### Allele gus-gus

**Mutation Type** missense  
**Chromosome** 14  
**Coordinate** 103,820,013 bp (GRCm38)  
**Base Change (assembly)** C ? T  
**Gene** Ednrb  
**Gene Name** endothelin receptor type B  
**Synonym(s)** ETb, ETR-b, Sox10m1  
**Chromosomal Location** 103,814,625-103,844,402 bp (-)  
**Accession Number** NCBI RefSeq: NM_007904.4, NM_001276296.1, NM_001136061.2; MGI: 102720  
**Mapped** Yes  
**Amino Acid Change** Glycine changed to Aspartic acid  
**Institutional Source** Beutler Lab  
**Phenotypic Category** lethality-postnatal, pigmentation, skin/coat/nails  
**Penetrance**  
**Alleles Listed at MGI** All alleles(45) : Targeted(10) Spontaneous(3) Chemically induced(14) Radiation induced(20)  
**Mode of Inheritance** Autosomal Recessive  
**Local Stock** Live Mice, Sperm  
**Repository** MMRRC: 37048  
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**Phenotypic Description**

![Image of gus-gus mouse](image1.png)

**Figure 1. Phenotype of the gus-gus mouse.** The homozygous gus-gus mice have a piebald appearance.

The hypopigmentation phenotype of gus-gus mice was initially identified among N-ethyl-N-nitrosourea (ENU)-induced G3 animals; the gus-gus homozygous mice have a piebald appearance (*i.e.*, a variable black and white spotting pattern; **Figure 1**). Although they appear healthy at birth, the gus-gus mice die prematurely at or around weaning; cause of death has not been determined.
Whole exome HiSeq sequencing of the G1 grandsire identified 61 mutations, one of which affected Ednrb, a gene known to cause pied spotting when mutated (1). The Ednrb mutation is a G to A transition at base pair 103,820,013 (v38) on Chromosome 14, or base pair 24,161 in the GenBank genomic region NC_000080 encoding Ednrb. The mutation corresponds to residue 1,337 in the mRNA sequence NM_007904.4 within exon 6 of 7 total exons or residue 1,523 in the mRNA sequence NM_001136061.2 within exon 7 of 8 total exons.

Genomic sequence is shown; numbering corresponds to NC_000080. The mutated nucleotide is indicated in red. The mutation results in a glycine (G) to aspartic acid (D) substitution at amino acid 371 in both endothelin receptor type B (ETB) isoforms.

**Protein Prediction**
**Figure 2.** The topography and domain structure of ET₉R. (A) ET₉R is a G-protein coupled receptor with seven transmembrane (TM) domains. Shown are the locations of the signal peptide and ET-1-induced cleavages of the N-terminus. The post-translational modifications that occur on ET₉R are also shown: N-linked glycosylation at N60, disulfide bond formation between C174 and C255, palmitoylation at C402, C403, and C405, the 13 phosphorylation (Ps) sites, and the location of Gᵢ coupling. The location of the *gus-gus* mutation within the TM7 is marked by a red asterisk. (B) The domain structure of ET₉R. The *gus-gus* mutation is a glycine to aspartic acid substitution at residue 371 within TM7. Abbreviations: TM, transmembrane domain; SP, signal peptide; CT, C-terminus, Ps, phosphorylation.

