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### Recommended Citation

Patra, Chinmoy; Monk, Kelly R.; and Engel, Felix B., "The multiple signaling modalities of adhesion G protein-coupled receptor GPR126 in development." *Receptors & Clinical Investigation*.1,3. e79. (2014).  
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## REVIEW

# The multiple signaling modalities of adhesion G protein-coupled receptor GPR126 in development

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Received: February 05, 2014  
Published online: March 03, 2014

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**The G protein-coupled receptor (GPCR) superfamily is the largest known receptor family in the human genome. Although the family of adhesion GPCRs comprises the second largest sub-family, their function is poorly understood. Here, we review the current knowledge about the adhesion GPCR family member GPR126. GPR126 possesses a signal peptide, a 7TM domain homologous to secretin-like GPCRs, a GPS motif and an extended N-terminus containing a CUB (Complement, Uegf, Bmp1) domain, a PTX (Pentraxin) domain, a hormone binding domain and 27 putative N-glycosylation sites. Knockdown and knockout experiments in zebrafish and mice have demonstrated that Gpr126 plays an essential role in neural, cardiac and ear development. In addition, genome-wide association studies have implicated variations at the *GPR126* locus in obstructive pulmonary dysfunction, in scoliosis and as a determinant of trunk length and body height. Gpr126 appears to exert its function depending on the organ system via G protein- and/or N-terminus-dependent signaling. Here, we review the current knowledge about Gpr126, which, due to the variety of its functions and its multiple signaling modalities, provides a model adhesion GPCR to understand general functional concepts utilized by adhesion GPCRs.**

**Keywords:** GPR126; heart; myelination; ear; GPS; GAIN; NTF; CTF; adhesion GPCR

*Receptors & Clinical Investigation* 2014; 1: e79. doi: 10.14800/rci.79; © 2014 by Chinmoy Patra, et al.

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## Introduction

The GPCR superfamily is the largest known receptor family in humans comprising around 4% of the entire protein-coding human genome<sup>[1,2]</sup>, and GPCRs can be grouped into five classes: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste 2, and Secretin<sup>[3,4]</sup>. These receptors are major targets of modern therapeutics, as they play important roles in organ development and disease<sup>[5]</sup>. The Adhesion class contains 33 receptors in

humans that can be classified in nine distinct families<sup>[6]</sup>. They are expressed in most organs playing important roles in development and disease. They contain extended N-termini containing various adhesion domains, which are separated from the 7TM domain by a GPCR autoproteolysis-inducing (GAIN) domain. Cleavage at the GPCR proteolysis site (GPS) in the GAIN domain results in an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that may or may not remain together at the cell surface. This property permits a variety of

signaling modalities including activation of CTF-dependent or -independent signals via agonist interactions at the NTF or the CTF. In addition, NTF can bind to partners at the surface of a neighboring cell or the extracellular matrix or NTF can be shed of to induce non-cell-autonomous signals in neighboring cells or in distant cells (reviewed in <sup>[7]</sup>). However, how these receptors mediate their physiological and pathological functions is poorly understood.

### Discovery and Structure of GPR126

*GPR126* was first identified by Fredriksson and co-workers by performing blast searches utilizing human sequences of known adhesion GPCRs; they identified *GPR123*, *GPR124*, *GPR125*, *GPR126*, *GPR127* and *GPR128* <sup>[8]</sup>. Phylogenetic analysis placed *GPR126* in a cluster with the previously identified *GPR64* (HE6), *GPR56* (TM7XN1), *GPR97* (Pb99), *GPR112* and *GPR114* (now designated Group VIII) <sup>[7, 8]</sup>. The first experimental evidence for a biological function was provided by Moriguchi and co-workers who cloned the full-length human and mouse *GPR126* cDNAs ("DREG" for developmentally regulated G protein-coupled receptor) utilizing human brain and human keratinocyte cDNA libraries <sup>[9]</sup>. While the groups of Moriguchi and Fredriksson identified *GPR126* based on sequence analyses <sup>[8,9]</sup>, Stehlik and co-workers isolated endogenously expressed GPR126 (vascular inducible GPCR, VIGR) from human umbilical vein endothelial cell (HUVEC) cultures by searching for differentially expressed secretory and membrane proteins in order to understand the role of the vascular endothelium in the process of acute inflammation <sup>[10]</sup>.

