Current concepts in RET-related genetics, signaling and therapeutics

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The receptor tyrosine kinase RET is expressed in cell lineages derived from the neural crest and has a key role in regulating cell proliferation, migration, differentiation and survival during embryogenesis. Germline and somatic mutations in *RET* that produce constitutively activated receptors cause the cancer syndrome multiple endocrine neoplasia type 2 and several endocrine and neural-crest-derived tumors, whereas mutations resulting in nonfunctional RET or lower expression of *RET* are found in individuals affected with Hirschsprung disease. This review focuses on the genetics and molecular mechanisms underlying the different inherited human neural-crest-related disorders in which RET dysfunction has a crucial role and discusses RET as a potential therapeutic target.

Introduction

The human gene *RET* is localized on chromosome 10 (10q11.2) and contains 21 exons [1]; alternative splicing generates three isoforms, which contain 51 (RET51), 43 (RET43) and 9 (RET9) amino acids in the carboxyl (C)-terminal tail [2]. RET51 and RET9 are the most prevalent and best-characterized isoforms *in vivo*; RET43 has not yet been characterized. The RET51 isoform shows the highest transforming and kinase activity *in vitro* [3], and several observations suggest that these isoforms have different tissue-specific effects during embryogenesis [4]. Monoisoformic RET9 mice are viable and normal, whereas the monoisoformic RET51 mice (which lack RET9) have kidney hypoplasia and lack enteric ganglia from the colon [4].

RET (Figure 1) is the receptor for members of the glial cell-derived neurotrophic factor (GDNF) family of ligands (GFLs): namely GDNF, Neurturin, Persephin and Artemin [5]. To stimulate RET, these GFLs first need to form a complex with their glycosylphosphatidylinositol (GPI)-anchored co-receptor, a member of the GDNF receptor-α family (GFRα1–GFRα4), after which the GFL–GFRα complex activates RET [6,7]. The GFRs differ in their specificity for GFLs (Figure 1).

During embryogenesis, RET is expressed in the developing excretory system, in all lineages of the peripheral nervous system, and in motor and catecholaminergic neurons of the central nervous system [8]. In addition, RET is also expressed in C-cells of the thyroid and tumors originating from these cells, in medullary thyroid carcinomas (MTCs), and in other tumors of neural crest origin such as pheochromocytomas and neuroblastomas [9].

The role of RET in cancers was first described when somatic rearrangements of *RET* (named *RET/PTC*) showing constitutive tyrosine kinase activity were found in papillary thyroid carcinomas (PTCs) [10]. These RET/PTC oncoproteins, of which over ten have been described, are all chimeric proteins in which the amino (N)-terminal region of different proteins is fused to the catalytic domain of RET [11]. Subsequently, germline mutations that give rise to constitutively activated RET proteins were discovered as the cause of the cancer syndrome 'multiple endocrine neoplasia type 2' (MEN2) [12]. By contrast, mutations causing loss of function of the RET protein were found to be associated with Hirschsprung disease: a developmental disorder characterized by the absence of enteric ganglia in the intestinal tract [13].

In this review, we focus on current concepts in the genetics and molecular mechanisms underlying the different inherited human neural crest disorders in which RET dysfunction plays a crucial role, and we further discuss new developments in which RET is a therapeutic target.

Wild-type RET activation and signaling

Wild-type RET signaling is crucial for the development of the enteric nervous system (ENS), kidney organogenesis and spermatogenesis [14]. Activation of the tyrosine kinase domain of RET occurs through transient homodimerization induced by the formation of a macromolecular GFL–GFRα–RET complex, which involves lipid rafts (reviewed by Saarma [15]).

Dimerization and activation of wild-type RET result in phosphorylation of its intracellular tyrosine residues, which act as docking sites for various adaptor proteins (Figure 2). For an excellent review that discusses in
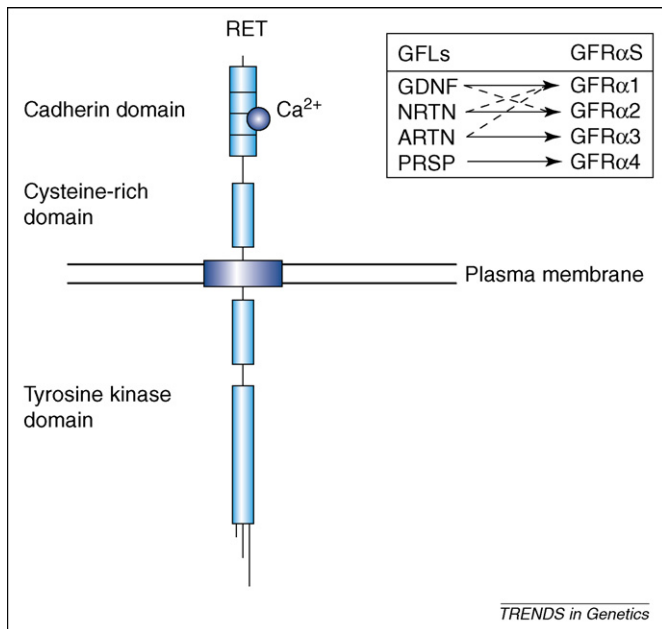
Figure 1. The RET protein, its functional domains, ligands and co-receptors. Left, functional domains of the three RET isoforms. Right, canonical (unbroken lines) and noncanonical (broken lines) interactions of the RET ligands GDNF, Neurturin (NRTN), Persephin (PSPN) and Artemin (ARTN) with their GFRα co-receptors. Lipid rafts are depicted as a purple box in the plasma membrane.

detail the signaling pathways activated by the wild-type RET receptor, we refer to Ichihara *et al.* [16]. GDNF-independent activation of RET has been also observed on binding of nerve growth factor to the receptor TRKA [17].

