# Mutagenetix Phenotypic Mutation 'bandit'

<table>
<thead>
<tr>
<th><strong>Allele</strong></th>
<th><strong>bandit</strong></th>
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<tr>
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<tr>
<td><strong>Coordinate</strong></td>
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<tr>
<td><strong>Base Change (assembly)</strong></td>
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</tr>
<tr>
<td><strong>Gene</strong></td>
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<tr>
<td><strong>Gene Name</strong></td>
<td>grainyhead-like 1 (Drosophila)</td>
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<tr>
<td><strong>Synonym(s)</strong></td>
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<tr>
<td><strong>Accession Number</strong></td>
<td>NCBI RefSeq: NM_001161406 (isoform 1), NM_145890 (isoform 2); MGI: 2182540</td>
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<tr>
<td><strong>Amino Acid Change</strong></td>
<td>Arginine changed to Stop codon</td>
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<td><strong>Penetrance</strong></td>
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<td><strong>Alleles Listed at MGI</strong></td>
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<td><strong>Lab Alleles</strong></td>
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<tr>
<td><strong>Science Writers</strong></td>
<td>Eva Marie Y. Moresco</td>
</tr>
<tr>
<td><strong>Authors</strong></td>
<td>Amanda L. Blasius, Xin Du, Bruce Beutler</td>
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Cite this information as follows: Amanda L. Blasius, Xin Du, Eva Marie Y. Moresco, Beutler B. Record for bandit, updated May 13, 2016. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

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(Generated on Jun 25, 2016)
Phenotypic Description

Figure 1. Bandit homozygote with patchy fur on the body and fur remaining on the face.

The *bandit* phenotype was detected in a G3 mouse and is characterized by small body size and patchy fur over the body, with fur remaining on the face (Figure 1). Homozygous *bandit* mice had normal serum iron concentration; occult blood was not detected in the feces. No abnormalities were found in the hematology profile.
Nature of Mutation

The *bandit* mutation was mapped to Chromosome 12. *Grhl1* was identified as a candidate within the critical region because knockout of the gene in mice causes a phenotype similar to *bandit* \(^{(1)}\). An A to T transversion was found at position 25,262,891 of the C57BL/6J mouse genomic DNA sequence of Chromosome 12, within exon 3 of the *Grhl1* gene (exons 1-3 are not included in the NCBI Genbank annotation of the genomic DNA sequence of *Grhl1*, region NC_000078). Two isoforms of *Grhl1* are expressed due to alternative splicing. Isoform 1 is the full length variant in which 16 exons are incorporated into a processed mRNA 3435 base pairs in length, encoding a 618 amino acid protein. Isoform 2 is transcribed to incorporate 14 exons (exons 4-16 plus one unique exon) and yields a processed mRNA 3066 base pairs in length, encoding a 536 amino acid protein. The residue mutated in *bandit* mice corresponds to nucleotide 366 in the mRNA sequence of *Grhl1* isoform 1. The mutation occurs in exon 3, which is absent from and therefore predicted not to affect isoform 2.

![Mutation Diagram](image)

The mutated nucleotide is indicated in red lettering, and converts arginine 72 of the Grhl1 protein (isoform 1) to a stop.

Protein Prediction

*Grhl1* is a member of the Grainyhead (GRH) family of transcription factors, named for a *Drosophila* gene that when mutated results in fly embryos with grainy and discontinuous head skeletons, flimsy cuticles, and patchy tracheal tubes \(^{(2)}\). GRH transcription factors, acting both
as repressors and activators, function in a wide variety of physiological processes during development and in mature organisms from fungi to humans (see Background). The family is divided into two subfamilies grouped by sequence similarity of their DNA binding and oligomerization domains (Table 1) (3;4). Some similarity also exists in the sequences of DNA binding domains between subfamilies, but not in the sequences of oligomerization domains (3). Drosophila Grainyhead (also designated NTF-1 or Elf-1) belongs to the GRH subfamily (2;5), while Drosophila CP2 aligns with the LSF/CP2 subfamily (4).

