Different Requirements for GFRα2-Signaling in Three Populations of Cutaneous Sensory Neurons

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Abstract

Many primary sensory neurons in mouse dorsal root ganglia (DRG) express one or several GFRα2’s, the ligand-binding receptors of the GDFN family, and their common signaling receptor Ret. GFRα2, the principal receptor for neurturin, is expressed in most of the small nonpeptidergic DRG neurons, but also in some large DRG neurons that start to express Ret earlier. Previously, GFRα2 has been shown to be crucial for the soma size of small nonpeptidergic nociceptors and for their target innervation of glabrous epidermis. However, little is known about this receptor in other Ret-expressing DRG neuron populations. Here we have investigated two populations of Ret-positive low-threshold mechanoreceptors that innervate different types of hair follicles on mouse back skin: the small C-LTMRs and the large Aβ-LTMRs. Using GFRα2-KO mice and immunohistochemistry we found that, similar to the nonpeptidergic nociceptors, GFRα2 controls the cell size but not the survival of both C-LTMRs and Aβ-LTMRs. In contrast to the nonpeptidergic neurons, GFRα2 is not required for the target innervation of C-LTMRs and Aβ-LTMRs in the back skin. These results suggest that different factors drive target innervation in these three populations of neurons. In addition, the observation that the large Ret-positive DRG neurons lack GFRα2 immunoreactivity in mature animals suggests that these neurons switch their GFRα signaling pathways during postnatal development.

Introduction

The primary somatosensory neurons responsible for conducting different modalities of information from the external world are located outside the CNS in the (spinal) dorsal root ganglia (DRG) and (cranial) trigeminal ganglia. In adult mice, most DRG neurons express the receptor tyrosine kinase Ret, the main signaling receptor for the GDNF family of neurotrophic factors [1]. This family consists of four ligands: GDNF, neurturin (NRTN), artemin, and persephin [2]. They signal through Ret via cognate receptors, three of which (GFRα1 through GFRα3) are expressed in partially overlapping sensory neuron populations [2]. Of these receptors, GFRα2, the principal receptor for NRTN [3,4], is the most widely expressed in DRGs [5]. Most Ret+/GFRα2+ DRG neurons are small, nonpeptidergic primary sensory neurons that extend unmyelinated C-fibers to their targets [1,5–7]. For the most part, these neurons consist of polymodal nociceptors that bind the plant isolectin B4 (IB4) and are known to switch from TrkA to Ret dependency during late embryonic and early postnatal development [1,5–8]. Additionally, a few other populations of small nonpeptidergic Ret+ DRG populations have been described [9–12]. In contrast to the nonpeptidergic neurons, a population of large Ret+/GFRα2+ DRG neurons starts to express Ret earlier during development [1,5,13–16]. These “early-Ret” neurons have been shown to constitute a group of myelinated, rapidly adapting (RA) low threshold mechanoreceptors (Aβ-LTMRs) [15–17].

GFRα2/Ret-signaling is known to provide essential trophic signals for proper soma size and epidermal innervation in the IB4-binding nonpeptidergic neurons [5,7,18,19]. However, the importance of GFRα2 mediated signaling in the other nonpeptidergic and in the early-Ret populations of neurons remains poorly known. Here, we have investigated the role of GFRα2-signaling in three different populations of Ret+/GFRα2+ DRG neurons that innervate the hairy skin: (1) small Mas-related G-protein-coupled receptor D expressing (MrgD+) polymodal nociceptors [9], (2) small tyrosine hydroxylase positive (TH+) nonpeptidergic C- low threshold mechanoreceptors (TH+/C-LTMRs) [11,12], and (3) large myelinated Ret+ neurons (putative Aβ-LTMRs) [15,16]. We found that GFRα2 is crucial for normal cell size in all the three neuronal populations. Surprisingly, although GFRα2-signaling is required for innervation of hairy skin epidermis by the MrgD+ neurons, our results show that it is dispensable for the innervation of hair follicles by the two types of low threshold mechanoreceptors.

Materials and Methods

Ethical Statement

The use of animals in this study was in accordance with the relevant EU directive and Finnish legislation (Directive 2010/63/
Animals, Tissue Processing and Immunohistochemistry

Adult GFRα2-knockout (KO) [20] and wild type (WT) littermate mice of both sexes in C57BL/6JOlaHsd background were used in this study. In addition, some newborn (P0) and adult MrgprdΔEGFP [9] and GFRα2-KO; MrgprdΔEGFP animals mice were used.