Neural crest disorders associated with *RET* mutations
MEN2
Germline missense mutations resulting in constitutive activation of RET cause MEN2, a dominant inherited cancer syndrome that affects neuroendocrine organs [12]. Depending on the tissues affected, three different clinical subtypes can be distinguished.

  (i) MEN2A, which is characterized by medullary thyroid carcinoma (MTC) in all affected individuals (100%); pheochromocytoma, a tumor of the adrenal medulla cells (50%); and hyperparathyroidism (15–30%) [18].
 (ii) MEN2B, which is also characterized by MTC (100%) and pheochromocytoma (∼50%); instead of hyperparathyroidism, however, affected individuals develop a more complex clinical phenotype including ganglioneuromas on the tongue, lips and eyelids, intestinal ganglioneuromas, thickened cornea nerves and a marfanoid habitus. MEN2B is considered to be the most aggressive subtype of MEN2 with the earliest age of onset [19].
(iii) Familial MTC (FMTC), usually considered to be the third MEN2 subtype, is characterized by the manifestation of only MTC in four or more members of an affected family.

Hirschsprung disease
Hirschsprung disease is a complex genetic disorder characterized by aganglionosis of variable length of the distal gastrointestinal tract. Genetic dissection has led to the identification of ten genes and four loci associated with Hirschsprung susceptibility [20], most of which cause
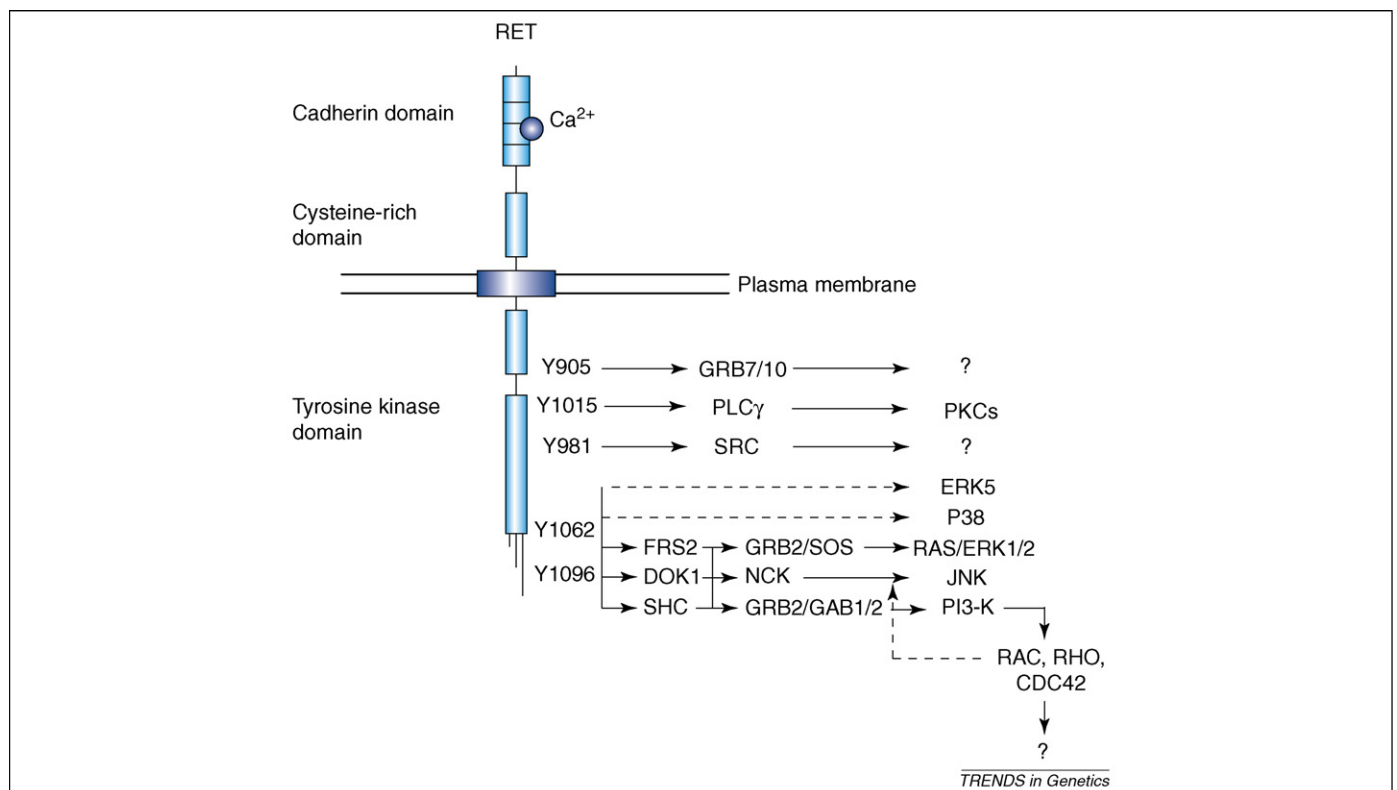

Figure 2. RET signaling pathways. Activation of RET leads to autophosphorylation of tyrosine residues in the cytoplasmic tail of the receptor that act as docking sites for several signaling transducers. Tyr1096 is present only in the RET51 isoform. Unbroken arrows indicate a direct functional interaction; broken arrows indicate indirect functional interactions.