*Ednrb* encodes endothelin receptor type B (ET₉R), a member of the endothelin (ET) receptor family of rhodopsin-like G protein-coupled receptors (GPCRs; see the record for *Bemr3*) (2-5). The rhodopsin-like GPCRs have seven helical transmembrane domains, three extracellular and three intracellular loops, an extracellular N-terminus, and a cytoplasmic C-terminus (Figure 2).
peptide comprised of the N-terminal 26 amino acids is cleaved after facilitating the translocation of ETßR across the ER membrane (6,7). At the cell surface, the N-terminus of the mature protein is further susceptible to ligand (ET-1)-induced cleavage between Arg65 and Ser66 by an unidentified extracellular soluble or membrane-bound metalloprotease (e.g., a member of a disintegrin and metalloprotease (ADAM) family (see the record for wavedx for information about Adam17) or matrix metalloprotease (MMP; see the record for cartoon for information about Mmp14)) (8-10). A mutant ETßR lacking the first 64 amino acids retained the functional properties of full-length ETßR when expressed in vitro, however, there was a 15-fold reduction in cell-surface expression (8). These data indicate that N-terminal proteolysis of ETßR regulates ETßR cell surface expression (8). A region containing 29 amino acids of the N-terminus adjacent to the first transmembrane domain (amino acids 74-101 in mouse) functions to stabilize the interaction between the endothelin ligand(s) and ETßR (11). Takasaka et al. propose that the 29 N-terminal amino acids may interact with another part of ETßR (e.g., the extracellular loops) to stabilize the receptor-ligand complex (11). For example, the binding of the ET ligand, ET-1, to ETßR is almost irreversible (11;12); when exposed to acid washes and 2% SDS, the receptor-ligand complex remains intact (13). The complex within living cells remains stable even when transported into late endosomal/lysosomal compartments (14).

The transmembrane domains (TM) and the intervening extracellular loops of ETßR regulate agonist selectivity and binding (15;16). The ligand binding domain of ETßR is Ile138-Ile197 within the second transmembrane domain (17).

The C-terminal tail of ETßR is essential for Gq protein coupling and the regulation of the stimulation of the epithelial sodium/hydrogen exchanger 3 (NHE3), a protein necessary for renal and intestinal sodium reabsorption (18;19). The amino acid sequence Ser-Cys-Leu-Cys within the C-terminal tail is conserved between ETßR and ETAßR, another member of the ET receptor family, and is proposed to be essential for signal transduction (18). The C-terminal tail of ETßR also directs the receptor’s intracellular trafficking route irrespective of ligand stimulation (20). ETßR is initially transported to the plasma membrane and then internalized to lysosomes where it undergoes proteolytic degradation (20). The C-terminal tail of ETßR mediates the interaction with ß-arrestin and subsequent internalization of the receptor, lysosomal sorting, and its degradation (20;21).

ETßR undergoes several posttranslational modifications: (i) Asn60 is within an N-linked glycosylation consensus sequence (Asn-X-Ser/Thr), however, this site is released upon the proteolytic processing of ETßR; no other glycosylation occurs on ETßR (3;6;22). (ii) Thirteen potential phosphorylation sites (most at the end of the C-terminus; aa 435-442) have been identified (15;23). ETßR phosphorylation is proposed to regulate receptor function as well as receptor desensitization by GPCR kinase or ß-arrestin-mediated phosphorylation (15;23). (iii) Three cysteines (Cys402, 403, and 405) within the C-terminal tail are potential palmitoylation sites (24). (iv) Cysteines 174 and 225 are proposed to cross-link extracellular loops one and two by a disulfide bond (25).


ETßR may form homodimers as well as heterodimers or higher order oligomers with ETAßR (15). Formation of the dimers or higher order oligomers is proposed to function in modifying ligand binding, receptor activation, desensitization and transmembrane signaling (15).
ET 

Background

There are two ET receptor subtypes expressed in mammals: ET_A R and ET_B R (4;5;31). A third ET receptor subtype, ET_C R, has been identified in Xenopus laevis; a mammalian homologue of ET_C R has not been identified (36;46). There are three ET ligands that associate with the ET receptors: ET-1, ET-2, and ET-3. Each of the ET receptors exhibit different affinities for the ET ligands: ET_A R has high affinity for ET-1 and ET-2, but low affinity for ET-3; ET_C R has higher affinity for ET-3 than for ET-1 and ET-2; while ET_B R exhibits equal affinity for all of the ET ligands (4;5;46).