Domain analyses revealed that GPR126 possesses a signal peptide, a 7TM domain homologous to secretin-like GPCRs, a GPS motif and an extended N-terminus containing a CUB (Complement, Uegf, Bmp1) domain, a PTX (Pentraxin) domain, a hormone binding domain and 27 putative N-glycosylation sites <sup>[8-10]</sup> (Figure 1). Overexpression experiments of wild-type and mutated forms of human and mouse GPR126 combined with antibody-based assays indicated that GPR126 is cleaved at the GPS motif into a CTF that locates to the plasma membrane and a NTF. Subsequently, the NTF is cleaved by furin at an additional cleavage site (S2 site) between the GPS motif and the PTX domain <sup>[9]</sup>, releasing a putative fragment from the rest of the NTF, which remains non-covalently associated with the CTF (Figure 1). Experiments utilizing tunicamycin, a potent inhibitor of N-glycosylation, confirmed that GPR126 is indeed highly glycosylated and indicated that glycosylation is not essential for the cellular localization of GPR126 <sup>[10]</sup>. Finally, the cytoplasmic domain contains a potential

palmitoylation site as well as several potential phosphorylation sites for cAMP dependent kinases/protein kinase G, protein kinase C, and casein kinase II, as well as a potential myristoylation signal and a microbodies C-terminal targeting motif <sup>[10]</sup>.