Mice were anesthetized with an overdose of pentobarbital (200 mg/kg, i.p.; Mebutam Vet, Orion, Finland) and transcardially perfused with 10 ml of cold PBS (pH 7.4), followed by 30 ml of 4% paraformaldehyde (PFA). Lumbar (L4, L5) and thoracic (T5–T8) dorsal root ganglia and sheets of back and hind paw hairy skin were dissected and post-fixed in 4% PFA for 1 hour. Newborn mice were killed by rapid decapitation, and tissue blocks containing the spinal cord and DRGs were immersion-fixed in 4% paraformaldehyde (PFA). For immunohistochemistry, the DRG sections and free-floating skin sections were blocked in 5% normal donkey serum (NDS) and incubated overnight in a primary antibody solution at room temperature. This was followed by another PBS washing step. The primary antibodies used were against GFRα2, Ret, tyrosine hydroxylase, neurofilament heavy chain (NFH), TbkB, GFP and PGP 9.5 (for more specific information, see Table S1). Donkey secondary antibodies were used in the investigation of DRG neurons and innervation of the glabrous epidermis [7].

Statistics

Differences between genotypes were compared by using the Mann-Whitney U-test (two-tailed) for average neuron numbers, and average innervated hair follicles. Neuronal size distributions were analyzed using the chi-square test. The statistical analyses were done using R (www.r-project.org). In the case of neuronal size distributions, some bins were merged to reach the required frequency of five or more in at least 80% of the bins. The threshold for statistical significance was set at p<0.05. The results are presented as mean ± SEM.

Results

Reduced MrgD+ Neuron Size and Deficient Epidermal Innervation in GFRα2-KO Hairy Skin

GFRα2-signaling is crucial for proper IB4+ nonpeptidergic neuron size and innervation of the glabrous epidermis [7]. To investigate if this phenotype extends to hairy skin, we used mice in which the Mrgprd gene locus had been targeted with an EGFP expressing construct [9]. This gene encodes MrgD, a Mas-related G-protein coupled receptor specifically expressed in nonpeptidergic nociceptive neurons that represent a great majority (75%) of the IB4+ DRG neurons [9]. As expected, nearly all (>97%) EGFP-positive (+ MrgD+) neurons were positive for GFRα2 in wild-type (WT) DRGs (Fig. 1A–D). We also analyzed the size distribution of these cells and found that the MrgD+ neurons were drastically smaller in KO mice than in WT animals (Fig. 1E, F, G).

Next, we studied the innervation density of MrgD+ fibers in the back and hind paw hairy skin. MrgD+ fibers were abundant in WT back skin epidermis and slightly sparser in the dorsal hind paw (Fig. 1H, J, L). Compatible with the phenotype found on GFRα2-KO glabrous epidermis [7], the KO animals showed a reduced density (30–70% loss) of MrgD+ innervation on both back and dorsal paw epidermis (Fig. 1I, K, L). A qualitatively similar
difference in back skin innervation was also observed using the pan-neuronal PGP9.5 immunostaining in additional WT and KO animals (Fig. S2).

**TH⁺** and Ret⁺/NFH⁺ Neurons Survive But Are Smaller in GFRα2-KO Thoracic DRGs

A distinct class of C-LTMRs that expresses vesicular glutamate transporter VGluT3 [11] and tyrosine hydroxylase (TH) [12] constitutes a subpopulation of nonpeptidergic C-fibers. This population of neurons has also been reported to express GFRα2 and Ret, but not to bind IB4 or express any of the Mas-related G-protein receptors, making them a separate population from the nonpeptidergic nociceptors [12]. Using immunohistochemistry and two different antibodies, we confirmed that a significant population of thoracic DRG neurons expresses TH in wild-type mice (Fig. 2A, E and Fig. S4). Co-staining showed that nearly all of the TH⁺ DRG neurons expressed GFRα2 (Fig. 2B–D). Vice versa, the TH⁺ neurons totaled to a roughly one third of all GFRα2⁺ neurons (Fig. 2D). To investigate if GFRα2 is required for TH expression or survival of these DRG neurons, we quantified the numbers of TH⁺ neurons in thoracic DRGs of both wild-type and GFRα2-KO animals. Firstly, intense TH⁺ immunoreactivity was found on a subpopulation of neurons in KO DRGs, indicating that GFRα2-signaling is not a requirement for TH expression (Fig. 2F). Secondly, no significant differences were found in the number estimates of TH⁺ neurons per DRG between the genotypes, suggesting that GFRα2 is not required for TH⁺ neuron survival (Fig. 2G). We then performed a cell size distribution

![Figure 1. MrgD⁺ DRG neurons in GFRα2-KO mice are smaller and deficient in epidermal innervation of hairy skin.](image_url)
analysis, which showed that the TH+ neurons were significantly smaller in the GFRalpha2-KO animals (Fig. 2H).

