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An Update on $P2Y_{13}$ Receptor Signalling and Function

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Abstract

The distribution of nucleotide P2Y receptors across different tissues suggests that they fulfil key roles in a number of physiological and pathological conditions. $P2Y_{13}$ is one of the latest P2Y receptors identified, a novel member of the Gi-coupled P2Y receptor subfamily that responds to ADP, together with $P2Y_{12}$ and $P2Y_{14}$. Pharmacological studies drew attention to this new ADP receptor, with a pharmacology that overlaps that of $P2Y_{12}$ receptors but with unique features and roles. The *P2RY12–14* genes all reside on human chromosome 3 at 3q25.1 and their strong sequence homology supports their evolutionary origin through gene duplication. Polymorphisms of $P2Y_{13}$ receptors have been reported in different human populations, yet their consequences remain unknown. The $P2Y_{13}$ receptor is versatile in its signalling, extending beyond the canonical signalling of a Gi-coupled receptor. Not only can it couple to different G proteins (Gs/Gq) but the $P2Y_{13}$ receptor can also trigger several intracellular pathways related to the activation of MAPKs (mitogen-activated protein kinases) and the phosphatidylinositol 3-kinase/Akt/glycogen synthase kinase 3 axis. Moreover, the availability of $P2Y_{13}$ receptor knockout mice has highlighted the specific functions in which it is involved, mainly in the regulation of cholesterol and glucose

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metabolism, bone homeostasis and aspects of central nervous system function like pain transmission and neuroprotection. This review summarizes our current understanding of this elusive receptor, not only at the pharmacological and molecular level but also, in terms of its signalling properties and specific functions, helping to clarify the involvement of $P2Y_{13}$ receptors in pathological situations.

Keywords
Cholesterol metabolism • GSK3 • MAP kinases • Nervous system • Neuroprotection • $P2Y_{13}$ receptor • Pain

Abbreviations

ADP	Adenosine 5′-diphosphate.
Ap_3A	P1,P3-Di(adenosine-5′) triphosphate.
Ap_4A	P1,P4-Di(adenosine-5′) triphosphate.
ATP	Adenosine 5′-triphosphate
cAMP	Adenosine 3′,5′-cyclic monophosphate.
CT1007900	(6-[1-(2-Dimethylaminopyrimidin-5-ylmethyl)-piperidin-4-yl]-2-morpholin-4-yl-pyrimidin-4-ol monohydrate).
GPCRs	G protein–coupled receptors.
GSK3	Glycogen synthase kinase 3.
HDL	High density lipoprotein.
2MeSADP	2-methylthio-adenosine 5′-diphosphate.
MAP kinases	Mitogen-activated protein kinases.
MRS2211	2-[(2-Chloro-5-nitrophenyl)azo]-5-hydroxy-6-methyl-3-[(phosphonooxy)methyl]-4-pyridinecarboxaldehyde.
MRS2179	2′-Deoxy-N6-methyl adenosine 3′,5′-diphosphate.
PLC	Phospholipase C.
PI3K	Phosphatidylinositol 3-kinase.
PPADS	Pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid).
RCT	Reverse cholesterol transport
SNP	Single-nucleotide polymorphism.

1 Introduction

$P2Y_{13}$ is a G-protein-coupled receptor (GPCR) that is included along with $P2Y_{12}$ and $P2Y_{14}$ in the P2Y subgroup of receptors, of which the main physiological agonist is ADP. GPCRs are a very large family of membrane proteins that account for approximately 2% of all the genes in the human genome. These receptors control a wide range of key physiological functions and they are also the pharmacological target to treat a large number of prevalent human diseases. P2Y receptors belong to the rhodopsin family, also known as Class A GPCRs, and based on sequence homology they have been included in the δ subfamily of Class A GPCRs, which also contains glycoprotein receptors, protease-activated receptors and olfactory receptors. Based on their pharmacology, signal transduction and structure, P2Y receptors are classified into two main subfamilies. The first subgroup are coupled to phospholipase C (PLC) via Gq proteins and it includes the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors. Conversely, the "$P2Y_{12}$-like" subfamily that contains the $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors preferentially signal through Gi proteins, and they mainly inhibit adenylate cyclase activity or they regulate ion channel activity (von Kugelgen and Hoffmann

2016). Currently, the only crystallographic structure available for these "$P2Y_{12}$-like" receptors is that of $P2Y_{12}$ (Zhang et al. 2014a; b), confirming to a large extent its inclusion in the δ subfamily receptors, although some of their specific features do not exactly match those of the δ or α subfamilies of Class A GPCRs. Whether the structural characteristics of $P2Y_{12}$ are shared by all P2Y family members, or if they are specific to the $P2Y_{12}$-like ADP-Gi coupled receptors, will require studies of the structure of these other receptors. Notably, the $P2Y_{13}$ receptor is closely related to $P2Y_{12}$.