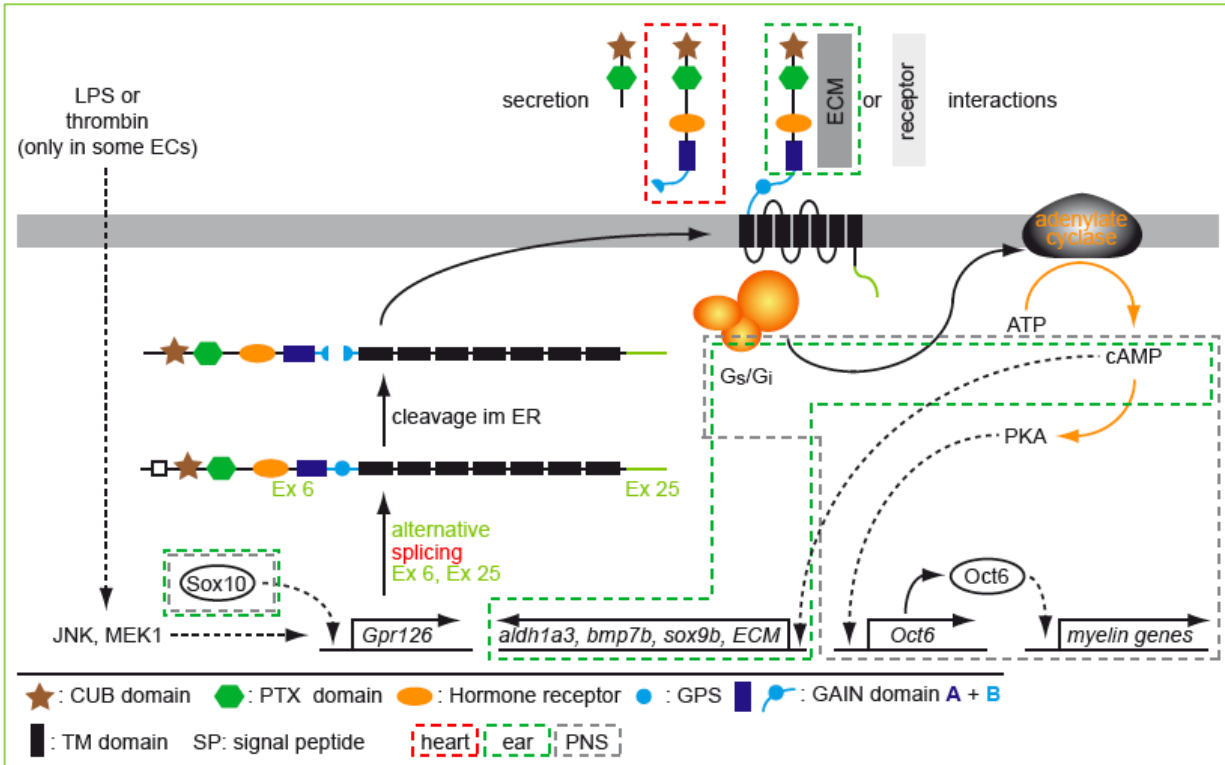
### Expression pattern of GPR126

Initially, it had been predicted based on human and mouse ESTs (expressed sequence tags) that *GPR126* is expressed in adult lung, liver, testis and skeletal muscle <sup>[6]</sup>. In recent years a large amount of expression data has been collected based on RT-PCR, *in situ* hybridization, and genetic labeling. *GPR126* is strongly expressed during mouse and zebrafish development predominantly in the somites, the frontonasal process, pharyngeal arches, the heart, the ear and the nervous system. In adult mouse tissues, *GPR126* has been detected at a high level in the lung and at low levels in a few other tissues (for details see Table 1). The broad and tightly regulated spatio-temporal expression pattern suggests that GPR126 might play important roles in development and disease.

### Gpr126 in peripheral nerve development

In 2009, the Talbot lab described the first known function of Gpr126 <sup>[11]</sup>. Starting with a forward genetic screen for mutations that affect the development of myelinated axons <sup>[12]</sup>, two allelic mutations of *gpr126* were uncovered. Myelin is the multilayered membrane generated by specialized glial cells that insulates and protects axons in the vertebrate nervous system; in the PNS, Schwann cells generate the myelin sheath by iteratively wrapping their membrane around a selected neuronal axon. In *gpr126* zebrafish mutants, peripheral myelination is specifically impaired: Schwann cells associate with axons but never spiral their membrane to generate the myelin sheath. Analysis of *Gpr126* constitutive knockout mice showed that the function of Gpr126 is conserved in mammalian myelination <sup>[13]</sup>. Like zebrafish mutants, Schwann cells in *Gpr126*<sup>-/-</sup> mice ensheath but do not myelinate axons. Unlike zebrafish mutants, all stages of Schwann cell development leading to axon ensheathment were significantly impaired in *Gpr126*<sup>-/-</sup> mice. Additional phenotypes observed in mouse knockouts but not zebrafish mutants included significant embryonic lethality, ataxia, limb abnormalities and pronounced axon degeneration <sup>[13]</sup>. These observations raised the question of which cell(s) require Gpr126 function for proper Schwann cell myelination.

To address the question of cellular autonomy in Schwann cell myelination, Mogha and co-workers recently described a conditional *Gpr126* mutant <sup>[14]</sup>. In this study, the conditional mutant was used to delete



**Figure 1: Summary of the multiple signaling modalities of adhesion GPCR Gpr126 in development.** Notably, Gpr126 is differently regulated (e.g. Sox10) and is utilizing different signaling modalities in neural (grey boxes), cardiac (red boxes) and ear (green boxes) development. Moreover, Gpr126 function is regulated by alternative splicing (Exon (Ex) 6 and Ex 25 visualized in green).

*Gpr126* in Schwann cell precursors at E12.5, and Schwann cell-specific *Gpr126* mutants recapitulated several defects observed in constitutive *Gpr126* knockouts. Specifically, Schwann cells completely failed to myelinate axons, and all stages of Schwann cell development leading to axon ensheathment were severely delayed. This data supports a model in which Gpr126 is required autonomously in Schwann cells for timely axon selection and myelination, consistent with genetic chimera analyses in zebrafish<sup>[11]</sup>. Interestingly, Schwann cell-specific *Gpr126* conditional mutants were viable and did not present with other defects observed in constitutive *Gpr126*<sup>-/-</sup> mutants, including ataxia, limb defects and axon degeneration. These data suggest that Gpr126 has essential functions in other cell types and underscore the need for further tissue-specific investigations of this adhesion GPCR.