Table 1. Genes involved in (syndromic) Hirschsprung disease[a]

Gene	Map position	Phenotype	Inheritance
RET	10q11.2	Non-syndromic	Dominant, incomplete penetrance
		Hirschsprung-MEN2A/FMTC	
GDNF	5p13	Non-syndromic	Non-Mendelian
Neurturin	19p13	Non-syndromic	Non-Mendelian
EDNRB	13q22	Shah–Waardenburg	Recessive
		Non-syndromic	Dominant (*de novo* in 80%)
EDN3	20q13	Shah-Waardenburg	Recessive
		Non-syndromic	Dominant, incomplete penetrance
SOX10	22q13	Shah-Waardenburg	Dominant (*de novo* in 75%)
ECE-1	1p36	Congenital heart malformation	*De novo* dominant
ZFHX1B	2q22	Mowat-Wilson	*De novo* dominant
KIAA1279	10q21.3-q22.1	Goldberg-Shprintzen	Recessive
PMX2b	4p12	Congenital central hypoventilation syndrome (CCHS)	Dominant (*de novo* in 90%)
Unknown	3p21	Non-syndromic, S-HSCR	Non-Mendelian
Unknown	9q31	Non-Syndromic, L-HSCR	Dominant, incomplete penetrance
Unknown	19q12	Non-syndromic, S-HSCR	Non-Mendelian
Unknown	16q23	Shah-Waardenburg	Non-Mendelian

[a]Reviewed in Ref. [20].

syndromic Hirschsprung disease (Table 1). Of the genes identified so far, *RET* has been shown to be the most important.

Various *RET* mutations have been identified and correlated with disease phenotype (Figure 3). The functional consequences of the different *RET* mutations have also been characterized [12,21,22] (Table 2). A recent update on all MEN2- and FMTC-associated *RET* mutations identified so far has been provided by Arighi *et al.* [14]. The *RET* mutations associated with Hirschsprung disease can be classified according to the dysfunctional mechanism of action of the RET protein (Figure 4, Table 3).

Oncogenic RET activation and signaling in MEN2

Despite the clear correlation between mutations found in MEN2 and their associated phenotypes, the molecular
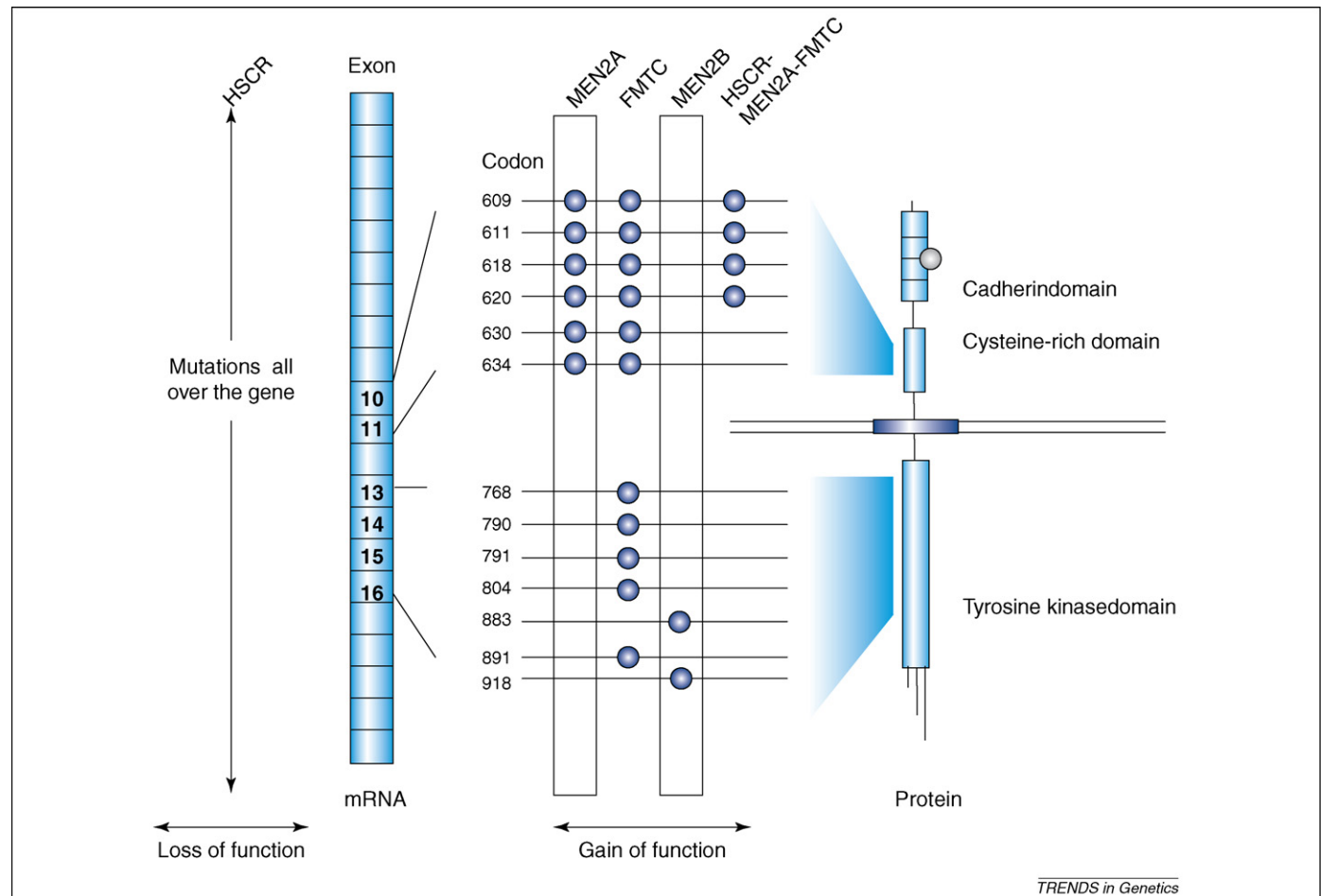