The ET receptors couple to heterotrimeric G-proteins (ET_A R: G_i and G_q; ET_B R: G_i and G_q) to activate several signaling components including phospholipase D (27;47;48), phospholipase C (PLC; (49;50)), phospholipase A_2 (51), cytosolic calcium (52;53), Na^+/H^+ exchange (54), cGMP production (55), cAMP production (49), tyrosine kinases (56;57), and mitogen-activated protein kinases (MAPKs) (58;59). The ET-activated ET receptors mediate several physiological actions including, but not limited to, vasoconstriction (60), vasodilation (61), activation of DNA synthesis and mitogenesis (62;63), induction of Fos transcription (64), stimulation of phosphatidylinositol (PI) hydrolysis in cerebellar granule cell neurons (63;65), depolarization of spinal neurons (66), and stimulation of substance P release (67). Several ET_B R-associated functions are discussed in more detail below.

Expression/Localization

During embryonic development, Ednrb is expressed in the pre-migratory and migrating neural crest (NC) cells as well as less abundantly in the surrounding mesenchyme (29;30). Ednrb is expressed in human melanoma cell lines; melanoma metastases expressed higher levels of Ednrb when compared to Ednrb expression in primary tumor samples (31).

In mouse and rat, ET_B R is expressed in most tissues (22;32) including vascular endothelial cells and vascular smooth muscle cells (VSMCs) (8;33), the cochlea (34), tubular epithelial cells, renal vessels, inner medullary collecting duct (CD), outer medullary CD, cortical CD, and other nephron segments of the kidney (35-38), melanocytes (31), chorioid and retina (39;40) as well as the myenteric plexus, mucosal layer, ganglia, and blood vessels of the submucosa of the colon (41-43).

ET_B R is localized to the plasma membrane in most cells. However, in subfractionated cardiac membranes, ET_B R was localized predominantly to intracellular membranes (44). Confocal microscopy of ventricular myocytes determined that ET_B R was localized to the nuclear membrane (44). The nuclear ET_B R was able to bind ligands and stimulated an increase in nuclear cisternal calcium content (44). Within the mouse intestine, ET_B R is localized to the nuclei of mucosal epithelial cells (45).

The ET_B-SVR isoform mRNA is expressed in the lung, placenta, kidney, and skeletal muscle; ET_B-SVR mRNA was not expressed in smooth muscle or endothelial cells (18).

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6 of 19
(Generated on Aug 30, 2018)
ET$_B$R-associated functions

Development of NC-derived cell lineages

ET-associated signaling (ET-1/ET$_A$R and ET-3/ET$_B$R) is essential for NC cell proliferation, migration, differentiation, and transformation (68-70). The embryonic NC gives rise to pluripotent cells that migrate to different locations within the embryo during development (71). The NC cells subsequently differentiate into several cell types including adrenomedullary cells, craniofacial skeletal tissue, glia, and some neurons of the peripheral nervous system, enteric neurons and glia, and melanocytes of the skin, hair and inner ear [reviewed in (71)].

ET-3/ET$_B$R-associated signaling is required between embryonic day (E)10-E12.5 in the mouse for the survival and migration of enteric ganglion neurons and melanocytes derived from trunk/vagal NC cells (1;69;70;72-74). Other studies indicate that ET$_B$R signaling may also stimulate melanocyte proliferation in the epidermis (75). ET-3/ET$_B$R-associated signaling maintains enteric NC-derived cells (ENCCs) in a proliferative state (76;77), inhibits their differentiation (77;78), and is required for the normal migration of ENCCs (73;76).