Historically the discovery of ADP receptors is closely associated with platelet function. There is an abundance of adenine nucleotides inside the dense granules of platelets, mainly ADP together with serotonin, and the release of the content of these granules induces platelet aggregation and clot formation. In fact, ADP was the first known aggregating agent and hence, a search began for specific ADP receptors in platelets. $P2Y_1$ was the first P2Y receptor family described in a platelet model and it was shown to be activated by ADP, changing the commonly accepted idea that ATP was its main agonist. This receptor was isolated by hybridization screening of a cDNA library generated from the embryonic chick brain (Webb et al. 1993). $P2Y_1$ is coupled to the Gq protein, activating the PLC pathway and mobilizing internal calcium stores in most cellular models, including platelets (Hechler et al. 1998; Savi et al. 1998). Platelets challenged with ADP also produce Gi-mediated inhibition of adenylyl cyclase, which lowers the cAMP available (Gachet et al. 1997). However, the specific ADP-receptor subtype coupled to this inhibition of adenylyl cyclase remained elusive until it was associated with clinical bleeding disorders, and to the absence of a response to the ADP-selective anti-aggregating drugs ticlopidine and clopidogrel (Cattaneo and Gachet 1999). Thus, the association of familial bleeding disorders with the anti-aggregant pharmacology was the key to hunt this ADP receptor.

The identification of a platelet ADP receptor targeted by antithrombotic drugs was preceded by the isolation of a functional P2 receptor that responded equally to ATP and UTP, leading to it being named P2U, currently $P2Y_2$ (Alexander et al. 2015; Lustig et al. 1993). $P2Y_{12}$ was the first nucleotide receptor to be associated with a genetic defect, a familial bleeding disorder (Hollopeter et al. 2001), and its identification facilitated the development of powerful antiplatelet aggregating agents that are among the most effective drugs in preventing cardiovascular diseases and ictus. Subsequently, the orphan GPCR SP1999 was shown to be the cognate receptor for ADP (Zhang et al. 2001), also corresponding in sequence to that described by Hollopeter and co-workers (2001). This receptor was linked to Gαi and it is expressed strongly in neural tissues and blood platelets. Since then the search for other homologous P2Y receptors became more intense.

Following a similar strategy as for $P2Y_{12}$, the orphan GPCRs GPR86 and SP174 were identified as ADP receptors and named $P2Y_{13}$ (Communi et al. 2001; Zhang et al. 2002). This new receptor couples to Gi proteins and it shares a high degree of sequence homology to $P2Y_{12}$, as well as a similar rank order of potency for ADP and analogues. The comparison of human and mouse $P2Y_{13}$ receptors demonstrated approximately 75% sequence homology and a similar pharmacological profile, although ADP and nucleotide analogues appear to act more potently on the murine receptor (Zhang et al. 2002). More extensive pharmacological characterization of the human $P2Y_{13}$ receptor has also been carried out, allowing further functional studies to discriminate the distinct P2Y-Gi-coupled receptors (Marteau et al. 2003).

The clinical and pharmacological relevance of $P2Y_{12}$ has somehow cast a shadow on the important physiological role of $P2Y_{13}$. Thus, in this review, we will try to provide an overview of this less well known family member, bringing together the data available regarding different aspects of this multi-faceted receptor. The review is organized in sections to make its content more comprehensible and accessible. The pharmacology of $P2Y_{13}$ with respect to other P2Y receptors represents a good starting point, which is followed by a comparative study of the sequence of $P2Y_{13}$ and its known single nucleotide polymorphisms (SNPs). The use of genetically

modified animals and a better understanding of the mechanisms that control its expression will be particularly useful to assign specific cellular responses to $P2Y_{13}$. This is particularly relevant when attempting to understand the signals generated by this receptor given the number of complex pathways it can activate. This signalling is clearly related to the wide range of essential functions that $P2Y_{13}$ performs in the control of relevant physiological functions, such as protection from oxidative and genotoxic stress, lipoprotein mobilization in cholesterol metabolism or pain, to mention just a few. All these examples indicate that $P2Y_{13}$ is a key receptor in the purinergic field, and that better understanding its physiology will provide us with useful tools to cope with pathophysiological situations that could be relevant to human disease.