In contrast to the small nonpeptidergic nociceptors and mechanoreceptors that switch from NGF- to Ret-dependency during late prenatal and early postnatal life, the large early-Ret population of DRG neurons expresses Ret and GFRalpha2 from early embryonic stages [15,16]. In accordance with this, we found GFRalpha2-immunoreactivity only in few large DRG neurons at P0 (Fig. S3A). These neurons were clearly a separate population from the MrgD+ neurons that make up the bulk of nonpeptidergic DRG neurons (Fig. S3B, C). Surprisingly, we were unable to detect GFRalpha2-immunoreactivity in adult DRG NFH+ neurons (Fig. S3D-F). Since the early-Ret neurons express NFH in addition to Ret [15,16], we used antibodies against these two markers to investigate this neuronal population in thoracic DRGs. Most Ret+ neurons in adult mouse thoracic DRGs are small and NFH-negative (Fig. 3A, D; arrowheads), and many large NFH+ neurons are negative for Ret (Fig. 3B, E; arrowheads). In addition, we observed a population of large Ret+/NFH+ cells in both WT and GFRalpha2-KO mice (Fig. 3C, F; double arrowheads). Quantification of the numbers of these double-positive neurons revealed no significant differences between the genotypes (Fig. 3G), but the cell size distributions indicated that the average size of these neurons was clearly smaller in the KO than in WT animals (Fig. 3H).

Figure 2. TH+ DRG neurons in GFRalpha2-KO mice are smaller. (A–C) TH and GFRalpha2 colocalize in a subpopulation of thoracic DRG neurons. (D) Nearly all TH+ neurons express GFRalpha2 (red bar). TH+ neurons comprise ~37% of all GFRalpha2 neurons (green bar). Analysis was done from three animals and three ganglia in total. (E–H) The number of TH+ neurons in thoracic DRGs is similar between the genotypes (G: WT 533 ± 59, KO 555 ± 24; positive neurons per DRG, n = 3 animals/genotype, U-test) but the size is smaller in the knock-out animals (H: WT 196 ± 14 μm2, KO 120 ± 8 μm2; three animals and 200 cells/genotype in total, chi-square test). Scale bar: 50 μm.

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GFRα2-Mice Have a Normal Pattern of Hair Follicle Innervation

To see if the diminished sizes of TH+ and Ret+/NFH+ neurons in GFRα2-KO mice reflected in changes of their peripheral nerve processes, we studied their end-organ innervation patterns in the hairy skin. The TH+/C-LTMRs have been shown to exclusively innervate the smaller types of hair follicles on mouse skin, forming LLEs around the outer root sheath [12,22]. These studies have used mice with a genetic TH-reporter to visualize the C-LTMR innervation. Since the cell bodies of TH+/C-LTMRs were readily visible with TH-antibodies, we examined if also the sensory TH+ fibers could be visualized by immunohistochemistry. The LLE complex is located between the origin of the arrector pili muscle on the hair follicle and the sebaceous gland associated with the follicle [23]. We used these landmarks to localize possible TH+ sensory endings on hair follicles. Indeed, TH+ LLEs were visible around the smaller hair follicles, but with only one of the two antibodies (rabbit anti-TH) (Fig. 4A). Because these follicles are also innervated by LLEs from another type of LTMRs, the Aδ-LTMRs, we used TrkB (a marker for the Aδ-LTMRs) [12,17] as a control for the specificity of our TH-antibody. Double-staining against TH and TrkB showed that these two markers were expressed in two separate populations of LLEs, demonstrating that our TH-antibody stains specifically C-LTMRs on the hair follicles (Fig. 4A–C).