Figure 3. Four signaling pathways are essential to melanocyte development. The Wnt/?-catenin, MC1R, Kit, and ET-3/ET$_B$R signaling pathways regulate the transcription of microphthalmia-associated transcription factor (Mitf) in NC-derived melanocyte precursors as well as regulate the phosphorylation of the melanocyte-specific MITF isoform (MITF-M). The Mitf promoter is regulated by the transcription factors PAX3, SOX10, Lef1/TCF, and CREB during melanocyte development. MITF-M regulates several target genes to mediate melanocyte survival (Bcl2 and Met), proliferation (e.g., Cdk2 and Tbx2), and differentiation (e.g., Tyr, Tyrp1, Slc45a2, Dct, Pmel, and Mc1r). In the Wnt signaling pathway, binding of the Wnt ligand to
a Frizzled/LRP-5/6 receptor complex leads to the activation of the cytosolic protein, Dishevelled. Dishevelled inhibits the ?-catenin degradation complex containing APC, Axin, and GSK3.

Stabilized hypophosphorylated ?-catenin subsequently interacts with TCF/Lef1 in the nucleus to activate transcription. In MC1R signaling, ? MSH activates MC1R, leading to GDP/GTP exchange on the G-protein. The GTP-bound G? subunit is released and activates adenylyl cyclase. Adenylyl cyclase catalyzes the production of cAMP, the activation of PKA and PKA-induced activation of the CREB family of transcription factors. The SCF/c-Kit signaling pathway modifies MITF post-translationally by phosphorylating Ser73 by the mitogen-activated protein kinase (Ras/Raf/MEK/ERK) pathway and Ser409 through RSK. RSK activation also results in CREB phosphorylation/activation. The ET-3/ETB R signaling pathway induces the phosphorylation of Ser298 on MITF through activation of the phospholipase C/PIP2/DAG/PKC pathway; the ET-3/ETB R merges with the ERK signaling pathway downstream of c-Kit. For a more comprehensive view of ETB R signaling please see Figure 4. See the text for more details about these signaling pathways. Abbreviations: PKC, protein kinase C; GSK, glycogen synthase kinase; APC, adenomatosis polyposis coli; cAMP, cyclic AMP; MITF, microphthalmia-associated transcription factor; MC1R, melanocortin 1 receptor; ? MSH, alpha melanocyte stimulating hormone; LRP, low-density lipoprotein receptor-related protein; MEK, mitogen-activated protein kinase kinase 1/2; H-Ras, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; c-Raf1, v-raf-1 murine leukemia viral oncogene homolog 1; RSK, ribosomal protein S6 kinase 90kDa; CREB, cAMP responsive element binding protein 1; PKA, protein kinase A; SCF, stem cell factor. Some protein structures are modeled after existing crystal structures: ?-catenin, PDB: 1I7W; MITF, PDB: 4ATH; PKC, PDB: 1CPK.
Figure 4. Overview of ET\textsubscript{B}R-associated signaling. ET-1/ET-3-mediated activation of ET\textsubscript{B}R can stimulate the dissociation of G\textsubscript{q}. G\textsubscript{q} activates PLC\textsubscript{\gamma}, leading to the hydrolysis of PtdIns(4,5)P\textsubscript{2} and the production of DAG and IP\textsubscript{3}. IP\textsubscript{3} leads to the cytosolic mobilization of Ca\textsuperscript{2+} from the endoplasmic reticulum. The cytosolic Ca\textsuperscript{2+} and DAG activate RAP-1A that, in turn, activates B-Raf. DAG also activates the PKC\textgamma/C-Raf pathway. B-Raf and C-Raf phosphorylate and activate MEK1/2, which phosphorylates ERK1/2. The ERK1/2 induces the activation of RSK, leading to the activation of CREB and ATF-1. CREB and ATF-1 induce cFos expression. Proliferation, cell migration, contraction, and vasoconstriction of vascular smooth muscle cells occur upon ERK1/2 stimulation. ET-1/ET-3-activated ET\textsubscript{B}R can also stimulate the dissociation of G\textsubscript{i}. The dissociated G\textsubscript{i} subsequently activates PI3K, which produces PtdIns(3,4,5)P\textsubscript{3} from PtdIns(4,5)P\textsubscript{2} (PIP\textsubscript{2}). PIP\textsubscript{2} recruits AKT, which phosphorylates eNOS, enhancing nitric oxide (NO) production. Enhanced NO production results in vascular relaxation of vascular endothelial cells. The activated G\textsubscript{i} subunit activates c-Src, which subsequently activates the SHC/Grb2/SOS/H-Ras/C-Raf pathway leading to contractility of vascular smooth muscle cells and proliferation. See text for more details. Abbreviations: ET-3, endothelin-3; ET\textsubscript{B}R, endothelin receptor type B; PI3K, phosphoinositide-3-kinase; AKT, v-akt murine thymoma viral oncogene homolog 1; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; DAG, diacylglycerol; IP\textsubscript{3}, inositol trisphosphate; B-Raf, v-raf murine sarcoma viral oncogene homolog 1; PKC\textgamma, protein kinase C epsilon; MEK, mitogen-activated protein kinase kinase 1; H-Ras, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; C-Raf, v-raf-1 murine leukemia viral oncogene homolog 1; RSK, ribosomal protein S6 kinase 90kDa; CREB, cAMP responsive element binding protein 1; ATF-1, activating transcription factor 1; cFos, cellular oncogene v-fos FBJ murine osteosarcoma viral oncogene homolog; ERK1/2, mitogen-activated protein kinases 3/1. Some protein structures are modeled after existing crystal structures: PLC\textgamma, PDB:3OHM; RAP-1A, PDB:1C1Y; B-Raf, PDB:1UWH; PKC\textgamma, PDB:1GMI; c-Src, PDB:1A07; C-Raf, PDB:1C1Y; SHC, PDB:1MIL; AKT, PDB:1H10; eNOS, PDB:1M9J.
Mutagenetix Phenotypic Mutation 'gus-gus'