2 Pharmacology of the $P2Y_{13}$ Receptor

Regarding the pharmacological profile of $P2Y_{13}$ receptors, they share a characteristic preference for ADP. ADP is the most potent agonist of the naturally occurring nucleotides that act on the $P2Y_{13}$ receptor, stimulating the receptor at an EC_{50} in the nanomolar range (Communi et al. 2001; Zhang et al. 2002; Marteau et al. 2003) a pharmacological feature shared with the $P2Y_{12}$ receptor (Hollopeter et al. 2001; Zhang et al. 2001). Another P2Y receptor that prefers ADP and that is potently activated by this nucleoside diphosphate is $P2Y_1$ (Leon et al. 1997). The $P2Y_{13}$ receptor also responds to adenine diphosphate analogues adenosine such as 2-methylthio-ADPβS (2MeSADP) or 5′-*O*-(2-thiodiphosphate) (Fig. 1) (Communi et al. 2001; Zhang et al. 2002; Marteau et al. 2003). In some cellular systems, 2MeSADP proved to be more potent than ADP, whereas under other experimental conditions both compounds were equipotent, possibly reflecting the distinct affinity for 2MeSADP or ADP of multiple active conformations of the $P2Y_{13}$ receptor, as well as differences in their preference for G proteins (Marteau et al. 2003). ATP and 2-methylthio-ATP appear to be partial and weak agonists of the $P2Y_{13}$ receptor (Marteau et al. 2003). Although the interaction of the $P2Y_{12}$ and $P2Y_{13}$ receptors with nucleotide analogues follows a similar pharmacological profile, inosine diphosphate is about fivefold more potent for human $P2Y_{13}$ than for $P2Y_{12}$ receptors. Moreover, inosine diphosphate acts more potently on murine $P2Y_{13}$ than human $P2Y_{13}$ and $P2Y_{12}$ receptors, with an EC_{50} of 9.2, 552 and 3180 nM, respectively (Zhang et al. 2002).

In addition to conventional mononucleotide agonists, dinucleotides have also been tested for their ability to stimulate the $P2Y_{13}$ receptor. Diadenosine triphosphate (Ap_3A) potently activates the $P2Y_{13}$ receptor, whereas higher diadenosine polyphosphate homologues (Ap_4A, Ap_5A and Ap_6A) are inactive (Zhang et al. 2002; Marteau et al. 2003). A similar profile has been observed with the $P2Y_1$ receptor (Patel et al. 2001), suggesting that selective sensitivity to Ap_3A is a common feature of ADP receptors. It has been hypothesized that dinucleoside triphosphates can structurally mimic nucleoside diphosphates (Shaver et al. 2005). Indeed, Ap_3A can be stored in secretory vesicles in neural and neuroendocrine tissues through the activity of a broad specificity vesicular nucleotide transporter capable of internalizing a wide variety of nucleotides, as well as the diadenosine polyphosphates (Gualix et al. 1997). Moreover, Ap_3A has been identified in microdialysis samples from the cerebellum of conscious, freely moving rats under basal conditions (i.e.: in the absence of any exogenously added stimuli). The extracellular concentration of Ap_3A in cerebellar interstitial fluid (10.5 nM) is double that of the other diadenosine polyphosphates detected (Ap_4A and Ap_5A) and it is in a range that allows this dinucleotide to interact with the $P2Y_{13}$ receptor (Gualix et al. 2014).

Regarding antagonists, the human $P2Y_{13}$ receptor is blocked by suramin, reactive blue-2 and high concentrations of the selective purinergic P2X antagonist, PPADS (Marteau et al. 2003). In recent years, PPADS analogues have been designed in an effort to identify more potent and/or selective $P2Y_{13}$ receptor antagonists. Among them, the 2-chloro-5-nitro