### GPR126 in heart development

Studies on *Gpr126* knockout mice have demonstrated that Gpr126 function is also required for cardiac development<sup>[15]</sup>. This was initially surprising, as previous reports analyzing the zebrafish mutant line *gpr126*<sup>st49</sup> did not report a heart phenotype<sup>[11]</sup>. Recently, the analysis of three different mouse lines has

demonstrated that disruption of *Gpr126* causes a heart phenotype and is embryonic lethal (E11 to E12.5). At E10.5 in *Gpr126* knockout mice, the ventricular cardiac wall is trabeculated and appears normal. In contrast, at E11.25, *Gpr126* knockout mice have a thinner ventricular myocardial wall, contain fewer trabeculae<sup>[15, 16]</sup> and exhibit bradycardia along with cardiac arrhythmia<sup>[16]</sup>. These data suggested that Gpr126 might be required for proper cell–cell contacts and electric coupling. However, immunohistological and electron microscopic analyses indicated that cell–cell contacts were maintained as the localization of cadherins and adherent junction proteins was not affected. In contrast, mitochondria in cardiomyocytes as well as in endocardial cells exhibited unusual characteristics such as a more complex shape, less well developed cristae and electron-dense precipitates, indicating mitochondrial dysfunction. This was further supported by the accumulation of lipid droplets and a decrease in glycogen deposits<sup>[16]</sup>. The accumulation of lipids might be the cause of the observed cardiac bradycardia as it has previously been demonstrated that mitochondrial respiratory dysfunction or the deficiency of lipid transporter in the mitochondrial membrane leads to the accumulation of lipids causing cardiac arrhythmia<sup>[17, 18]</sup>.