Figure 3. Ret+/NFH+ DRG neurons in GFRα2-KO mice are also smaller. (A–F) Ret and NFH colocalize in a population of thoracic DRG neurons in both wild-type and GFRα2-KO mice. Single arrowheads in (A, B, D, E) point to examples of single-positive neurons. Double arrowheads in (C, F) point to examples of double positive neurons. (G) GFRα2-KO thoracic DRGs have normal numbers of Ret+/NFH+ neurons (WT 124±21 (n = 4 animals), KO 149±26 (n = 3 animals); double-positive neurons per ganglion, U-test). (H) The size distribution of Ret+/NFH+ thoracic DRG neurons has shifted toward smaller sizes in KO animals (WT 757±21, KO 557±12 μm2; three animals/group, 250 cells per genotype, chi-square -test). Scale bar: 50 μm. doi:10.1371/journal.pone.0104764.g003
Figure 4. C- and Aδ-LTMR innervation of hair follicles in GFRα2-KO mouse back skin remains unchanged. (A–C) The images show two small caliber hair follicles with TH+ and TrkB+ LLEs. Immunoreactivity for the markers is localized in different populations of endings (C). The asterisks in (B) mark the two separate hair shafts. (D, E) The images demonstrate TH+ sensory innervation around small caliber hair follicles in wild-type (D) and KO (E) back skin. The arrowheads indicate obliquely cut hair follicles with TH+ LLEs. The small arrows mark hair follicles with few or no visible endings. Sebaceous glands (sg) around the follicles are indicated. No changes are obvious in the morphology of TH+ LLEs around GFRα2-KO hair follicles. (I, left side) The percentage of small hair follicles that are innervated by TH+ LLEs is similar between the genotypes (WT 87.8±6.4%, KO 90.6±1.1%; three animals/genotype, 60–80 small hair follicles/animal, U-test). (F, G) The morphology of NFH+ LLEs (arrowheads) appears similar between the genotypes. Double arrowheads point to nerve structures innervating the touch dome (located outside of the image). (I, right side) NFH+ LLEs remain in KO back skin (WT 88.7±0.8%, KO 81.6±3.6%; innervated follicles/total follicles, three animals/genotype, 60–80 small hair follicles/animal, U-test). Scale bars: 10 μm.

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Due to the similar phenotypic changes in TH⁺ and MrgD⁺ neuron somas in GFRα2-KO animals, we hypothesized that the density of TH⁺/C-LTMR innervation around hair follicles could also be reduced in the KO mice. Surprisingly, we found an apparently similar pattern and morphology of TH⁺/C-LTMR innervation in WT and KO skin (Figure 4 D, E). A thorough quantification indicated no difference in the proportion of hair follicles innervated by TH⁺ fibers between the genotypes (Fig. 4H, left).

Lastly, we proceeded to investigate hairy skin innervation by the Ret⁺/NFH⁺ DRG neurons. In the back skin the RA Aβ-LTMRs form LLEs around the larger awl/auchene and guard types of hair follicles [15]. In concurrence with this, we observed NFH⁺ longitudinal and circular endings around most large hair follicles in WT back skin. In the smaller hair follicles, only circular NFH⁺ endings were visible (not shown). A previous study has suggested that these RA Aβ-LTMRs are critically dependent on Ret-signaling for target innervation [16]. As these neurons express GFRα2 during development, we expected to see a lacking phenotype in the GFRα2-KO mice. However, NFH⁺ endings were present around large hair follicles in the GFRα2-KO mice (Fig. 4G), and quantification of the percentage of large hair follicles with NFH⁺ LLEs indicated no difference between the WT and GFRα2-KO animals (Fig. 4H, right). Also, the morphology of NFH⁺ LLEs and circular endings appeared similar between the genotypes (Fig. 4F, G).

Discussion

The key finding of the present study is that GFRα2 is required for proper size of both TH⁺/C-LTMR and RA Aβ-LTMR neurons, but is expendable for their target innervation of hair follicles, in contrast to the MrgD⁺ neurons that depend on GFRα2 for both cell size and target innervation of the epidermis.