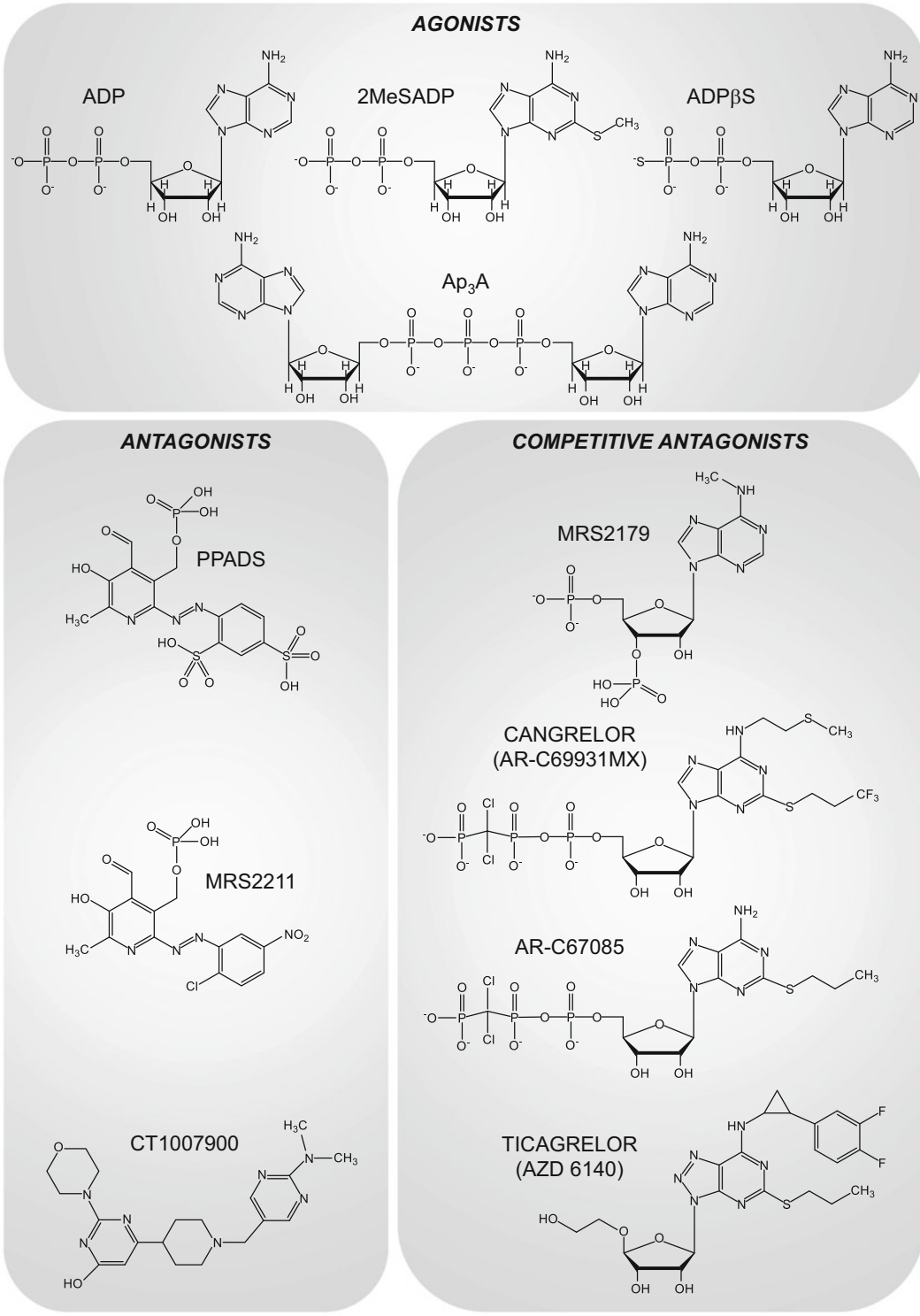

Fig. 1 Structures of agonists and antagonists of ADP receptors

analogue MRS2211 (2-[(2-Chloro-5-nitrophenyl) azo]-5-hydroxy-6-methyl-3-[(phosphonooxy) met hyl]-4-pyridinecarboxaldehyde) proved to be 45-fold more potent than PPADS as a competitive antagonist of the human $P2Y_{13}$ receptor, with a pA_2-value of 6.3 (Kim et al. 2005). Moreover, MRS2211 is >20-fold more selective as an antagonist at the $P2Y_{13}$ receptor than of the $P2Y_1$ and $P2Y_{12}$ receptors, cangrelor (Kim et al. 2005). The $P2Y_{12}$ antagonists and AR-C67085 also block the human $P2Y_{13}$ receptors, and cangrelor apparently acts 100 times more potently than AR-C67085. By contrast to its competitive interaction with the $P2Y_{12}$ receptor, cangrelor depressed the maxima of the agonist dose-response curves in studies on the recombinant human $P2Y_{13}$ receptor, compatible with a non-competitive interaction (Marteau et al. 2003). The rat $P2Y_{13}$ receptor is also blocked by nanomolar concentrations of cangrelor but not by the selective $P2Y_1$ antagonist MRS2179 (2′-Deoxy-N6-methyl adenosine 3′,5-′-diphosphate), even when used at a concentration as high as 100 μM (Fumagalli et al. 2004). However, cangrelor is a partial agonist of the mouse $P2Y_{13}$ receptor, enhancing $P2Y_{13}$-mediated high density lipoprotein (HDL) endocytosis by hepatocytes more potently than its endogenous agonist, ADP (Jacquet et al. 2005). Ticagrelor is an antagonist of the $P2Y_{12}$ receptor approved for the prevention of thromboembolic events in patients with acute coronary syndrome. Ticagrelor and its active metabolite, TAM, act as $P2Y_{13}$ antagonists in a transfected cell system *in vitro*, although they had no impact on $P2Y_{13}$-regulated pro-platelet formation by human megakaryocytes in culture (Bjorquist et al. 2016). Ap_4A has also been described as a complete antagonist of the human $P2Y_{13}$ receptor, with an IC_{50} of 216 nM (Marteau et al. 2003).

Given the important roles that $P2Y_{13}$ and $P2Y_{12}$ receptors play, it will be crucial to obtain selective ligands that can discriminate between them, capable of distinguishing the influence of $P2Y_{13}$ receptors on lipid transport and metabolism, and on bone formation, as deduced from studies on knockout animals (see Sect. 4). Due to the similarities between the $P2Y_{12}$ and $P2Y_{13}$ receptors in terms of their activation by nucleotide analogues, possibly reflecting a similarity in their agonist binding sites, allosteric effectors would be one possible approach to develop selective modulators of the $P2Y_{13}$ receptor, as has already been achieved for other GPCRs (May et al. 2007; Melancon et al. 2012).