Figure 3. Germline missense mutations in *RET* associated with MEN2 and Hirschsprung disease (HSCR). Shown are the structure of the RET mRNA and protein. The codons mutated, the associated clinical entities, and the location of these mutations in relation to the exons and structural domains are indicated.

Table 2. Subdivision of *RET* mutations[a]

Phenotype		Codons mutated/position of the mutation	Consequences of the mutation
MEN2	Level 1	C609, E768, L790, Y791, V804, S891	Mild activating *RET* mutations predisposing to FMTC
	Level 2	C611, C618, C620, C634	Moderate activating *RET* mutations predisposing to FMTC or MEN2A
	Level 3	A883, M918	Aggressive *RET* mutations predisposing to MEN2B
HSCR	Class 1	Mutations in the extracellular domain	Mutations disrupting RET maturation and/or transport to the membrane
	Class 2	C609, C611, C618, C620	Moderate activating *RET* mutations predisposing to a combination of Hirschsprung disease and FMTC or MEN2A
	Class 3	Mutations in the catalytic domain	Mutations altering the catalytic activity of RET
	Class 4	Mutations surrounding residue Y1062	Mutations interfering with RET substrates binding to this tyrosine
	Class 5	Mutations in regulatory sequences	Changing the expression of the *RET* gene

[a]Mutations are subdivided according to the predisposing phenotype, the position in the coding sequence and the consequences of the mutations for the encoded proteins [21–23]. The levels of the MEN2 mutations represent a scale of aggressiveness of the associated phenotype (level 1 represents the least aggressive and level 3 the most aggressive phenotype).

mechanisms that connect the mutated receptors with their different clinical subtypes are far from understood. Mutations affecting the extracellular cysteine-rich domain of RET result in the replacement of a crucial cysteine residue and the loss of an intramolecular disulfide bond, thereby facilitating the formation of an inter-molecular disulfide bond between two (mutated) RET monomers and producing a constitutively activated receptor. Intracellular mutations, associated usually with FMTC and always with MEN2B, signal independently of GDNF as monomeric oncoproteins. These oncoproteins show not only an altered catalytic activity but also an altered substrate specificity because, unlike wild-type RET, they phosphorylate substrates that are usually preferred by cytoplasmic tyrosine kinases such us SRC and ABL [3]. It has been shown, however, that GDNF can further enhance RET activity of most MEN2A proteins and in MEN2B [23–25]. Furthermore, a different pattern of RET receptor autophosphorylation has been found for MEN2A-mutated (RET-MEN2A) and MEN2B-mutated

(RET-MEN2B) RET proteins [26,27]. As a consequence, different sets of phosphotyrosine-mediated downstream signaling pathways will be triggered by RET mutants associated with specific disease phenotypes, which might explain the distinct clinical features observed.