Wnt/?-catenin pathway regulates the transcription of microphthalmia transcription factor (Mitf) in NC-derived melanocyte precursors by activating the TCF/LeF1 transcription factor; the transcription factors SOX10 (see the record for Dalmatian) and PAX3 also regulate the expression of melanocyte-specific Mitf [reviewed in (79)]. SOX10 is required for the survival and the maintenance of pluripotency of migrating NC progenitors (80) and also functions in melanocyte differentiation by regulating Tyrp2/Dct (81;82). MITF regulates several target genes to mediate melanocyte survival (Bcl2 and Met; (83;84)), proliferation (e.g., Cdk2 and Tbx2; (85; 86)), and differentiation [e.g., Tyr (see the record for ghost), Tyrl (see the record for chi), Scl45a2 (see the record for cardigan), Dct, Pmel, and McIr (see the record for deer); reviewed in (87)]. Melanocyte stimulating hormone (MSH)-mediated activation of the melanocortin 1 receptor (MC1R) elevates cyclic-AMP levels to subsequently activate MAP kinase pathways and increase Mitf and Sox10 promoter activities as well as activates the CREB family of transcription factors (88;89). ET-3 (or ET-1)/ET\(_{\beta}\)R signaling activates a phosphatidylinositol-calcium second messenger system following binding and activation of G proteins (Figure 4). The GTP-bound G\(_{\text{q}}\) subunit activates phospholipase C\(_{\gamma}\) (PLC\(_{\gamma}\) ), leading to the hydrolysis of Ptdins(4,5)P\(_2\) and the production of diacylglycerol (DAG) and inositol trisphosphate (IP\(_3\) ). IP\(_3\) leads to the mobilization of Ca\(^{2+}\) in the cytosol, while the DAG and cytosolic Ca\(^{2+}\) activate RAS-related protein-1a (RAP-1A), a member of the RAS oncogene family. Activated RAP-1A activates v-raf murine sarcoma viral oncogene homolog B1 (B-Raf). DAG also activates the PKC?/H-Ras and C-Raf1 pathway (91). B-Raf and/or C-Raf1 phosphorylate and activate MEK1 and MEK2, which subsequently phosphorylate ERK1/2 (91). ERK1/2 induces the activation of the p90 ribosomal S6 kinase (p90Rsk). The activation of the p90Rsk proteins leads to the phosphorylation of cAMP responsive element binding protein 1 (CREB1) and activating transcription factor 1 (ATF-1) (92;93). The transcriptional activity of ATF-1 and CREB1 induces cFos expression and subsequent cellular proliferation and migration as well as the induction of the Mitf promoter during melanocyte development. The Kit (see the record for Pretty2) and ET-3/ET\(_{\beta}\)R signaling pathways merge at the level of Ras activation and subsequently induce the phosphorylation of MITF in mature melanocytes. ET-3/ET\(_{\beta}\)R signaling pathways cooperate for the development of the enteric nervous system and melanocytic NC cell lineage (71). See the “Vasodilation” section for additional information about ET\(_{\beta}\)R signaling.

