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<th><strong>Allele</strong></th>
<th><em>gimpy</em></th>
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</tr>
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<tr>
<td><strong>Gene</strong></td>
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<tr>
<td><strong>Gene Name</strong></td>
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<td><strong>Science Writers</strong></td>
<td>Eva Marie Y. Moresco</td>
</tr>
<tr>
<td><strong>Authors</strong></td>
<td>Karine Crozat, Bruce Beutler</td>
</tr>
<tr>
<td><strong>Illustrators</strong></td>
<td>Diantha La Vine, Katherine Timer</td>
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Phenotypic Description

The *gimpy* phenotype was identified as a limb/digit malformation defect on the *domino* background. *gimpy* mice display malformed and/or extra digits, and occasionally malformed limbs. These phenotypes segregated in Mendelian ratios separately from the *domino* phenotype. *gimpy* mice are sterile, but otherwise healthy.

Nature of Mutation

The *gimpy* mutation corresponds to a T to A transversion at position 1275 of the *Wnt7a* transcript on Chromosome 6, in exon 4 of 4 total exons.

1260 GTCAAGTGTAACACGTCAGCGACGCACGGAG
334  -V--K--C--N--T--C--S--E--R--T--E--

The mutated nucleotide is indicated in red lettering, and causes a cysteine to serine substitution of amino acid 339 in the Wnt7a protein.

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The Wnt7a gene encodes a 349-amino acid secreted protein growth factor of the Wnt family, whose members are defined by sequence rather than by functional properties (1,2). All Wnts contain an N-terminal signal sequence, and 23 or 24 cysteine residues distributed in a highly conserved manner across the length of the protein. Little is known about the structure of Wnts, as they have been notoriously difficult to solubilize due to their recently discovered palmitoylation on a conserved cysteine residue (3). This palmitoylation renders Wnt3 hydrophobic, and is essential for Wnt3 signaling ability. It has been speculated that palmitoylation helps target Wnts to the plasma membrane. Wnt proteins are glycosylated, although this modification does not appear essential for Wnt1 function (4).

The gimpy mutation results in a cysteine to serine change at position 339 of Wnt7a, in the 23rd of the 24 conserved cysteines. The cysteines have been hypothesized to participate in intramolecular disulfide bonds (2). Thus, the gimpy mutation may disrupt proper folding of Wnt7a. How Wnt7a expression is affected by the gimpy mutation is unknown.

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Wnts are expressed throughout embryonic development and in the adult, and several share similar temporal patterns of expression in mice (5). Wnt7a is expressed in the distal dorsal ectoderm and reproductive tract during embryogenesis (6;7), and in the lung and brain in adult mice (5). Brain expression is predominantly in the cerebellum (8) and olfactory bulb (GNF SymAtlas).

Wnts are secreted proteins: they have an amino-terminal signal sequence, are present in the secretory pathway and act non-cell autonomously (2). Active Wnts can be harvested from the medium of cultured cells (9;10). At the same time, they can be detected bound to the cell surface in close association with glycosaminoglycans (11;12). Studies of Drosophila Wingless (Wg, the prototypical Wnt), suggest that Wnts are endocytosed preferentially in cells anterior versus posterior to the Wg source (13), setting up a morphogenetic gradient that regulates epidermal patterning in the fly embryo (14).

Background

Wnts, found in invertebrates and vertebrates, regulate cell fate, proliferation, migration, polarity and death during embryonic development, and contribute to homeostasis and some cancers in adulthood. There are 19 WNT genes in humans and at least 18 Wnt genes in mice (2). Wnts have a well-studied role in vertebrate limb development, which proceeds through the stages of limb bud initiation, early limb patterning, and late limb morphogenesis (15;16). After limb bud formation, patterning depends on signals from the apical ectoderm ridge (AER), the zone of polarizing activity (ZPA), and the non-AER ectoderm. These signaling centers specify the proximal-distal, anterior-posterior and dorsal-ventral axes, respectively, and mediate their signaling in part through Wnt proteins (15).

Studies of Wnt7a mutant mice demonstrate that Wnt7a signaling regulates dorsal-ventral and anterior-posterior limb pattern formation. Wnt7a is expressed in the dorsal ectoderm (7), and mice lacking Wnt7a activity show dorsal-to-ventral cell fate transformations in the limb mesoderm, indicating that Wnt7a provides a dorsalizing signal (17). In particular, dermal thickenings representing footpads are found on the dorsal surface of Wnt7a-deficient limbs, and these thickenings also lose hair and acquire striations (17). Ventral tendons are duplicated dorsally in Wnt7a mutants, preventing flexion of the digits (17). Anterior-posterior patterning is affected in Wnt7a mutants, as demonstrated by a specific loss of posterior skeletal elements such as digit five and the ulna (17). Interestingly, these patterning defects appear to be restricted to the distal portion of the limb, suggesting that Wnt7a-independent mechanisms control more proximal dorsal-ventral specification.