This hypothesis has been partially corroborated by several studies over the past few years. For example, Liu *et al.* [26] have shown that, as compared with wild-type RET, RET-MEN2B mutants lack phosphorylation at Tyr1096, directly leading to a decrease in binding of GRB2 to RET [26]. RET-MEN2B oncoproteins also trigger higher levels of activated PI3K and its downstream signaling molecules [24]. In addition, expression of RET/PTC2-MEN2B, in contrast to that of wild-type RET/PTC2, results in activation of the cytoskeleton protein Paxillin [28]. Furthermore, RET-MEN2B mutants trigger higher activation of Jun N-terminal kinase (JNK) [29]. These data suggest that the high levels of activated AKT and JNK contribute to the MEN2B phenotype. Along the same lines, phosphorylation of Tyr1062 of RET and its association with
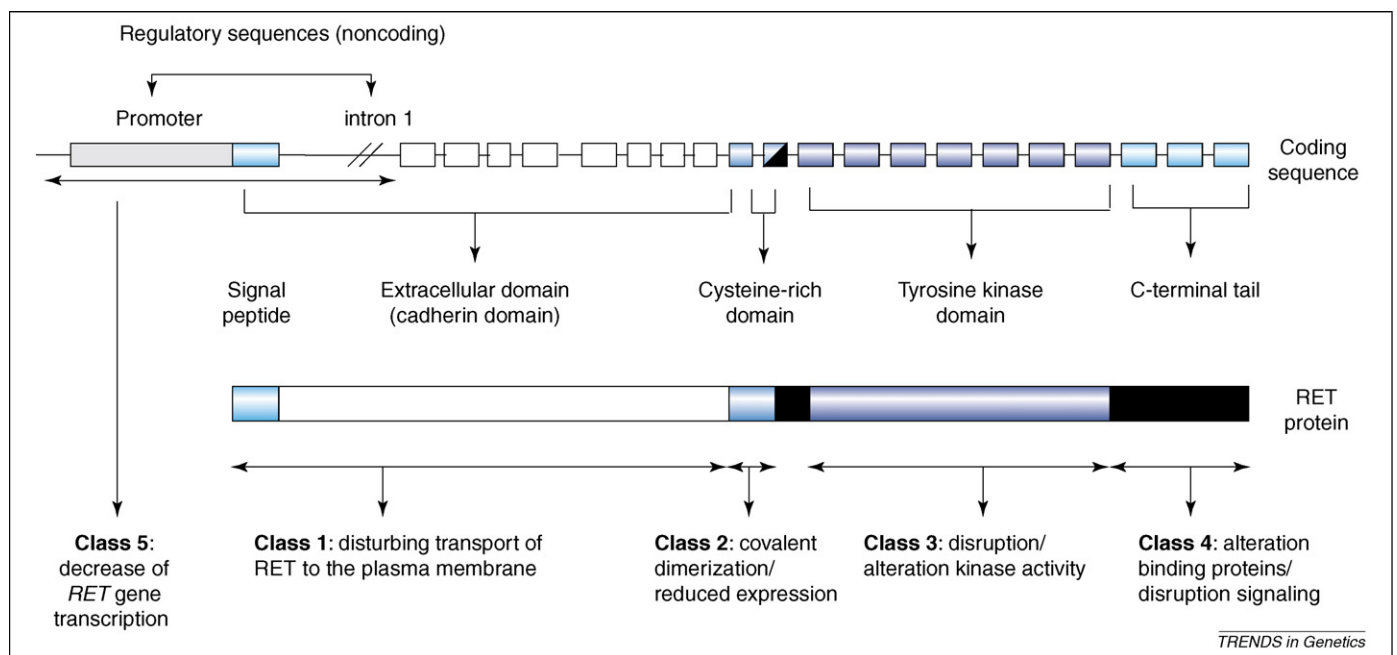

Figure 4. Classification of Hirschsprung-associated *RET* mutations. Shown are the different types of *RET* mutation associated with Hirschsprung disease, the domains of the gene or protein affected, and their functional consequences. The mutations are classified as follows. Class 1, mutations affecting coding sequences in the extracellular domain of *RET*, resulting in disturbed transport of RET to the plasma membrane. Class 2, mutations affecting the cysteine-rich domain of *RET*, resulting in covalent dimerization of the RET proteins and reduced expression of RET at the plasma membrane. Class 3, mutations targeting the kinase domain of *RET*, causing disruption or alteration of the catalytic activity of the receptor. Class 4, mutations located at the C-terminal tail, affecting protein-binding sites and thus disrupting signaling. Class 5, mutations located in regulatory sequences (promoter and intron 1), resulting in a decrease in *RET* mRNA levels.

Table 3. Biological properties of different RET mutant proteins in NIH-3T3 cells and mice

Codon mutated in *RET*	Transforming activity in NIH-3T3	Maturation of the protein	Promoter/knock-in /knockout	Mouse phenotype	Refs
C6090Y	+	++			[78]
C611Y	+	++			[78]
C618Y	+	++			[78]
C620R	++	++			[78]
C630R	++				[78]
C634R	+++/+++++	++++	Calcitonin gene related peptide/calcitonin promoter	MTC	[79]
C634R			Moloney murine leukemia virus repeat	C-cell hyperplasia/MTC in 50% of mice; mammary or parotid gland adenocarcinoma	[80]
A883F	+++++				[81]
M918T	+++++		Dopamine-hydroxylase promoter	Benign neuroglial tumors	[81–82]
			Knock-in (heterozygous) Calcitonin promoter	C-cells and chromaffin cells hyperplasia	[83]
				C-cells and chromaffin cell hyperplasia (early onset) and male reproductive defects MTC (in 3 out of 8)	[84]
RET			Knockout	Renal dysgenesis and lack of enteric neurons; C-cells affected	[85]

SHC are stronger for RET-MEN2B than for RET-MEN2A mutants, resulting in higher activation of the Ras/ERK and the PI3K/AKT signaling pathways [25]. Also, interestingly, the ligand-induced activation of AKT by wild-type RET is dependent on the localization in lipid rafts, and this regulatory mechanism is bypassed by RET-MEN2A [30].

Finally, we should mention STAT3, a latent transcription factor that is involved in the activation of many cytokine- and growth-factor-inducible genes but that, when aberrantly activated, contributes to cancer development [31]. Transactivation of STAT3 by the RET-MEN2A mutant RETC634R is required for cellular transformation in a process that is independent of JAKs and SRC [32]. By contrast, the FMTC-associated mutants RETY791F and RETS891A implicate SRC and JAKs in constitutive activation of STAT3 [33]. Moreover, Yuan *et al.* [34] have demonstrated that RET-MEN2B mutants interact with and activate STAT3 more strongly than do RET-MEN2A mutants. These data support previous findings showing that RET-MEN2B mutants are specifically associated with SRC, of which STAT3 is one of the best-characterized targets [35]. Notably, we have found that STAT3 is activated only by gain-of-function MEN2-associated RET mutants, and not by ligand-stimulated wild-type RET (Ivan Plaza-Menacho, unpublished). The different mechanisms of receptor activation by wild-type RET and MEN2-associated RET mutants are shown in Figure 5.