Using a NC cell-specific Ednrb knockout (NCC ETB KO) mouse model, Zaitoun et al. determined that ganglia are absent from the distal third of the NCC ETB KO colon, and are decreased in number and size in the proximal and distal mid-colon (74). In the ganglionated region of the NCC ETB KO colons, there was an increase in vasoactive intestinal polypeptide (VIP) and nitric oxide synthase (nNOS) with a concomitant decrease in choline acetyltransferase (ChAT) in comparison to the wild-type mouse; these molecules in the enteric ganglia regulate motility of the gut (74). The expression of nNOS, VIP, and ChAT were inversely related to neuronal density (74).

In ENCCs, the RET receptor tyrosine kinase is activated by the glial cell-line-derived neurotrophic factor (GDNF) and GDNF family receptor ?1 (GFR\(_{\text{1}}\)) complex [reviewed in (94)]. RET-associated signaling promotes the survival, proliferation, migration, and differentiation of enteric neurons through the activation of Akt, ERK and MAPK P38 (95;97; reviewed in (94)). In vitro and in vivo studies have demonstrated crosstalk between the RET and ET-3/ET\(_{\beta}\)R signaling pathways during enteric nervous system development, however the molecular bases of this crosstalk is unknown (76;98;99).

Vasoconstriction
ET-1-mediated activation of ET\(_{\alpha}\)R and/or ET\(_{\beta}\)R on vascular smooth muscle cells (VSMCs) induces vasoconstriction and VSMC proliferation (100). During vasoconstriction, ET-1/ET\(_{\beta}\)R and/or ET-1/ET\(_{\alpha}\)R stimulate the phosphorylation of ERK1/2 (Figure 4; (101)). ERK1/2 is a known regulator of cellular proliferation, differentiation, migration, and vasoconstriction (34;101).

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10 of 19
(Generated on Aug 30, 2018)
during hypertension-induced remodeling in conduit arteries (102). In an Ednrb knockout (Ednrb+/−) mouse with complete ligation of the right common carotid artery, vascular remodeling was accelerated while the levels of nitric oxide (NO; a component of the dilator response) release were decreased compared to wild-type mice (103). Murakoshi et al. propose that the loss of ETB R-mediated apoptosis of VSMCs leads to neointimal hyperplasia (i.e., thickening of the blood vessel) and vascular stenosis (i.e., abnormal narrowing of the blood vessels) in the proximal carotid artery of the Ednrb+/− mouse after ligation (103). They also suggest that a decrease in endothelium-derived relaxation factors and NO may enhance vascular remodeling in the KO mice after ligation (103).

The ET-1 ligand is elevated in patients with hypertension and is considered a key mediator of hypertension (60). ETB R mediates the clearance of circulating ET-1 by tightly binding the ligand and transporting it into lysosomal compartments (104-106). The endocytosed ET-1 subsequently negatively regulates ET-1 gene transcription (107). The decrease in circulating ET-1 also reduces the amount of ET-1 that can interact with ETAR, subsequently leading to a decrease in ETAR-mediated vascular contraction (106;108).