MrgD⁺ and TH⁺/C-LTMRs Require GFRα2-Signaling for Size But Not for Survival

We found that both MrgD⁺ and TH⁺ DRG neurons are clearly undersized in the GFRα2-KO animals. These results support the previous conclusion that GFRα2-signaling is an important factor in controlling the soma size of nonpeptidergic sensory neurons; over 80% of IB4⁺ DRG neurons express GFRα2, and these cells are visibly smaller in GFRα2-KO mice [7]. This phenotype of diminished cell size has also been shown in peripherin expressing DRG neurons [5], Ret⁺ nociceptors [19] and Ret⁺/IB4⁺ and Ret⁺/IB4⁻ neurons [18] in different Ret conditional knock-out (cKO) mouse models. The importance of neurotphin on neuron or neurturin has not been reported. In addition to GFRα2 signaling, many other repulsive or attractive molecules influence the innervation patterns of nerve fibers [27]. For example, Nogo receptor 2 [NgR2] [28] is expressed on the IB4-binding nonpeptidergic neurons and regulates their epidermal innervation by interacting with the repulsive proteoglycan, versican [29]. Also, neurturin has been shown to prevent axon growth cone collapse caused by another repulsive molecule, semaphorin 3A [30]. These findings suggest that the axon guidance effects of neurotrophic factors could be partially dependent on inhibiting repulsive cues. Since the MrgD⁺ neurons terminate as free nerve endings in the epidermis, whereas the TH⁺/C-LTMR endings terminate just below the epidermis, “anti-repulsive” signaling through GFRα2 is one possible explanation for the different dependence of these endings on GFRα2.

Anti-TH Antibodies as C-LTMR Markers

We have used here antibodies against TH as a marker for the C-LTMR neurons and their target innervation around hair follicles, whereas the previous studies used genetic labeling of TH⁺ or VGlut3-expressing neurons to visualize the C-LTMR endings in hairy skin [12,31]. In our hands, two different anti-TH antibodies labeled an identical subpopulation of DRG neurons (Fig. S4) but only one of them, namely the rabbit anti-TH antibody (Table S1), labeled the LLEs (although both antibodies labeled sympathetic nerve fibers) in the skin. The reason for this difference is presently unclear, but this antibody should be a valuable tool for further studies on these neurons in different species.

Large Ret⁺ Neurons Require GFRα2 for Proper Soma Size Development But Downregulate GFRα2-Expression after Adolescence

Our results show that Ret⁺/NFH⁺ DRG neurons have diminished in size in GFRα2-KO mice. This is in line with the observation that myelinated axons in the saphenous nerves are thinner in GFRα2-KO than in WT mice [25]. Furthermore, in NRTN-overexpressing mice, the largest myelinated fibers in the saphenous nerves are selectively hypertrophied, and these fibers likely correspond to the Ret⁺/NFH⁺ neurons [24]. Collectively these findings indicate that NRTN/GFRα2-signaling plays a significant role in the regulation of cell body and axon size in the Ret⁺ myelinated DRG neurons.

Ret mRNA expression in the mouse DRG starts already during the second week of embryonic development [32]. These large, early-Ret neurons express NFH from P0 onwards [16] and have been shown to express GFRα2 in the mouse [15,16,26] at least until P14 [15]. Consistent with this, we saw GFRα2⁺ immunoreactivity at P0 only in large DRG neurons that did not express the nonpeptidergic marker MrgD, in agreement with the view that GFRα2 is expressed only in the early-Ret neurons at this point in

TH⁺/C-LTMRs Do Not Require GFRα2 for Target Innervation

We found that the density of MrgD⁺ fibers is reduced in GFRα2-KO back skin epidermis, consistent with the scarcity of nonpeptidergic innervation in the glabrous skin of these mice [7] and in both glabrous and hairy skin of Ret cKO mice [3,18]. In striking contrast, the percentage of TH⁺ innervated hair follicles and the morphology of TH⁺ LLEs in the GFRα2-KO mice were similar with WT mice, indicating that target innervation by TH⁺/C-LTMRs is independent of GFRα2-signaling. In adult mice, TH⁺/C-LTMRs do not express other Ret co-receptors in addition to GFRα2 [3], but whether the formation of these endings requires Ret or neurturin has not been reported. In addition to neurotrophic factors, many other repulsive or attractive molecules influence the innervation patterns of nerve fibers [27].
development [15]. Interestingly, we saw no immunoreactivity against GFRα2 in the large NFH+/DRG neurons in adults, suggesting that GFRα2 is downregulated in the Ret+/NFH+ neurons after P14 in mice. This also suggests that the large, early-Ret neurons depend on GFRα2 for their size during development but not in adults.

Our observation that the large Ret+/NFH+ neurons survive in GFRα2-KO supports the earlier finding that the number of myelinated axons in the saphenous nerve of these mice is normal [25]. Furthermore, both in neurturin-KO and in another GFRα2-KO mouse, the large Ret+ DRG neurons appear intact at P0 [15]. However, the dependency of these large neurons on Ret-signaling for survival is somewhat unclear. Normal numbers of DRG neurons were reported in one Ret cKO mouse line at P14 [5], but in another Ret cKO mouse model, the number of NFH+ DRG neurons was reported to be reduced by 15% at P15.5 [16]. Also, in a RetGFP/KO mouse line, about one-third of GFP+ DRG neurons were reported lost at E14.5 [26]. Consequently, it remains possible that Ret is required for the survival of a subset of myelinated Ret+ DRG neurons.