Taken all together, these studies indicate that specific signaling profiles are connected with specific MEN2-associated *RET* mutations. A better understanding of the molecular mechanisms underlying this cancer
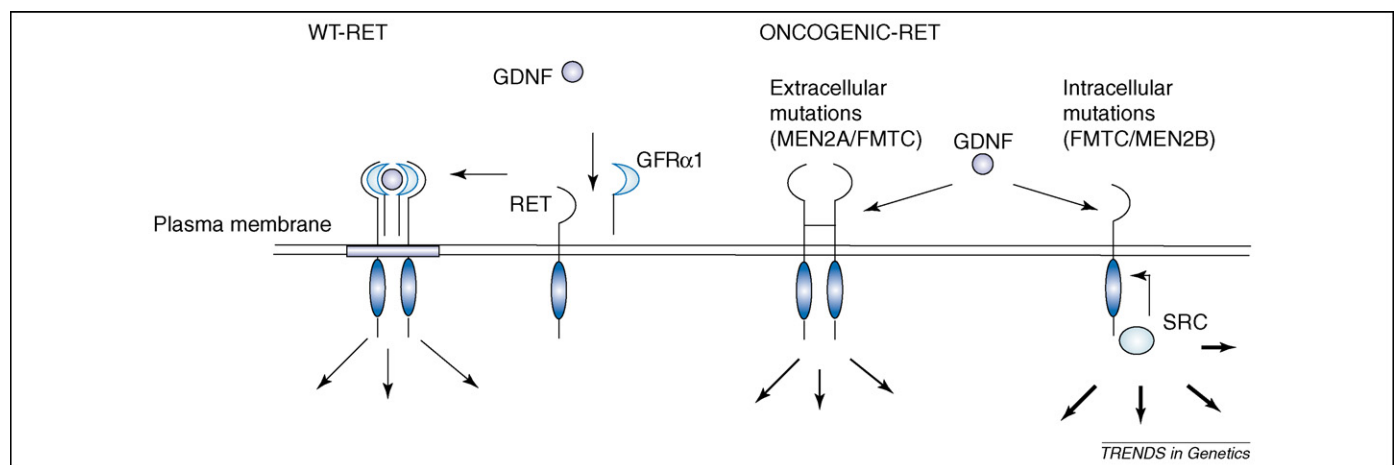

Figure 5. Possible mechanisms of activation of wild-type RET and MEN2-associated *RET* mutations. **(a)** Activation of wild-type RET: the ligand (GDNF) first binds to the GPI-anchor co-receptor 1 (GFRα1); RET is then recruited to form a macromolecular complex receptor. **(b)** Constitutive activation of RET by mutations affecting the cysteine-rich domain that cause covalent dimerization of the (mutant) receptor. **(c)** Aberrant activation of mutations affecting the tyrosine kinase domain of RET, resulting in monomeric oncoproteins with altered catalytic activity and altered substrate specificity that preferentially recognize substrates of cytoplasmic tyrosine kinases such as SRC or ABL. GDNF further enhances both RET-MEN2A and RET-MEN2B activity.

syndrome might ultimately help us to design new therapeutic strategies for this disease.

Polymorphisms and haplotypes in RET-associated disorders

Not only do high penetrant germline *RET* mutations have a key role in disease development, but also *RET* polymorphisms and haplotypes, containing disease-associated mutations, exist that are believed to be genetic modifiers and might be associated with an increased relative risk for the development of disorders derived from neural crest cells. These polymorphisms might interact with other genetic variants or with disease-associated germline mutations, modulating the disease phenotype or age of onset. How these polymorphisms contribute to disease development is largely unknown, but there are several possibilities: missense polymorphisms might alter the function of the protein, silent (coding) polymorphisms might be involved in aberrant splicing, and noncoding variants could regulate the level of expression. Because polymorphisms are comparatively common in the population, they could bestow a much higher attributable risk on the general population as compared with rare mutations in high-penetrance disease susceptibility genes such as *RET*.

MEN2 and FMTC

It has been suggested that the *RET* polymorphisms G691S and S904S have a modifier effect on the age of onset of MEN2A [36], and the same has been concluded for the single-nucleotide polymorphism (SNP) L769L in combination with the FMTC germline mutation Y791F [37]. It has also been proposed that the polymorphism G691S might be functional because it probably creates a new phosphorylation site affecting downstream signaling [36], or it might change the secondary structure of *RET* [38].

Sporadic MTC

Several *RET* polymorphisms have been described in association with sporadic MTC. The SNPs S836S [39,40] and IVS1–126G → T have been found to be overrepresented and associated with the somatic mutation M918T; however, other studies have not confirmed this finding [37,41, 42]. A specific haplotype (the so-called 'CGGATGCCAA haplotype') containing the SNPs G691S and S904S has proved to be associated with sporadic MTC [38]. Both G691S and S904S had been previously associated with sporadic MTC and MEN2A [36,43].