Vasodilation

ETB R-associated signaling can also induce vasodilation (33). Vascular endothelial cells (ECs) exclusively express ETB R, which mediates vasodilation upon ET-1 or ET-3-associated ETB R activation (109;110). Mechanistically, ET-1 or ET-3-induced ETB R activation promotes Gαs dissociation from the Gαs subunit of the heterotrimeric G-protein (Figure 4). The dissociated Gαs subsequently activates phosphatidylinositol 3 kinase (PI3K) to produce PtdIns(3,4,5)P3 (PI(3,4,5)P3). PI(3,4,5)P3 recruits and activates AKT, which subsequently phosphorylates eNOS, leading to increased NO production, the release of prostacyclin (a vasodilator and inhibitor of platelet aggregation), and vasodilation (103;111-113). In an EC-specific Ednrb knockout mouse (EC ETB KO), ETB R-mediated vasodilation was impaired and the plasma concentration of ET-1 was increased; other ETB R-mediated functions in non-ECs (i.e., enteric nervous system development and pigmentation) were not affected (114).

Regulation of natriuresis and diuresis

ETB R-associated signaling in the medullary CD, outer medullary CD, and cortical CD regulates systemic blood pressure (36). ET-1/ETB R-associated signaling reduces vasopressin-stimulated cAMP accumulation (115-117) and osmotic water permeability (118;119) in the CD. CD-specific knockout of Ednrb (CD ETB KO) resulted in hypertension on a normal-sodium diet, which was further exacerbated on a chronic high-sodium diet; the mice had no other gross morphological abnormalities (36). Upon acute sodium loading, the CD ETB KO mice exhibited impaired sodium excretion (36). CD-derived ET-1 functions through the activation of ETB R to promote natriuresis (excretion of sodium in the urine) and diuresis (urine production by the kidney) to subsequently lower blood pressure (120-123). Interestingly, CD-specific ET-1 knockout mice displayed more severe hypertension than CD ETB KO mice, suggesting that ET-1 may also signal in a paracrine manner to ETB R and ETAR outside the CD to regulate natriuresis and diuresis (36).

Inflammatory pain and cutaneous inflammation

Inflammatory pain, tissue swelling, and neutrophil infiltration are mediated by ET-1/ETB R-associated signaling (124). An Ednrb knockout mouse was treated with pharmacological agents phenylbenzoquinone (induces algesia, a sensitivity to inflammatory pain) and arachidonic acid (induces pruritus and cutaneous inflammation) (124). The Ednrb null mice did not display sensitivity to inflammatory pain when compared to wild-type mice; noninflammatory pain was equivalent between the null and wild-type mice (124). In addition, null and heterozygous mice exhibited reduced arachidonic acid-induced cutaneous inflammatory responses and neutrophil infiltration compared to wild-type mice (124).

Human diseases associated with ETB R defects
type 4A [WS4A; OMIM: #277580; (126)], and susceptibility to Hirschsprung disease 2 [HSCR2; OMIM: #600155; (10;126;127)]. Patients with ABCD syndrome exhibit albinism, retinal depigmentation, bilateral deafness, and aganglionosis of the large intestine together with total absence of neurocytes and nerve fibers in the small intestine (125). Patients with WS4A also exhibit pigment abnormalities of the hair, skin, and ears due to a lack of melanocytes, and congenital sensorineural hearing loss due to a lack of melanocytes in the cochlea, as well as Hirschsprung disease, a congenital absence of ganglion cells distal portion of the gastrointestinal tract (10;126-128).

A link between ET<sub>B</sub>R-associated signaling and melanoma has been described [reviewed in (92)]. Increased EDNRB expression has been detected in melanoma metastases (compared to primary tumors) (129). EDNRB mutations that impair ET<sub>B</sub>R function have been detected in melanoma patients (130).

