* | | | | 11 | | |
| $Foxn1^{nu\text{-}Bc}$ | nude | SELH[c] (from *sph-2Bc* line) | 1983 | 11 | ETnII insertion in intron between exon 1b and exon 2 | [8] |
| $Tyr^{c\text{-}Bc}$ | tyrosinase | SELH[c] | 1989 | 7 | Deletion of >2 cM including at least exons 1–3 of *Tyr* | [9] |
| $Tyr^{c\text{-}Bc2}$ | tyrosinase | SELH[c] | 1989 | 7 | Deletion of exons 1–3 | [9] |
| $Tyr^{c\text{-}Bc3}$ | tyrosinase | SELH[c] | 1990 | 7 | ETnII insertion in exon 1 | [8,9] |
| — | "lens rupture" | SELH[c] | 1990 | — | Not known | Juriloff, unpublished |
| *wam* | whiskers amiss | SELH/Bc | 1993 | 11 | Not known | [10] |
| — | "white belly spot" allele of $Kit^{W}$ | SELH/Bc | 1994 | 5 | Not known | Juriloff, unpublished |

[a] Symbols in italic are current mutation symbols according to the Mouse Genome Database [17]. Symbols in quotation marks are original mutation symbols, not currently listed in MGD.
[b] Data in present report.
[c] Descended from the *sph-2Bc* line, a continuous lineage in transition from BALB/cGa (carrying the $lg^{Ga}$ mutation) while becoming the SELH/Bc inbred strain.