3 Sequence Analysis of the $P2Y_{13}$ Receptor

Considering that the $P2Y_{12}$ and $P2Y_{13}$ receptors exhibit similar pharmacological features, we looked into the molecular structure of this receptor subfamily. Alignments of amino acid sequences of human $P2Y_{13}$ with either $P2Y_{12}$ or $P2Y_{14}$ receptors reveal approximately 40–45% identity (Fig. 2, Table 1). Moreover, the *P2RY12*, *P2RY13* and *P2RY14* genes reside on human chromosome 3 at 3q25.1, which would be consistent with gene duplication having led to their evolutionary origin. Conversely, Gq-coupled P2Y receptors share less than 20% identity with $P2Y_{13}$ receptors, even the $P2Y_1$ receptor that has a similar pharmacological profile and chromosomal location (the *P2RY1* gene is situated at human chromosome 3q25.2, in close proximity to the *P2RY12*, *P2RY13* and *P2RY14* genes). Notably, the $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors cluster with *GPR87* in the same region of human chromosome 3, an orphan GPCR that shares 38%, 36% and 44% amino acid identity with the $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors, respectively (Fig. 3). Although the protein encoded by the *GPR87* gene is still to be identified, it would not be a surprise if it were a new P2Y-like receptor, as occurred with GPR17. From a phylogenetic point of view, GPR17 lies in an apparently intermediate position between P2Y and cysteinyl leukotriene receptors, as GPR17 can bind both uracil-nucleotide sugars (UDP, UDP-galactose and UDP-glucose) and cysteinyl leukotrienes (LTD4, LTC4 and LTE4) (Marucci et al. 2016). Interestingly, inhibition of GPR17 by

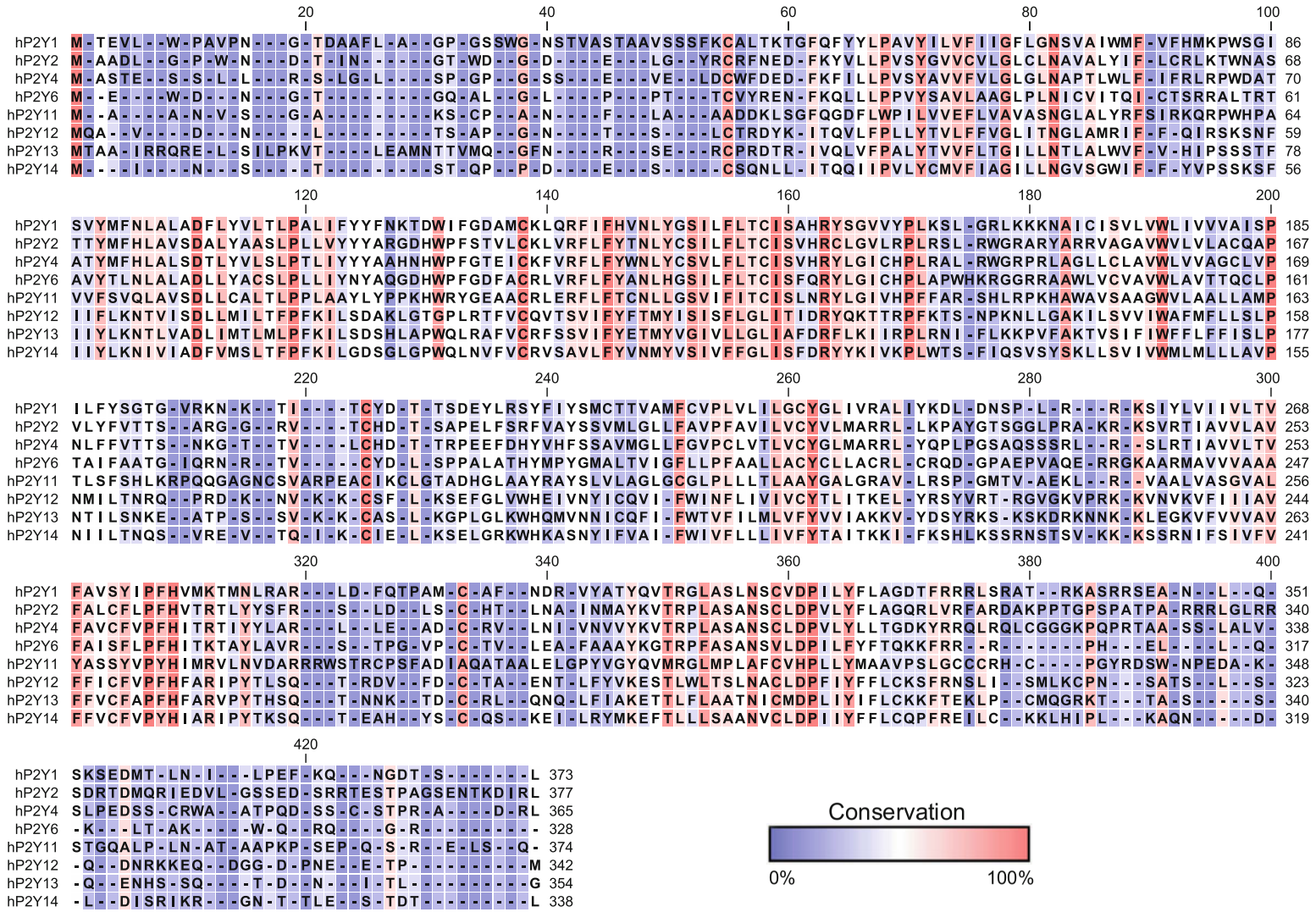

Fig. 2 Comparative alignment of the human P2Y receptor amino acid sequences

Table 1 Similarity (*above the diagonal*) and Jukes-Cantor distance (*below the diagonal*) in globally aligned human P2Y receptor amino acid sequences