Interestingly, SNP IVS14–24G → A, which was originally interpreted as a Hirschsprung mutation [44], has now been observed more frequently in sporadic MTC [37]. Another study has found, however, that IVS14–24G → A is not associated with either Hirschsprung or sporadic MTC [45]. Finally, a haplotype with a protective effect for sporadic MTC has been recently identified [38].

Sporadic pheochromocytoma

A low-penetrance *RET* haplotype comprising the wild-type allele at IVS1–126 and IVS1–1463 and a 16-base-pair intron-1 deletion 5′ of these SNPs is strongly associated with and overrepresented in sporadic pheochromocytoma

[46]. The significant association between an individual's age of diagnosis and genotype suggests that the additive effect of the haplotypes can modulate the age of onset of the disease.

In summary, several different *RET* polymorphisms and haplotypes have been associated with different MEN2-related endocrine tumors. For almost all polymorphisms analyzed however, the data are inconsistent, most probably because of the small sample sizes analyzed. Thus, the significance and mechanism of action of these potential genetic modifiers remain to be demonstrated.

Hirschsprung disease

Inactivating coding sequence mutations in *RET* are responsible for a dominant form of Hirschsprung disease (with incomplete penetrance), and mutations are found in up to 50% of the familial and 15% of the sporadic cases [47]. However, ample evidence indicates that, in addition to coding sequence mutations, noncoding regions of the *RET* gene also have a chief role in the development of this disease.

This was concluded from association studies on Hirschsprung populations living in The Netherlands, Italy, France, Spain, USA and Hong Kong. The same disease-associated haplotype was observed in the 5′ region of the *RET* locus in 56–62% individuals of European descent (including Americans) [48–55]. The same haplotype was also present in Asians, although at a much higher frequency (85%) [52,54]. The common haplotype spans a 27-kb region (4 kb of the 5′ UTR, exon 1, intron 1 [23 kb] and exon 2 of *RET*) and strongly indicates the existence of a regulatory mutation or mutations located either in the promoter region or in intron 1 of *RET*. In addition, most of the individuals found to carry the Hirschsprung haplotype were homozygous for it. Because individuals who are homozygous for the disease-associated haplotype have a tenfold higher risk of developing the disease than heterozygous carriers, a dose-dependent action of the unknown mutation has been suggested [51].

Several groups have characterized candidate disease-associated polymorphisms [49,55–57]. Two promoter SNPs, rs10900296 and rs10900297 (also named SNP-5G > A and SNP-1A > C, respectively), located just upstream of the *RET* transcriptional start site, have been shown to reduce *RET* promoter activity in luciferase reporter assays [49,55], although others have shown that this effect is dependent on the cell lines studied [57].

Two groups have described two closely located SNPs, rs2435357 and rs2506004, in intron 1 as disease-causing candidates on the basis of association studies, functional assays and comparative genomics [52,56]. In particular, the last approach seems to be useful for judging the functional relevance of noncoding mutations, because it shows that the two disease-causing candidates are located in regulatory binding sites, which in turn are in regions that are conserved among different vertebrate species. Furthermore, mutant alleles from rs2435357 and rs2506004 show the highest associated values in affected individuals, and Emison *et al.* [52] have found that the region containing both of these SNPs reduces promoter activity in reporter assays. In addition, Grice *et al.* [58] recently generated

LacZ reporter mice that express the MCS+9.7 *RET* intron 1 region containing both rs2435357 and rs2506004 in the context of the *hsp68* promoter. Interestingly, they found that the MCS+9.7 region drives a pattern of *LacZ* expression that resembles the temporal and spatial expression of *RET* during embryonic development, which is a good indication of the relevance of this particular region for regulating *RET* expression. The transcription factor that binds to the sequence studied by Grice *et al.* [58], however, remains unidentified.

Nevertheless, many affected individuals do not have any (RET) mutations. In these individuals, other disease susceptibility factors must be present in the form of rare noncoding *RET* mutations or mutations at other loci either in coding or regulatory sequences.

RET as a central factor in Hirschsprung development

So far, almost all Hirschsprung-causing mutations are found in genes encoding proteins involved in the RET and endothelin receptor type B (EDNRB) signaling pathways [20]. At first, these two signaling pathways seemed to be completely unrelated. Now, however, growing evidence indicates that these two key signal transduction routes interact directly in the development of the ENS, at both the genetic and the biochemical level [53,59,60]. In a population of Old Order Mennonites, for example, it has been shown that specific alleles of the *RET* and the *EDNRB* loci are nonrandomly associated with Hirschsprung disease and are jointly transmitted to individuals much more often than expected [53,61].

To test the functional connection between the RET and EDNRB pathways, McCallion *et al.* [59] generated intercrossed mice from existing strains, resulting in offspring that were compound heterozygous for a null *RET* allele (truncating deletion at Lys748) and loss-of-function *EDNRB* alleles (*s*, piebald; *s*^{l}, piebald lethal) [59]. It was thought, in particular, that the combination of *Ret* and *Ednrb* genotypes (*Ret*$^-$/*Ret*$^+$; *Ednrb*^{s}/*Ednrb*^{s}) explained better the Hirschsprung transmission and phenotype observed in humans than did the independent mutants, thereby indicating interactions between the two pathways. McCallion *et al.* [59] also showed that 100% of the male offspring with this genotype were affected; similarly, all females manifested variable length of an aganglionic colon, although 30% had reduced clinical expression of Hirschsprung disease, which to some extent explains the sex bias observed in humans. Furthermore, compound genotypes did not have strong effects on melanocyte or renal development as compared with isolated genotypes, showing that the interaction of these genes might be specific to ENS development [59].