Table 1. Mammalian GRH transcription factors

<table>
<thead>
<tr>
<th>Official symbol (mouse)</th>
<th>Synonyms</th>
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<tr>
<td><strong>LSF/CP2 subfamily</strong></td>
<td></td>
</tr>
<tr>
<td>Tcfcp2</td>
<td>CP2, LSF, LBP-1c</td>
</tr>
<tr>
<td>Ubp1</td>
<td>LBP-1a, NF2d9</td>
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<td>Tcfcp2l1</td>
<td>LBP-9, CRTR-1</td>
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<tr>
<td><strong>GRH subfamily</strong></td>
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</tr>
<tr>
<td>Grhl1</td>
<td>LBP-32, MGR</td>
</tr>
<tr>
<td>Grhl2</td>
<td>BOM</td>
</tr>
<tr>
<td>Grhl3</td>
<td>SOM, Get1</td>
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GRH transcription factors from both subfamilies typically possess DNA binding, oligomerization, and transactivation domains as determined by deletion and mutational analysis of CP2 and Grainyhead, the best studied members of the family (6-10). The DNA binding regions of Grainyhead and CP2 encompass ~250 amino acids in the C-terminal and N-terminal halves of these proteins, respectively (6;7;9). Although the Grainyhead DNA binding domain was reported to show minimal similarity to a region of MyoD and myogenin with helix-loop-helix structure (22% over 90 amino acids) (5;11), the Grainyhead/CP2 domain is much larger. No similarity to other known DNA binding motifs is observed, but computer modeling using fold recognition and protein structure prediction programs suggests a fold homologous to that of the p53 core domain (12). Characterization of the preferred binding site of CP2 based on the known sequences of binding sites within the ?-globin and SV40 gene promoters demonstrated that CP2 binds to a pair of directly repeated sequences with consensus CNRG-N_6^-CNRG, where N is any nucleotide and R is a purine (13-15). The spacing of the repeated
elements is restricted to position the repeats on the same face of the DNA helix. Grainyhead binds to a similar repeated sequence, CTGG, within the gene promoters of Ultrabithorax (Ubx), dopa decarboxylase (Ddc), and fushi tarazu (ftz) (5).

GRH proteins function as homodimers or oligomers of members from the same subfamily. Using deletion mutants in crosslinking, GST pull-down, and yeast two-hybrid experiments, the oligomerization domains of Grainyhead and CP2 were mapped to an ~80-100 amino acid region that may partially overlap with the C-terminal portion of the DNA binding domain (6;7;9). In CP2, the domain displays similarity to the sterile alpha motif (SAM), which has been shown to mediate homo- and hetero-oligomerization (12;16). Despite 29% sequence identity in their oligomerization domains, Grainyhead has been shown to form dimers (9) whereas CP2 forms tetramers on DNA (6). These findings are consistent with intrinsic (perhaps structural) differences between the oligomerization domains of proteins from the two GRH subfamilies, which do not hetero-oligomerize across subfamily boundaries (4;7). Similarity between the subfamilies may reflect similarity in recognition of the consensus binding site within the DNA.

Mapping of the transactivation domain of Grainyhead using deletion constructs demonstrated that an N-terminal 56 amino acid region functions as the minimal activation domain (9). Grainyhead has been reported to interact with components of the TFIID complex to mediate transactivation (17).

Figure 2. Domain structure of Grhl1. The position of the bandit mutation is indicated. TAD, transactivation domain; DBD, DNA binding domain.

Grhl1 is a member of the GRH subfamily, and bears 37% identity and 52% similarity in
sequence to Grainyhead (Figure 2) (4). Alternative splicing results in expression of two Grhl1 isoforms. Isoform 1 is the full length isoform (618 amino acids); isoform 2 (536 amino acids) begins with 11 unique N-terminal amino acids encoded by a distinct exon not present in isoform 1, followed by exons 4-16 of isoform 1 (4). Isoform 2 utilizes its own set of promoter sequences (GC box, TATA box, 5' CAP site) and translation start codon. Both Grhl1 isoforms contain a DNA binding domain (amino acids 248-486 in isoform 1) and a dimerization domain (amino acids 512-618 in isoform 1) that are 48% and 39% identical in sequence to those of Grainyhead. Grhl1 has been shown to bind in vitro to known Grainyhead binding sites in the regulatory regions of the Drosophila Ddc and proliferating cell nuclear antigen (PCNA) genes, and to dimerize with Grhl2 but not CP2 or LPB-1a (4). The amino acids excluded from isoform 2, encoded by exons 1-3 of isoform 1, comprise a transcriptional activation domain as defined by deletion mutants in a transcriptional reporter assay (4). The domain bears no similarity to that of Grainyhead or other known transactivation domains. Isoform 2 has been postulated to function in dominant negative fashion as a repressor of transcriptional activation by isoform 1. The consensus DNA target sequence of Grhl1 (AACCGGTT) is the same as that for Grhl3 and Grainyhead (4;18), but the in vivo targets of Grhl1 regulation are unknown.