	$P2Y_1$	$P2Y_2$	$P2Y_4$	$P2Y_6$	$P2Y_{11}$	$P2Y_{12}$	$P2Y_{13}$	$P2Y_{14}$
$P2Y_1$		33.25	37.21	32.19	25.62	19.39	19.00	20.31
$P2Y_2$	1.15		53.03	35.17	24.13	21.30	19.95	22.51
$P2Y_4$	1.03	0.65		3693	27.20	22.28	22.74	22.99
$P2Y_6$	1.19	1.09	1.04		23.48	22.03	19.73	20.86
$P2Y_{11}$	1.45	1.52	1.38	1.56		16.28	14.60	15.68
$P2Y_{12}$	1.79	1.67	1.62	1.63	2.02		45.43	45.06
$P2Y_{13}$	1.82	1.76	1.59	1.77	2.18	0.81		40.72
$P2Y_{14}$	1.73	1.61	1.58	1.70	2.08	0.82	0.93	

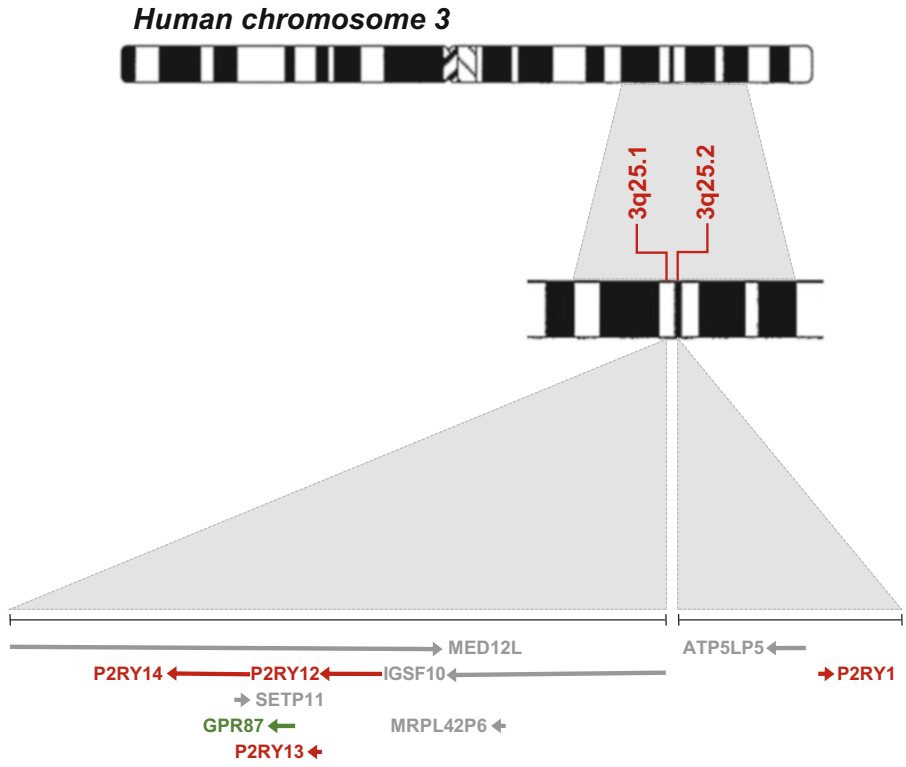

Fig. 3 Chromosomal location of the P2RY genes on human chromosome 3. *P2RY12*, *P2RY13* and *P2RY14* genes cluster on the long arm (band 25.1) of chromosome 3, together with the orphan GPCR *GPR87*. The *P2RY1* gene is also located nearby (band 25.2) (Modified from (Milewicz and Seidman 2000))

montelukast, a well-known anti-asthmatic drug that antagonizes CysLT1R, reduces neuroinflammation, it elevates hippocampal neurogenesis and it improves learning and memory in aged rats (Marschallinger et al. 2015).

Regarding interspecific variation, the alignment of $P2Y_{13}$ receptors from 13 different species reveals that both their nucleotide and amino acid sequences are highly conserved, especially in mammals (Fig. 4). Humans, great apes (chimpanzee *Pan troglodytes*, orangutan *Pongo pygmaeus*, and gorilla *Gorilla gorilla*), and old world monkeys (rhesus macaque *Macaca mulatta*) show more than 95% identity at both the nucleotide and amino acid level. Human and new world monkeys (Ma's night monkey *Aotus*