Formation of Aβ-LTMR Longitudinal Lanceolate Endings Is Independent of GFRα2-Signaling

Our finding that NFH+ LLEs are normal in GFRα2-KO mice suggests that signaling mediated through GFRα2 is not essential for target innervation by this subpopulation of RA Aβ-LTMR neurons. On the other hand, Pacinian corpuscles, the end organs of these two RA Aβ-LTMR neurons, were reported to be almost completely lost in NRTN-KO mice and in another GFRα2-deficient mouse line [15]. Furthermore, the formation of these two RA Aβ-LTMR end organ types appears to be critically dependent on Ret-signaling, as NFH+ LLEs [16] and Pacinian corpuscles [15] are compromised in different Ret cKO models. These results imply that subpopulations of early-Ret neurons differ in their GFRα2 requirements for target innervation. In addition to our finding that GFRα2-immunoreactivity is not detectable in the large NFH+/Ret+ neurons, other studies on both mice and rats have indicated that GFRα1 but not GFRα2 (or GFRα3) is expressed in the largest Ret+ DRG neurons in adult animals [33,34]. Together, these results suggest that the early-Ret neurons might switch from GFRα2 to GFRα1 signaling during postnatal maturation.

Conclusions

We show that GFRα2 is critical for controlling the neuron size (but not survival) of three different subpopulations of DRG neurons in mice: small MrGD+ nociceptors, small TH+ C-LTMRs and large (Ret+/NFH+) Aβ-LTMRs. However, in contrast to the MrGD+ neurons that require GFRα2 to innervate the epidermis, GFRα2 is not required for the innervation of hair follicles by the two populations of LTMRs. Thus, factors other than GFRα2 drive target innervation in these neurons.

Supporting Information

Figure S1 Bright-field images from wild-type mouse back skin. (A) The image shows several small caliber hair follicles representative of the type used in the analysis of TH+ LLE innervation. The black arrowhead points to a sebaceous gland next to a hair follicle. Note that the small hair follicles generally form closely associated pairs (asterisks). (B) The image shows several small and one large caliber hair follicle. The large hair follicle (asterisk) is representative of the type used in NFH+ LLE innervation analysis. Note the considerably larger diameter and singular hair shaft (yellow arrows) in the large follicle compared to a small hair follicle (green arrows). Scale bars: 100 μm.

Figure S2 Epidermal innervation in GFRα2-KO mouse back skin is reduced. (A) WT mouse back skin epidermis is densely innervated by PGP9.3+ nerve fibers. (B) Epidermal innervation is sparse in KO back skin epidermis. Arrowheads in (A) and (B) exemplify the difference in innervation density between the genotypes. Asterisks mark large caliber hair shafts. The images are maximum projections from conventional microscopic stacks. Scale bar: 25 μm.

Figure S3 GFRα2 is not detectable in large NFH+ DRG neurons in adult mice. (A–C) Conventional microscopic images demonstrating that GFRα2 expression is restricted to a minor population of large DRG neurons at P0 (A), while MrGD is expressed in a major population of smaller neurons (B). (C) GFRα2 and MrGD do not colocalize in newborn mouse DRGs (arrowheads point to single-positive GFRα2+ neurons). (D–F) Maximal projection images showing that GFRα2 is abundantly expressed in small to medium diameter neurons in adult mouse thoracic DRGs (D) and that NFH is expressed in many medium to large sized DRG neurons (E). (F) GFRα2 and NFH do not colocalize in adult mouse DRGs. Scale bars: 50 μm.

Figure S4 Two different anti-TH antibodies stain the same population of DRG neurons. (A, B) Representative images from a WT mouse thoracic DRG section stained with (A) rabbit anti-TH and (B) sheep anti-TH antibody. (C) The merged image shows that the two antibodies label the same cell population. This virtually 100% colocalization was confirmed by analysis of 356 TH+ cells (in five DRGs from two animals). Scale bar: 50 μm.

Table S1 Primary antibodies used in immunohistochemistry.

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Author Contributions

Conceived and designed the experiments: JK MSA. Performed the experiments: JK. Analyzed the data: JK. Contributed to the writing of the manuscript: JK MSA.

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