**Table 1. GPR126 expression data.**

Organ/Tissue	Age	Detection	Species	References	KO/KD Phenotype
<b>embryo</b>	E11/E15/E17	RT-PCR	mouse	[9]	Yes [15, 16]
<b>bone</b> , spine, vertebrae	E16.5	ISH	mouse	[25]	TBD
jaw	48 hpf	ISH	zebrafish	[11]	TBD
head, pectoral fin	48/72 hpf	ISH	zebrafish	[19]	TBD
<b>cartilage</b>	adult	RT-PCR	human	[25]	TBD
<b>ceratobranchials</b> (gill arches)	72 hpf	ISH	zebrafish	[19]	TBD
<b>ear</b> , otic vesicle	E9.5/E11.5	ISH	mouse	[16]	No [13]
otic vesicle	24 hpf	ISH	zebrafish	[19]	Yes [19]
	48/72/96 hpf	ISH	zebrafish	[19]	
	30/48/54 hpf	ISH	zebrafish	[11]	
canal projections	48 hpf	ISH	zebrafish	[19]	
<b>heart</b>	E9.5/E11.5	ISH	mouse	[16]	Yes [15, 16]
endocardium	E9.5/E11.5	multiplex ISH	mouse	[16]	Yes [16]
	E10/E11	ISH	mouse	[9]	
	adult	RT-PCR	mouse	[9]	Yes [16]
	E11	RT-PCR	rat	[16]	
	14/23 somite stage	ISH	zebrafish	[19]	
	24/48 hpf	ISH	zebrafish	[16, 19]	
<b>intermediate mesoderm</b>	14 somite stage	ISH	zebrafish	[19]	TBD
<b>kidney</b>	adult	RT-PCR	mouse	[9]	TBD
collecting duct	adult	microarray	mouse	[41]	TBD
<b>liver</b>	adult	northern blot	human	[10]	TBD
<b>lung</b>	P14	ISH	mouse	[14]	TBD
	adult	RT-PCR	mouse	[9]	TBD
<b>macula</b>	48 hpf	ISH	zebrafish	[19]	TBD
<b>nervous system</b> , forebrain	E15	RT-PCR	mouse	[42]	TBD
Bergmann glial cells	P6	RT-PCR	mouse	[42]	TBD
DRG neurons	P4, P21	ISH, RT-PCR	mouse	[11, 14]	Yes [13]
sciatic nerve	P4	RT-PCR	mouse	[11]	No [13]
brain	adult	RT-PCR	mouse	[9]	
olfactory epithelium	24 hpf	ISH	zebrafish	[19]	TBD
olfactory bulb	32 hpf	ISH	zebrafish	[11]	TBD
<b>frontonasal process</b>	E9/E10	ISH	mouse	[9]	TBD
<b>nose</b>	48/72 hpf	ISH	zebrafish	[19]	TBD
brain	adult	RT-PCR	zebrafish	[11]	TBD
PLLn	3 dpf, adult	ISH, RT-PCR	zebrafish	[11]	TBD
Schwann cells of PLLn	30 hpf to 4 dpf	ISH	zebrafish	[11]	Yes [11, 15, 16]
Schwann cells of ALLn	48 hpf, 54 hpf	ISH	zebrafish	[11]	TBD
<b>neural crest</b>	14/23 somite stage	ISH	zebrafish	[19]	TBD
<b>pancreas</b>	adult	ISH	human	[10]	TBD
<b>pericardium</b>	48 hpf	ISH	zebrafish	[16]	TBD
<b>pharyngeal arches</b>	E9/E10	ISH	mouse	[9]	TBD
<b>placenta</b>	adult	RT-PCR	human	[31]	No [15]
placenta	adult	northern blot	human	[10]	
trophoblast giant cells	E10/E11	LacZ reporter	mouse	[15]	No [15]
<b>presomite mesoderm</b>	E9	ISH	mouse	[9]	TBD
<b>post-otic neural crest</b>	24 hpf	ISH	zebrafish	[19]	TBD
<b>skeletal muscle</b>	adult	RT-PCR	mouse	[9]	TBD
<b>somites</b>	E9.5/E11.5	ISH	mouse	[16]	No [15]
	E10/E11	ISH	mouse	[9]	
	E10.5 to E 12.5	LacZ reporter	mouse	[15]	
<b>spleen</b>	adult	RT-PCR	mouse	[9]	TBD
<b>tail fin tip</b>	48/72 hpf	ISH	zebrafish	[19]	TBD
	48 hpf	ISH	zebrafish	[11]	TBD
<b>testis</b>	adult	RT-PCR	mouse	[9]	TBD
<b>uterus</b>	adult	RT-PCR	human	[31]	TBD
<b>Cultured Cells</b>					
<b>Vascular cells</b> (aortic ECs, HUVEC)		RT-PCR	human	[10]	No [15]
IFRS1 cell line, primary DRG neurons, Schwann cells		antibody	rat	[43]	NA

KO: knockout; KD: knockdown; ISH: *in situ* hybridization; EC: endothelial cell; PLLn: posterior lateral line nerve; ALLg: anterior lateral line ganglia; TBD: to be determined; NA: not applicable.



The mitochondrial phenotype, but not the accumulation of lipid deposition, could be recapitulated in zebrafish utilizing morpholinos that deplete Gpr126<sup>[16]</sup>.

The major common phenotype between *Gpr126* knockout mice and Gpr126-depleted zebrafish is cardiac ventricular hypotrabeculation<sup>[15, 16]</sup>. With the help of domain-specific morpholinos, rescue experiments in Gpr126-depleted morphants and an *in situ* protein binding assay using mGpr126-NTF on mouse cardiac sections, Patra and co-workers demonstrated that the NTF fragment NTF<sup>AGPS</sup> (amino acid 1–783) of Gpr126 is important for cardiac trabeculation<sup>[16]</sup>. Moreover, these data suggested that mGpr126-NTF acts in a paracrine fashion. However, the molecular mechanism utilized by Gpr126 to regulate heart development remains elusive.