In addition to having defective limb development, Wnt7a mutants are sterile due to abnormal oviduct and uterus development in females, and a failure of Müllerian ducts to regress in males (6). Postnatal uterine patterning of the female Wnt7a-deficient reproductive tract is abnormal, concomitant with loss of hoxa-10 and hoxa-11 expression (18). Wnt7a mutants display defects in cerebellar development (8). Cerebellar granule cells secrete Wnt7a and mossy fibers exhibit a delay in synapse formation on granule cells in Wnt7a-deficient
mice, suggesting that Wnt7a promotes synaptogenesis (8).

Figure 4. Wnt Signaling Pathways. Wnt glycoproteins are palmitolated by porcupine family proteins (Porcn) and secreted into the extracellular matrix with the assistance of the retromer complex. In the extracellular matrix, heparan sulfate proteoglycans bind to Wnt proteins, stabilizing them for binding to the seven transmembrane Frizzled receptor and coreceptor LRP5 or LRP6. Several Wnt inhibitors, including Dickkopf (Dkk), Wnt-inhibitor protein (WIF), soluble Frizzled-related proteins (SFRP), Cerberus, Frzb, and Wise, bind Wnts or their receptors directly and prevent Wnt from interacting with LRP5/6 and Frizzled. Canonical Wnt/?-catenin pathway: In the absence of Wnt, ?-catenin is constantly degraded. ?-catenin is phosphorylated by glycogen synthase kinase 3 (GSK3) and casein kinase 1? (CK1?) in a destruction complex that also contains adenomatous polyposis coli (APC) and Axin. Phosphorylation allows association with ?-TrCP, an E3 ubiquitin ligase subunit that targets ?-catenin for proteasome-mediated degradation. Thus, ?-catenin cannot travel to the nucleus and Wnt target genes are repressed by lymphoid enhancer-binding factor 1/T cell-specific transcription factor (LEF/TCF) proteins. Wnt binding to Frizzled and LRP5/6 results in recruitment of Dishevelled (Dsh) and Axin, and LRP5/6 phosphorylation by GSK3 and CK1?. Dsh

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is also phosphorylated by casein kinase 1/2 (CK1/2), metastasis associated kinase (MAK), protein kinase C (PKC), and Par1. These events disrupt the β-catenin destruction complex, thereby permitting the stabilization of β-catenin, which accumulates in the cell and translocates into the nucleus where it associates with and coactivates LEF/TCF to stimulate target gene expression. The two non-canonical Wnt signaling pathways control cell polarization and migration and do not require LRP5/6 nor act through β-catenin. In the Planar Cell Polarity Pathway, Wnt-activated signals arising from Frizzled recruit Dsh to the cell membrane. Dsh activates cytoskeletal regulatory pathways, either directly (for Rac) or through Dishevelled associated activator of morphogenesis 1 (Daam1) (for Rho and profilin). In addition to cytoskeletal regulation, Rac also controls transcription through activation of JNK. In the Wnt/Ca²⁺ Pathway, binding of Wnt to Frizzled recruits G-proteins that activate Dsh. Several molecules subsequently control Ca²⁺ release from the endoplasmic reticulum, including Protein Kinase G (PKG) that inhibits Ca²⁺ release, and phospholipase C (PLC) that stimulates Ca²⁺ release through the generation of IP₃. Diacylglycerol (DAG), also generated by PLC, together with Ca²⁺, activates protein kinase C (PKC), leading to control of tissue separation through Cdc42. Ventral fate is regulated by the Wnt/Ca²⁺ pathway through the action of calcineurin and nuclear factor of activated T cells (NFAT). The Wnt/Ca²⁺ pathway also inhibits the canonical Wnt pathway through calcium/calmodulin-dependent protein kinase II (CamKII), TAK1, and NEMO.

The best studied Wnt signaling pathway is the canonical Wnt/β-catenin pathway, which signals through stabilization of β-catenin to regulate transcription factors controlling gene expression (Figure 1) [reviewed in (1;2)]. The data support a role for the Wnt/β-catenin pathway in cell fate determination during development. This signaling cascade is initiated by binding of Wnt proteins to receptors of the Frizzled (Fzd) and LDL-receptor-related (LRP) protein families (for a mutation in Lrp5, please see r18), and may be modulated by cell surface proteoglycans that are hypothesized to cluster Wnts for presentation to receptors. Wnt signals are also modulated by secreted proteins that bind Wnts directly and prevent their binding to Fzd receptors. These include the Frizzled-related protein (FRP or FrzB), Wnt-inhibitory factor-1 (WIF-1), Cerberus-1 (Cer1), and Dickkopf (Dkk). Cytoplasmic β-catenin levels are normally kept low by continuous proteasome-mediated degradation, which is controlled by a multi-protein complex including glycogen synthase kinase-3 (GSK3), Adenomatous Polyposis Coli (APC) and Axin. APC facilitates phosphorylation of β-catenin by GSK3, which allows binding of an E3 ubiquitin ligase complex member and ubiquitination. When Wnt binds to Fzd or LRP, the scaffolding protein Dishevelled (Dsh) disrupts the GSK3/APC/Axin complex and prevents phosphorylation and ubiquitination of β-catenin, leading to stabilization of β-catenin in the cytoplasm and nucleus. Nuclear β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1/T cell-specific transcription factor (LEF/TCF) to control target gene expression.