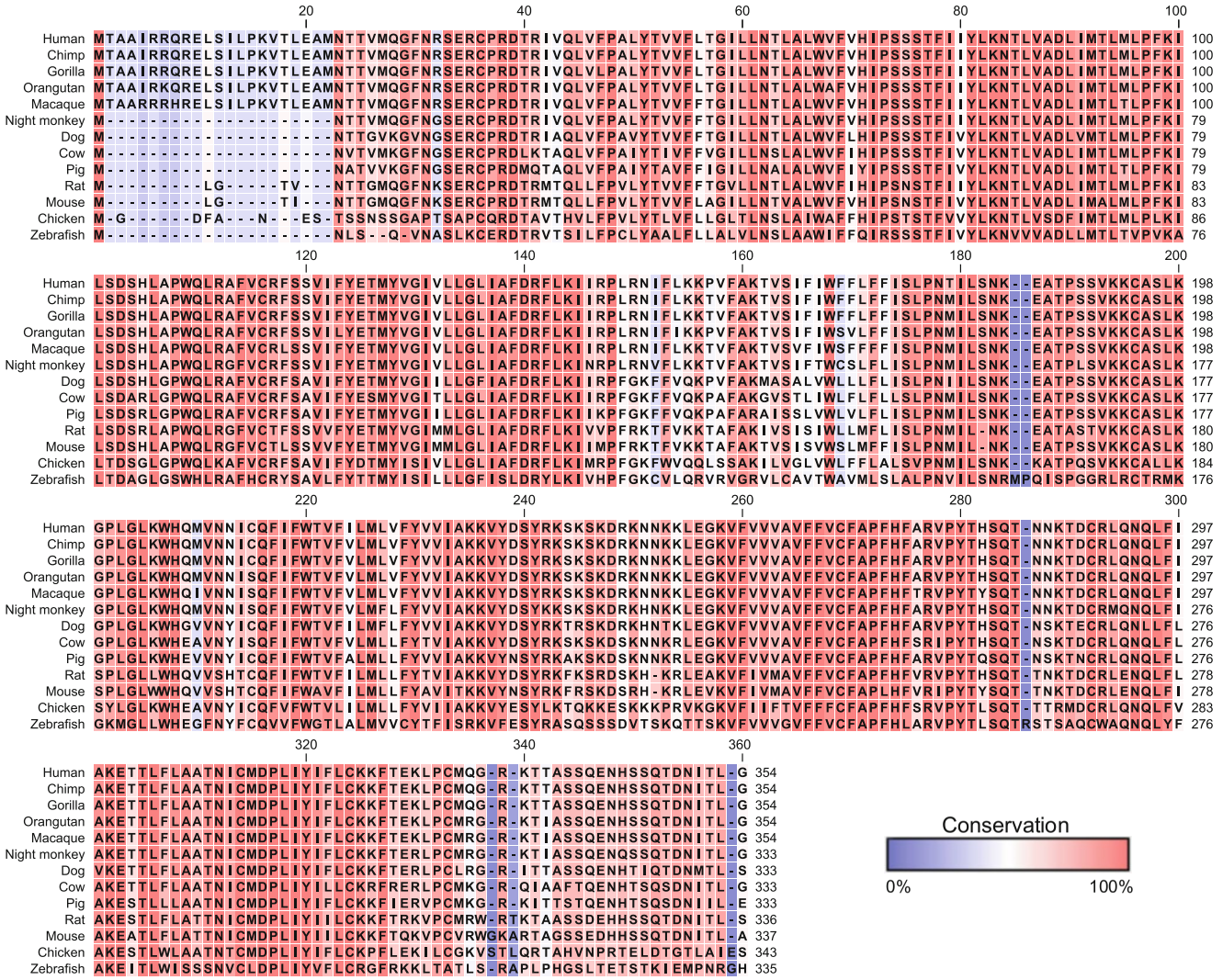

Fig. 4 Alignment of the P2Y$_{13}$ receptors from 13 different species, including mammals, birds and fish

nancymaae), rodents (rat *Rattus norvegicus* and mouse *Mus musculus*) and other mammals with diverse diets (carnivorous dog *Canis familiaris*, herbivorous cow *Bos taurus* and omnivorous pig *Sus scrofa*) show approximately 75–85% sequence identity. Finally, humans and birds (chicken *Gallus gallus*) or fish (zebrafish *Danio rerio*) share less than 60% and 45% identity, respectively (Table 2).