### GPR126 in ear development

In 2013, Geng and co-workers reported that Gpr126 plays a role not only in heart and PNS development, but also in inner ear development<sup>[19]</sup>. The three semicircular canals of the vertebrate inner ear detect angular acceleration (rotational movements). These canals are formed via the movement and fusion of sheets of epithelium (reviewed in<sup>[20]</sup>). In zebrafish, finger-like projections of epithelium grow into the center of the otic vesicle, where they fuse<sup>[21]</sup>. In *gpr126* mutants, these projections overgrow and fail to fuse correctly, implicating Gpr126 in the control of projection outgrowth, contact recognition and fusion in the developing ear. A closer analysis revealed that the major difference of *gpr126* mutants compared to wild-type zebrafish were changes in the extracellular matrix<sup>[19]</sup>. These data suggest that Gpr126 plays an important role in cell adhesion, signaling and cell-cell or cell-matrix interactions (Figure 1). In the future it will be important to determine in more detail how Gpr126 regulates the cell behavior in zebrafish inner ear development and if this function is conserved between fish and mammals.

### Emerging roles of GPR126 in disease

To date, there is no human disease known to be caused by a mutation in *GPR126*. However, genome-wide association studies have implicated variations at the *GPR126* locus as a determinant of trunk length and body height<sup>[22-24]</sup>, adolescent idiopathic scoliosis<sup>[25]</sup> as well as pulmonary function<sup>[26]</sup>. Body height is determined by trunk and leg length. In contrast to trunk length, leg length is positively associated with nutritional intake in childhood. This suggests that final upper and lower body size is controlled by different pathways<sup>[22, 27]</sup>. To search for loci influencing adult height, several groups have independently associated *GPR126* with body height. A

genome-wide analysis identified associations with trunk length at SNP rs6570507 in *GPR126* ( $P$ -value =  $4 \times 10^{-5}$ ) based on 299,216 single nucleotide polymorphisms (SNPs) and a group of 12,611 adults of Caucasian origin<sup>[22]</sup>. Notably, this SNP was associated in a separate study with adolescent idiopathic scoliosis utilizing 1,819 cases and 25,939 controls ( $P$ -value =  $2.25 \times 10^{-10}$ ) as well as Han Chinese and European-ancestry populations (combined  $P$ -value =  $1.27 \times 10^{-14}$ )<sup>[25]</sup>. The association of *GPR126* with height is further supported by a study including 11,536 individuals composed of Australian twins, family members, and unrelated individuals (~550,000 genotyped SNPs)<sup>[23]</sup> and in a study based on a European American pediatric cohort (8,184 children)<sup>[24]</sup>. Finally, *GPR126* has also been associated with obstructive pulmonary dysfunction based on the analysis of 20,890 participants of European ancestry identifying 69 SNPs with the top SNP (rs3817928) having a  $P$ -value of  $2.60 \times 10^{-10}$ <sup>[26]</sup>.

### Regulation of GPR126 expression

In order to understand the function and physiological role of GPR126 and how to modulate it in disease it is important to understand how its expression is regulated. In zebrafish, the expression pattern of *gpr126* during development in ear and PNS development suggested that *gpr126* might be regulated by the transcription factor Sox10 (Figure 1), which is known to be expressed in the neural crest and ear<sup>[11, 13, 19]</sup> and to be important for ear development<sup>[28]</sup> as well as Schwann cell development<sup>[29, 30]</sup>. Importantly, Geng and co-workers showed that *gpr126* expression is partially lost in colorless (*sox10*) zebrafish mutants. A detailed analysis of these mutants suggested that *gpr126* expression is in the ear and the PNS but not in the heart and posterior mesoderm *sox10*-dependent<sup>[19]</sup>. In the future it will be important to elucidate the relationship between Sox10 and Gpr126.

Very little is also known about signaling pathways that induce GPR126 expression. Stehlik and co-workers presented the first and so far only evidence that *GPR126* expression can be induced<sup>[10]</sup>. They demonstrated that stimulation of HUVECs with lipopolysaccharide (LPS) or thrombin resulted in transiently increased *GPR126* mRNA levels peaking at 12 h and declining to background levels at 24 h of LPS treatment. However, this effect was cell-type dependent. While no *GPR126* expression was observed in primary skin microvascular endothelial cells (ECs), constitutive expression was detectable in aortic ECs. Recently, it has also been shown that *Gpr126* is expressed in endocardial cells in the developing mouse heart<sup>[16]</sup>. Induction of *GPR126* expression in HUVECs was independent of the major pro-inflammatory transcription factor NF- $\kappa$ B as well as

p38 mitogen-activated protein kinase. However, specific inhibition of JNK and MEK1 completely abolished *GPR126* expression<sup>[10]</sup> (Figure 1).