At least two non-canonical Wnt signaling pathways exist, which function independently of β-catenin (Figure 1) [reviewed in (2)]. Activation of the “Wnt/Ca²⁺” pathway results in increased intracellular Ca²⁺ and stimulation of PKC and a heterotrimeric G protein. The biological function of this pathway is unknown. The “Wnt/polarity” pathway controls the polarity of cells through regulating cytoskeletal organization. The intracellular signaling members of this pathway are under investigation in Drosophila, and so far include...
Mutagenetix Phenotypic Mutation 'gimpy'

DFzd1 (*Drosophila* Fzd), Dsh, DrhoA, Drok (rho-associated kinase), Jun N-terminal kinase (JNK), myosin II, and myosin VIIA. Polarized cell movements during gastrulation and neurulation are thought to require the Wnt/polarity pathway.

Humans with Fuhrmann syndrome (OMIM #228930) have limb malformations characterized by limb aplasia/hypoplasia and joint dysplasia. The Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome (OMIM #276820) results in more severe limb truncation phenotypes. Homozygous mutations in *WNT7A* were found in patients with these syndromes, with loss-of-function mutations causing Fuhrmann syndrome and null mutations causing Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome (19).

**Putative Mechanism**

How *Wnt7a* regulates dorsal-ventral limb patterning involves at least two other transcription factors, the homeobox-containing *Engrailed (En)-1*, and the LIM-homeodomain transcription factor *Lmx1b*. *En-1* represses *Wnt7a* transcription in the ventral ectoderm, thus explaining dorsal ectoderm-specific *Wnt7a* expression (20). *Wnt7a* induces *Lmx1b* expression in the dorsal mesenchyme to specify dorsal mesodermal cell fate (21). However, factors other than *Wnt7a* regulate *Lmx1b*, as *Lmx1b* mutants display ventralization of features extending along the entire anterior-posterior axis, and to a more proximal level than observed in *Wnt7a* mutants (22).

It remains unclear whether *Wnt7a* signals through a canonical or non-canonical Wnt signaling pathway to regulate limb patterning. *Wnt7a* present in the dorsal ectoderm signals to the underlying mesoderm to maintain dorsal cell fate. Loss of ?-catenin in the limb results in dorsal and not ventral transformation, suggesting that *Wnt7a* may not utilize the canonical Wnt/?-catenin pathway (23). However, genetic interactions exist between *Wnt7a* and *Lrp6*, a canonical Wnt receptor (24), and *Wnt7a* mutant limb patterning phenotypes are alleviated by reducing *Dkk* expression in mice (25). These findings suggest instead that *Wnt7a* signals through the canonical Wnt/?-catenin pathway.
**Gimpy** 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. This protocol has not been tested.

**Primers for PCR amplification**

Gimpy(F): 5’- TCTGAAAGATCAAGAAGCCCCCTGTCC -3’
Gimpy(R): 5’- GCCGTAGAAAAACTTTGTTCCAGCCC -3’

**PCR program**

1) 94°C 2:00
2) 94°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**

Gimpy_seq(F): 5’- TCGAGAAGTCACCCAATTACTGTG -3’
Gimpy_seq(R): 5’- TCTGAGAGATGCTTCCCACG -3’

The following sequence of 557 nucleotides (from Genbank genomic region NC_000072 for linear DNA sequence of Wnt7a) is amplified:

```
45224  tctgaag atcaagaag
45241  ccctgtccta cgcagacgccc atggacacgtg acctgtgta tategagaag tcaccaattat
45301  acgtggaag ggacccagtg acagggcgacg tgggtaccca gggcggagcc tgcataaga
45361  cagccccccta ggcagggcgtg tgcagctcga tgcgtgagtg cgcgtgtcac aacacacacc
45421  atgtactggcc gggtgtggcag tgcaacggtc aattcctggt cggcttcaggt gtaaagtga
45481  acagctgagc cagagggcagc gagatgtata cgtgcaaggtg atggcgggtca caggcagat
45541  caccaggcctg atacacgttgc cctgcagggc actgtgctgga tgtcacagg gaagaacca
45601  cagaacacag gcttcggctt ttcgggtga ggggagggcgt gtttgtggtt ttctctcagag
45661  acctcccgagc cagagcgttggct cgcaggtggc gcgcacacgt tccacagggc atgtgtgtca
45721  gccacccctccagccagggc gcgcagctc ctgcagcggc ggaacaaggt ttttacagcgc
```

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

**References**

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