**Putative Mechanism**

Mice homozygous for targeted as well as naturally occurring Ednrb null mutations (e.g., MGI:1856148, MGI:1856149, MGI:1857161, and MGI:3795226) exhibit a piebald appearance due to the absence of NC-derived melanocytes in the epidermis (1;73;114;128;131-133). Ednrb null mice also exhibit an absence of choroidal melanocytes; neuroectoderm-derived pigment epithelium melanocytes develop normally (1). Ednrb null mice display early postnatal lethality [from ~postnatal day 15 to up to seven weeks after birth; (1;114;128;133)], distended and aganglionic colon [i.e., megacolon; (1;73;128;133;134)], reduced inflammatory response to topical application of arachidonic acid (124), and deafness due to a loss of strial intermediate cells and degeneration of the organ of Corti (128). A naturally occurring deletion in ET<sub>B</sub>R occurs in the spotting lethal (sl) rat model (135). Similar to the mouse models described above, homozygous sl rats exhibit abnormal epidermal melanocyte and enteric nervous system development as well as premature postnatal death following intestinal aganglionosis and obstruction (135). Homozygous sl rats that express a dopamine-?-hydroxylase-ET<sub>B</sub>R transgene (ET<sub>B</sub>R is expressed in the adrenal glands and other adrenergic neurons) survive to adulthood and exhibit normal enteric development (121;136). The sl rats that express the ET<sub>B</sub>R transgene are deficient in ET<sub>B</sub>R expression in the kidney, vascular endothelium, and vascular smooth muscle; they did not exhibit vasoconstriction, vasodilation, or diuretic action (135).

Similar to spontaneous and targeted Ednrb knockout mouse models, the gus-gus mice exhibit a piebald appearance and early postnatal lethality. The Ednrb knockout mouse models have established that the observed pigmentation defects are due to the absence of NC-derived melanocytes in the hair bulbs of non-pigmented areas (1;73;114;128;131-133). Other phenotypes associated with Ednrb deficiency have not been examined in the gus-gus mouse.
Gus-gus 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.

PCR Primers
Gus-gus(F): 5’- CCCATCACATATATCGCAAGGCAGG -3’
Gus-gus(R): 5’- CCTCCCTCGCTTCCAAATGAGAATC -3’

Sequencing Primer
Gus-gus_seq(F): 5’- GGGAAAATTAGCAATCTTCCAGCTC -3’

PCR program
1) 94°C 2:00
2) 94°C 0:30
3) 55°C 0:30
4) 72°C 1:00
5) repeat steps (2-4) 40X
6) 72°C 10:00
7) 4°C

The following sequence of 517 nucleotides is amplified (Chr.14: 103819795-103820311, GRCm38):

```
1  cccatcaca atatcgcaga gcagggaaaaa ttagcaatct tccagcttc tctagatagc
61  aatattgtgt tgttttttaa agcctatatt tgtgtcagag tgtcgaggaa cattttatat
121  tagaagagtt tcttacctta aagcagtttt tgtaagctttt cctcaccaaa aatgagcga
ttgatttgg cagaggatcc aagaagccca tgttgata cc aatgtagtcc aaaaaccaac
241  aaaaaagtcga aagaaaccaca agttgtcccc aaagattacca tccctcccaag tgtgtatatta
tagctcaccct ttgtgttttt atgaagaaag cccaaatcacc tctacagc aaatatatat
301  taccctgatga ttgctcaagct gagttgcctg tgtgtctctc tgcgtgcctg tctctctc
tttctctct ctgagctggt gcagccctct cactcccttc cccaccaatat gcagcatttc
481  ttgcaatatgatagc
```

Primer binding sites are underlined and the sequencing primer is highlighted; the mutated nucleotide is shown in red text (C>T, Chr. + strand; G>A, sense strand).

References
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16 of 19
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18 of 19
(Generated on Aug 30, 2018)