The bandit mutation, a premature stop, lies in exon 3 of the full length isoform 1, within the region defined by transcriptional reporter assay as the transactivation domain.

**Expression/Localization**

*In situ* hybridization in mouse embryonic tissue demonstrates Grhl1 expression predominantly in the epidermis (4). Grhl1 is detectable in the surface ectoderm at embryonic day 10.5 (19), and in the skin and developing hair germs by embryonic day 15.5 (1). In postnatal animals, expression of Grhl1 is restricted to the suprabasal layers of the skin and to the hair follicles (1). Grhl1 is presumed to localize in the nucleus, like Grainyhead (11) and Grhl3 (20).

**Background**

*Drosophila Grainyhead*

Grainyhead was first identified in *Drosophila* through its ability to bind to a cis element mediating transcriptional activation of the *dopa decarboxylase (Ddc)* gene (5;11;21). The gene, initially named Elf-1 (element I-binding activity) or NTF-1 (neuronal transcription factor 1), was
later shown to be identical to *Grainyhead* (*grh*) (2), a previously identified embryonic lethal locus on Chromosome 2 (22). Grainyhead mutant flies die at the end of embryogenesis with normal segmentation but altered head skeleton and cuticular structures. The head skeleton has a granular appearance, and the cuticle is bulging, distended, and easily ruptured (2). Other cuticular structures such as mouth hooks and denticles are also affected. The tracheal network appears patchy, resulting from failure of an overextended and elaborated tracheal tree to fill with gas (23). Consistent with an important role for Grainyhead in cuticle formation, impaired wound healing of the larval cuticle is observed in *grh* mutant flies (24).

During development of the newly fertilized fly embryo, Grainyhead is initially involved in dorsal/ventral patterning through repression of *decapentaplegic* and *zerknüllt* gene transcription (25). Grainyhead also mediates localized repression of the *tailless* gene in the central portion of the embryo, restricting *tailless* expression to the poles during early embryogenesis (26). Embryonic patterning defects are not observed in *grh* mutant embryos due to maternal provision of *grh* mRNA to oocytes and early embryos (25;26). Instead, the cuticular defects observed later in embryonic development are thought to arise from aberrant regulation of Grainyhead targets in the epidermis (responsible for embryonic cuticle secretion) where Grainyhead is predominantly expressed (11). The epidermal expression of *Ddc*, required for production of dopamine and subsequently quinones that crosslink cuticular proteins, is abrogated in *grh* mutant embryos, resulting in weakening of the cuticle and defective cuticle wound healing (24;27). However, expression of *engrailed* (28), *ftz*, and *Ubx* (5;29), genes responsive to Grainyhead regulation *in vitro*, was not affected in *grh* mutant fly embryos (2). Grainyhead has been shown to regulate PCNA expression *in vitro* (30), and to cooperate synergistically with Suppressor of Hairless [Su(H)] to activate transcription in response to Notch signaling during cell fate decisions *in vivo* (31).

Central nervous system (CNS) development in *Drosophila* requires Grainyhead. An isoform of Grainyhead generated by alternative splicing is specifically expressed in neuroblasts during embryogenesis (11;32), and is activated there during late stages of neuroblast lineage development (33). Deficiency of this isoform results in pupal and adult lethality with flies demonstrating uncoordinated movements (32). Grainyhead is the last gene to be activated in the temporal sequence of transcription factor activation in neuroblasts, and has been shown to regulate neuroblast mitotic activity during larval stages, in particular to either terminate (in the abdomen) or prolong proliferation (in the thorax) (33-36).
Mammalian GRH proteins

Members of the LSF/CP2 subfamily of GRH transcription factors are ubiquitously expressed and regulate transcription in a variety of settings. The most extensively studied member, CP2, was identified as a factor binding to elements in the simian virus 40 (SV40) late promoter and in the human immunodeficiency virus type 1 (HIV-1) promoter (37;38). Further study demonstrated that CP2 binds near the transcription start site of the HIV-1 long terminal repeat (LTR) and represses transcription by inhibiting the binding of TFIID to the TATA box (8;39), and by preserving higher order nucleosome structure through the recruitment of histone deacetylase 1 in cooperation with the transcription factor YY1 (40). In addition, CP2 can restrict HIV-1 transcription at the level of elongation by reducing RNA polymerase processivity (41). The repression of HIV-1 transcription by CP2 has been postulated as a mechanism underlying the establishment of virological latency in vivo.