Alternative splicing is one important way gene function can be regulated to increase proteome diversity in higher eukaryotic organisms. GPR126 is a complex protein. It is encoded by 26 exons in mice<sup>[16]</sup>, is widely expressed in many tissues and plays distinct roles in several organs. Thus, it appears likely that different splice isoforms of *GPR126* exist. Indeed, several alternatively spliced forms have been described in mouse that differ in the presence of exon 6 (including: isoform 1; excluding: isoform 2) and exon 25 changing the usage of termination codons (including:  $\alpha$  isoforms; excluding:  $\beta$  isoforms)<sup>[9]</sup> (Figure 1). Subsequently, the existence of different splice isoforms has been confirmed in human, monkey<sup>[31]</sup>, and zebrafish<sup>[16]</sup>. However, the importance of these splice-isoforms remains unclear. In the future, it will be interesting to determine how splice-isoforms exist, where they are expressed, and how they differ in their signaling modalities and function.

### GPR126 signaling

Until recently, it was unclear whether the class of adhesion-GPCRs functioned primarily as adhesion molecules or if members could also function as traditional GPCRs which couple to heterotrimeric G proteins to elicit downstream signaling cascades. In recent years, however, great strides have been made to elucidate signaling capabilities of adhesion GPCRs<sup>([32]; reviewed in [7, 33])</sup>, and it is now accepted that many adhesion-GPCRs are indeed capable of G protein-coupling.

The first piece of evidence suggesting that Gpr126 could function as a canonical GPCR came from pharmacological studies in zebrafish. Addition of forskolin, an adenylate cyclase activator which increases cAMP levels, suppressed defects in *gpr126* mutants and restored myelination<sup>[11]</sup>. Furthermore, studies using transgenic zebrafish demonstrated that expression of activated PKA in Schwann cells could also rescue myelination in *gpr126* mutants<sup>[34]</sup>. Similarly, cAMP elevation and PKA activation could suppress myelin defects in *Gpr126*<sup>-/-</sup> mouse mutants<sup>[14]</sup>. These studies supported a model in which Gpr126 elevates cAMP, presumably through interactions with  $G\alpha_s$ ; recently, more direct biochemical evidence for this model has been reported. Using heterologous cells, Mogha and co-workers showed that GPR126-transfected COS-7 cells showed a concentration-dependent increase in cAMP levels, strongly supporting  $G_s$ -protein coupling capabilities for this receptor<sup>[14]</sup>. In the same study,

chimeric G-protein assays demonstrated that GPR126 can couple to  $G_s$ -proteins as well as  $G_i$ -proteins<sup>[14]</sup> (Figure 1). Interestingly, Schwann cell-specific *Gpr126* mutants also phenocopy Schwann cell-specific *Rac1* mutants<sup>[14, 35-37]</sup>, and future work is required to determine the relationship between Gpr126 and Rac1.

A role for cAMP in Schwann cell development and myelination has long been hypothesized, as increased levels of this second messenger promote Schwann cell differentiation *in vitro* (e.g. [38, 39]); thus, the ability of Gpr126 to couple to  $G\alpha_s$  and to elevate cAMP as well as the results of the phenotypic analysis of mutants in zebrafish and mouse strongly supports the hypothesis that Gpr126 is the major, if not the receptor, that modulates cAMP in Schwann cells to promote myelination. cAMP elevation can also rescue the ear defects observed in *gpr126* zebrafish mutants, supporting a model in which  $G_s$ -protein interactions are critical for canal projection development in this tissue<sup>[19]</sup>. Given that *Gpr126* is expressed in a wide range of tissues, it will be interesting in the future to determine the function of  $G_s$ -protein and  $G_i$ -protein coupling in other organ systems.