Work of Barlow *et al.* [60] in mice has elaborated on the spatial and temporal regulation of neural crest migration and ENS formation by the RET and EDNRB transduction routes. Seventy per cent of double homozygotes for *Ret*^{51} and *Et-3*^{ls} (the null allele of the ligand of Ednrb) developed total intestinal aganglionosis, again indicating a strong interaction between these two loci [62]. cAMP-dependent protein kinase A (PKA) has been pinpointed as a likely candidate that mediates interactions between the RET and EDNRB pathways, because inhibition of PKA mimics the effect of ET-3 (proliferation and inhibition of migration) on neural crest cells (NCCs) [60]. One of the possible mechanisms of PKA action on RET signaling is via Ser696 of RET. Phosphorylation of this residue by PKA promotes lamellipodia formation in neuroectodermal cells and thereby stimulates migration [63].

When the genetic and biochemical evidence is taken together, the central role of *RET* in ENS development and Hirschsprung disease is unquestionable. Nevertheless, regulation of RET signaling by other receptors, growth and transcription factors along the developing gut is certainly necessary for proper development of the ENS.

Hirschsprung combined with MEN2

Mutations of extracellular residues Cys609, Cys611, Cys618 and Cys620, which were primarily thought to be specific for MEN2A and FMTC, have also been identified in individuals with (sporadic) Hirschsprung disease and in MEN2A families in which Hirschsprung sometimes segregates with MEN2A or FMTC [64]. These mutations have a dual impact on RET. On the one hand, they constitutively activate RET through the formation of covalent dimers; and on the other hand, they result in a marked reduction of RET expression at the plasma membrane. This leads to uncontrolled proliferation of thyroid C-cells, as seen in MEN2A and FMTC, and in apoptosis of enteric neurons, as seen in Hirschsprung disease. The reason why C-cells hyperproliferate and enteric neurons undergo apoptosis is probably due to differences in sensitivity to GDNF. MEN2A mutations such as C634R are responsive to GDNF, whereas Hirschsprung–MEN2A and Hirschsprung–FMTC mutations of *RET* (e.g. C620R) do not respond to GDNF [65]. Insensitivity to GDNF renders cells more prone to apoptosis, and these features are shared by all Hirschsprung-associated mutations of *RET* [66].

RET as a therapeutic target

Using drugs to target various (constitutively activated) tyrosine kinases has been a recent success in the fight against cancer; for example, Herceptin, Imatinib and Gefitinib have been successfully used in breast cancer, gastrointestinal stromal tumors and non-small-cell lung cancers, respectively [67]. This success has directed attention to RET as a possible therapeutic target in MEN2 and FMTC because so far no systematic treatment for individuals with MEN2 is available [68]. All of the crucial steps in the activation and signaling of RET might be subject to specific inhibitors.

The most potent therapeutic drugs are expected to come from tyrosine kinase inhibitors. Imatinib (Glivec or Gleevec) shows inhibitory activity against RET-MEN2A and RET-MEN2B in MTC-derived cell lines and induces degradation of RET; however, the concentrations needed to inhibit cellular proliferation are high [69,70]. Encouraging results have been obtained with another tyrosine kinase inhibitor, BAY43–9006 [71], although resistance might become a problem, as has been previously demonstrated for RET [72].

Another elegant approach to targeting RET might be the inhibition of RET dimer formation. The neutralizing, nuclease-resistant D4 aptamer can bind and inhibit

wild-type RET and RET-MEN2A on the cell surface [73]. The efficacy of D4 as a therapy for RET-associated tumors remains to be established.

Another approach might be to use SHP-1, a cytoplasmic phosphatase that can associate with mutated RET, thereby reducing its degree of autophosphorylation and consequently suppressing growth-promoting signals mediated by the RET-induced MAPK pathway [74,75]. This reduction in autophosphorylation is activated by somatotropin release-inhibiting factor [74].

Another option that has been explored is to target RET with monoclonal antibodies. Yano *et al.* [76] have generated an antibody that can induce internalization of RET, but its efficacy has not been demonstrated. Alternatively, gene therapy might become available. Inhibition of oncogenic RET signaling by expression of a dominant-negative RET mutant has been investigated and reviewed by Putzer *et al.* [68]. Another gene therapy approach might be the introduction of a RET-selective ribozyme that specifically cuts mutant *RET* mRNA and blocks RET-mediated cell growth and transformation [77].

In addition to the above approaches, which all focus on RET, one might of course target downstream signaling proteins of (mutant) RET. In summary, the RET receptor represents a potential theraupetic target in the neuroendocrine tumors observed in MEN2, in which RET dysfunction has a crucial role.

Conclusions

In conclusion, *RET* provides an excellent example of how mutations, either alone or as part of a polygenic model, can give rise to different (inherited) human diseases by altering the signaling properties and transcriptional regulation of the protein encoded. Unraveling the genetic and molecular mechanisms underlying the different RET-related neural crest disorders not only has been a success in the history of genetic medicine but also has helped us to understand how these different diseases develop and might contribute to the development of new therapeutic strategies.

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