CP2 binds to the regulatory regions of diverse genes. For example, CP2 activates transcription of multiple erythroid-specific genes and all globin genes (10;14;42;43), and mediates hemoglobin switching as part of the stage selector protein complex that regulates developmental globin expression (44;45). In T cells, CP2 DNA-binding activity is increased upon cell activation with PMA/ionomycin, an effect that may be regulated by ERK phosphorylation of CP2 (46;47). CP2 has also been shown to bind to the interleukin (IL)-4 promoter in T helper cells (48). Notably, CP2 deficient mice grow and develop normally, and display normal hematopoietic differentiation, globin gene expression, and immunological responses to mitogenic T cell and B cell stimulation (49). LBP-1a (also known as Ubp1 and NF2d9), which is 72% identical to CP2 in amino acid sequence and can bind to known CP2 consensus sites, is thought to compensate for the loss of CP2 function. LBP-1a deficient mice die at embryonic day 11.5, with defects in blood vessels of the placenta (50). CP2 is reported to bind in vitro to the promoters of c-fos, ornithine decarboxylase (47), major histocompatibility class II (51), thymidylate synthase (5), and P450scc genes (52).

The closest mammalian homologues of Drosophila Grainyhead are Grhl1, Grhl2, and Grhl3, only recently identified by database searches for mammalian proteins with sequence similarity to Grainyhead (4;20;53). These three proteins form the GRH subfamily. Similar to Grainyhead, the mammalian proteins are not ubiquitously expressed, but display restricted expression predominantly in tissues derived from the ectoderm; all three genes are highly expressed in the
embryonic epidermis, although at differing levels and times (19). The function of Grainyhead in the development and maintenance of the *Drosophila* cuticle is conserved in mice by Grhl3. *Grhl3*−/− mice display defective skin barrier function, deficient wound repair, and reduced expression of multiple genes linked to epidermal terminal differentiation and barrier function, including transglutaminase I, the key enzyme involved in crosslinking structural components of the superficial epidermis (18;54;55). Thus, the role of Ghrl3 is analogous to that of Grainyhead in the regulation of *Ddc* in the fly cuticle. Expression of the three Grhls is also observed in non-ectoderm-derived tissues, including brain, heart, lung, and kidney (19;53). Grhl3 is essential for neural tube closure during mouse embryonic development (56).
Figure 2. Deficiency of Grhl1 leads to impaired anchoring of the hair shaft to the hair follicle. Desmosomal cadherins, comprising multiple subtypes of desmocolins (Dsc1-3) and desmogleins (Dsg1-4), are the adhesion molecules of desmosomes, intercellular junctions that anchor the hair shaft to the hair follicle. Within desmosomes, the extracellular domains of desmocolins and desmogleins from neighboring cells form heterophilic interactions in the extracellular space, while intracellularly they are linked to plakoglobin, plakophilins, and desmoplakin. Desmoplakin binds to keratin intermediate filaments, thereby tethering the intermediate filaments to the plasma membrane to physically strengthen the junction. Grhl1 controls the expression of Dsg1 through direct binding to the Dsg1 promoter, but not to the...
promoters of Dsg2, Dsg3, or Dsg4. Both Dsg1 and Grhl1 are expressed in the inner root sheath, but not in the dermal papilla cells of the hair follicle. Thus, mice deficient in Grhl1 easily lose hairs in tape-stripping tests, and these hairs are bared of the inner root sheath, companion layer, and outer root sheath tissues that remain adherent to wild type hairs removed in the same way.