While evidence is accumulating that Gpr126 signals through G-proteins, the consequence of this is poorly understood. The analysis of zebrafish mutants has demonstrated that *gpr126* expression is required for *oct6* expression in Schwann cells, which is required for the expression of *krox20* and *mbp* myelin basic protein<sup>[11, 34]</sup> (Figure 1). The analysis of *Gpr126* knockout mice showed that this function of regulating Oct6 (also known as Pou3f1) is conserved in mammals<sup>[13]</sup>. During ear development loss of Gpr126 function results in the misregulation of genes that encode extracellular matrix (ECM) proteins (*hapln1a*, *hapln3*, *vcana*, *vcamb*) or ECM-modifying enzymes (*chsy1*, *ugdh*, *has3*). In addition, *gpr126* mutants were characterized by substantial changes in the expression of other semicircular canal marker genes, including *aldh1a3*, *bmp7b* and *sox9b*<sup>[19]</sup> (Figure 1). It will be important to identify the direct targets of Gpr126 signaling resulting in the observed phenotypical changes such as ECM modulation.

The function of the NTF of adhesion GPCRs has been implicated in adhesion, cellular migration and dimerization of adhesion GPCRs<sup>[40]</sup>. However, although most different protein domain types found in adhesion GPCRs have the ability to mediate contact with cellular or matrix-associated molecules, there is little information available about the functional role of NTFs (reviewed in [7]). The importance of the NTF has been indicated through the detailed analysis of why the zebrafish mutant *gpr126*<sup>st49</sup> exhibits no heart phenotype<sup>[16]</sup>. The *gpr126*<sup>st49</sup>

mutant allele carries a single point mutation that introduces a stop codon immediately before the GPS motif<sup>[11]</sup>. This suggested that the NTF of Gpr126 might function during heart development independently of its CTF, which plays an important role in Schwann cell myelination. Rescue experiments in Gpr126-depleted morphants demonstrated that the NTF fragment NTF<sup>ΔGPS</sup> (amino acid 1-783) rescued the cardiac phenotype but not the myelin nor ear phenotype<sup>[16]</sup>, which both depend on G-protein signaling<sup>[11, 14, 19]</sup>.

How the NTF functions during heart development is poorly understood. Based on multiplex *in situ* hybridization experiments on mouse heart sections, *Gpr126* is expressed in the endocardium. This appears to secrete the NTF that binds to the myocardium, as incubation of tissue sections with recombinant Gpr126-NTF<sup>ΔGPS</sup> resulted in cardiomyocyte-specific binding in the heart<sup>[16]</sup>. As endocardium and myocardium exhibit defects in *Gpr126* knockout mice, it is possible that the NTF is required for proper cardiomyocyte function while the CTF might be required for endocardial function (Figure 1). However, it is also possible that the endocardial phenotype is due to the disruption of endocardial-cardiomyocyte interactions. In the future it will be important to delete *Gpr126* in the heart with cell-type-specificity and to perform rescue experiments utilizing the CTF and NTF.

## Conclusions

Gpr126 is expressed in a variety of organs and tissues. It regulates neural, cardiac and ear development via G-protein- and/or N-terminus-dependent signaling. In addition, genome-wide association studies have implicated variations at the *GPR126* locus in obstructive pulmonary dysfunction, scoliosis and as a determinant of trunk length and body height. The variety of functions of Gpr126 and its multiple signaling modalities identify Gpr126 as model adhesion GPCR to better understand general functional concepts utilized by adhesion GPCRs.

## Acknowledgements

This work was supported by the Alexander von Humboldt Foundation (Sofja Kovalevskaja Award to FBE) and by the National Institutes of Health (R01 NS079445 to KRM).

## Conflicting interests

The authors declare that they have no conflict of interest.

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**To cite this article:** Patra C, et al. The multiple signaling modalities of adhesion G protein-coupled receptor GPR126 in development. *Receptor Clin Invest* 2014; 1: e79. doi: 10.14800/rci.79.