Consistent with the prominent expression of Grhl1 in the suprabasal layers of the epidermis and in hair follicles, mice with a targeted deletion of Grhl1 exhibit skin and hair phenotypes (1). Over 30% of Grhl1−/− mice are devoid of hair at weaning, although whiskers, nails and teeth are normal. Hair growth is delayed in all mice, and older mice exhibit hair loss resulting from poor anchoring of the hair shaft in the follicle. Thus, grooming behavior by cage mates causes regional hair loss that is not observed in mice housed in isolation. Reduced numbers of abnormal desmosomes are observed in the interfollicular epidermis of Grhl1 mutants; however, no defects in skin barrier or wound healing are evident. Sequence analysis, DNA binding, and chromatin immunoprecipitation experiments demonstrate that the promoters of desmoglein 1 (Dsg1), encoding several isoforms of a desmosomal cadherin, are direct targets of Grhl1. Reduced expression of desmoglein 1 in hair follicles and epidermis likely underlies some of the phenotypes of Grhl1 mutants (Figure 2). In support of this hypothesis, mice lacking Dsg3 display hair anchoring defects (57), and Dsg3−/− mice in which two Dsg1 isoforms have been inactivated exhibit a specific loss of anagen hair upon tape stripping (58). Human mutations in DSG1 cause the dominantly inherited disorder type I striate keratosis palmoplantaris (PPKS1; OMIM #148700), characterized by hyperkeratosis at sites of pressure and abrasion such as the palms and soles. Grhl1−/− mice also develop palmoplantar keratoderma, a thickening of the stratum corneum (the most superficial layer of epidermis) on the bottom of the paws. The molecules that regulate Grhl protein function remain unknown.
Putative Mechanism

The *bandit* mutation creates a premature stop codon in the transactivation domain of the full length Grhl1 isoform 1. The shorter isoform 2 is not predicted to be affected by the *bandit* mutation, but protein expression of this isoform has not been examined in *bandit* mice. The phenotype of homozygous *bandit* mice closely resembles that of Grhl1 null mice, in which targeted deletion of exons 1 and 2 results in a confirmed lack of expression of both Grhl1 isoforms. This is consistent with a complete lack of protein expression resulting from the early stop codon in *bandit* homozygotes. The function of isoform 2, if expressed, is apparently insufficient to rescue the phenotype of homozygous *bandit* mice.

Genotyping

*Bandit* 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.

**Primers**

bandit (F): 5’- TGCCTGTTTTGGACCATGTCTCAGC -3’
bandit (R): 5’- GGGTCTCAATGCAGATGAGCAAACAC -3’

**PCR program**

1) 95°C 2:00
2) 95°C 0:30
3) 56°C 0:30
4) 72°C 1:00
5) repeat steps (2-4) 29X
6) 72°C 7:00
7) 4°C ?

**Primers for sequencing**

bandit_seq(F): 5’- CCCAGCATTTAGGAGCATTG -3’
bandit_seq(R): 5’- ACCAGTAGAGGTTGGTGCT -3’

The following sequence of 941 nucleotides (NCBI Mouse Genome Build 37.1, Chromosome 12, bases 25,262,288 to 25,263,228) is amplified:
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tg cctgtttggga ccatgtctca gctggggtttaa
tgtcagctgtg taccagtgtc atttttccag ctttttcagct ctggggtttaa gaaaaaggga
gtcagacaag gagtggaaatg gaggccaagaga gatggccccag catttaggag cattgggtggc
tcttttcagag gccctgtggt cagtttccag caccacatag gttgactcata gccatctcata
actgcaagtc cagggtatcc gatgcattct tctgcacctg ggcaccattc acacatgtgg
ataatcagaca taggtagagg ccaacacattt atacatgaaa aaaccaaat acatacctta
aatatatagag aagaaagaaa tctttactcc cactataaag taaagtttga gataccgcag
cttatgtttcc agttgtctct caccggttgc tgtgggtgatc aagcgttgag cttcttgaggg
gccagagtggc cccaggggttc agcaggtatg caggagatga gtgaacccaa gagcatcttg
agaatgttgg cctgtttccttc tcaagttggag aagcattttcc cccctgaaata ggacgtgagtc
cctctcattg atgttgacac tttgagtttcc cagagagcgc cggctccagt cccctgaagcc
cgagggagag cacccagagc cagacagaca gaaagttgga tgtgcctcctta caggacacaca
gcagcagaca ggtggtacac agtgagacag caggcttttg ccaagcagac cccggaagct
cctgctgttgt gccaccctctag gaggacctct gcacccctcag gttgggtgatc actgtggttg
ttgccctctgg aagtttattgt tttatttattgct tgtggttcccc ttcccagaaa acatgagcac
caacccctcagc tgggtccggag gcaggtttta ataaagccct ggagttggcgt tccctctgca
ttgagacac

Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated A is indicated in red.

References

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J. 370, 953-962.


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