As mentioned previously, the first crystallographic assessment of a "$P2Y_{12}$-like" subfamily member of purinergic receptors was that of human $P2Y_{12}$ receptor, both the agonist- and antagonist-bound structures (Zhang et al. 2014a; b). Key residues involved in both 2MeSADP and AZD1283 binding were Y^{105}, F^{106}, L^{155}, N^{159}, N^{191}, R^{256}, Y^{259} and K^{280}. Other residues specifically participate in 2MeSADP binding, including R^{19}, R^{93}, C^{97}, S^{156}, T^{163}, C^{175}, K^{179}, H^{187} and Q^{263}, whereas V^{102}, Y^{109}, V^{190}, Q^{195}, F^{252} and L^{276} contribute to the interaction with AZD1283. In addition to the AZD1283 binding site (pocket 1), an analysis of the extracellular interface revealed an adjacent ligand-binding region (pocket 2). Both pockets seem to be required for Ap_4A recognition and binding to the receptor. One nucleotide may bind in pocket 1 while the second half of the dinucleotide molecule was predicted to reach pocket 2, the polyphosphate moiety occupying a highly cationic region of the binding site (Zhang et al. 2014a, b). Crystal structure data can be very helpful in optimizing receptor models for structure-guided drug design (Jacobson et al. 2015). Thus, a model of ticagrelor binding to the human $P2Y_{12}$ receptor and homology models of the human $P2Y_{14}$ receptor for ligand docking have recently been described (Kiselev et al. 2014; Paoletta et al. 2015). A similar strategy could be used to study the $P2Y_{13}$ receptor, since neither crystal structure nor homology models of this receptor are as yet available. Remarkably, alignment of the human $P2Y_{12}$ and $P2Y_{13}$ amino acid sequences reveals that most of the key residue positions implicated in agonist/antagonist binding to the $P2Y_{12}$ receptor are conserved in the $P2Y_{13}$ receptor, with the exception of $F^{106}E$, $L^{155}I$ and $T^{163}S$. It is noteworthy that the relative distances between all the residues are also conserved (Fig. 5, Table 3). It is not inconceivable that the change of these 3 amino acids is involved in the pharmacological differences observed between $P2Y_{12}$ and $P2Y_{13}$ receptors.

4 Single Nucleotide Polymorphisms (SNPs) of the Human *P2RY13* Gene

Many human diseases have a causative association with genetic components and recent advances have significantly improved our understanding of the causal effects of genetic changes associated with a growing number of diseases. There is currently a great deal of information regarding the SNPs in genes associated with disease, including those affecting the genes encoding human purinergic receptors. SNPs are widespread in genes encoding ionotropic nucleotide receptors, particularly the *P2RX7* gene (Caseley et al. 2014), variants of which are associated with altered chronic pain sensitivity, cardiovascular risk, susceptibility to affective mood disorders, multiple sclerosis, childhood febrile seizure, osteoporosis, tuberculosis, toxoplasmosis and sepsis. Furthermore, SNPs in the *P2RX2* gene are associated with susceptibility to hearing loss, and a *P2XR4* gene variant is related to high pulse pressure and age-related macular degeneration.

Concerning P2Y receptors, non-synonymous SNPs in the *P2RY1* and *P2RY12* genes are mainly associated to alterations in platelet aggregation. Thus, the A1622G mutation of the *P2RY1* gene could contribute to an inadequate platelet response to anti-coagulants, which would be associated with a higher risk of cardio- and cerebrovascular diseases (Lordkipanidze et al. 2011; Timur et al. 2012). Haplotype variants of the *P2RY12* gene have also been related to platelet aggregation, peripheral arterial disease, venous thromboembolism, myocardial infarction and cerebrovascular accidents (Lordkipanidze et al. 2011; Cavallari et al. 2007; Kim et al. 2013; Li

Table 2 Similarity (%) in globally aligned nucleotide coding sequences (CDS, *above the diagonal*) and amino acid sequences (*below the diagonal*) of the $P2Y_{13}$ proteins/genes from 13 different species

	Human	Chimp	Gorilla	Orangutan	Macaque	Night monkey	Dog	Cow	Pig	Rat	Mouse	Chicken	Zebrafish
Human		99.44	99.34	98.22	96.62	90.05	80.28	80.09	81.41	74.91	74.79	63.57	50.83
Chimp	99.15		99.53	98.22	96.81	90.23	80.09	80.09	81.60	74.72	74.60	63.48	51.11
Gorilla	98.87	99.72		98.12	96.71	90.14	80.00	80.00	81.31	74.72	74.60	63.38	50.65
Orangutan	96.89	97.18	96.89		96.15	89.86	79.81	79.53	80.85	74.44	74.51	63.29	51.02
Macaque	95.20	96.05	95.76	95.48		89.95	79.62	79.44	80.94	74.53	74.79	62.83	51.02
Night monkey	87.57	88.42	88.14	88.14	87.57		84.85	83.65	85.14	77.90	77.47	63.44	53.33
Dog	79.38	78.81	78.53	78.25	77.40	83.78		83.53	85.83	75.71	75.49	63.93	54.85
Cow	77.40	77.68	77.40	77.40	77.12	81.38	82.88		88.12	74.04	73.63	64.12	55.53
Pig	75.71	75.71	75.42	75.14	74.86	78.98	84.08	87.39		75.81	75.00	64.80	55.14
Rat	75.77	76.06	75.77	75.21	75.21	78.70	74.85	74.56	73.67		89.35	62.01	54.35
Mouse	73.31	73.60	73.31	73.03	73.60	74.93	71.68	69.91	69.91	87.54		61.44	52.56
Chicken	56.86	56.86	56.58	56.02	55.74	56.85	60.35	61.52	60.64	57.10	55.07		52.49
Zebrafish	43.45	43.45	43.18	42.90	42.90	44.67	46.15	45.86	46.75	42.69	41.40	46.82	

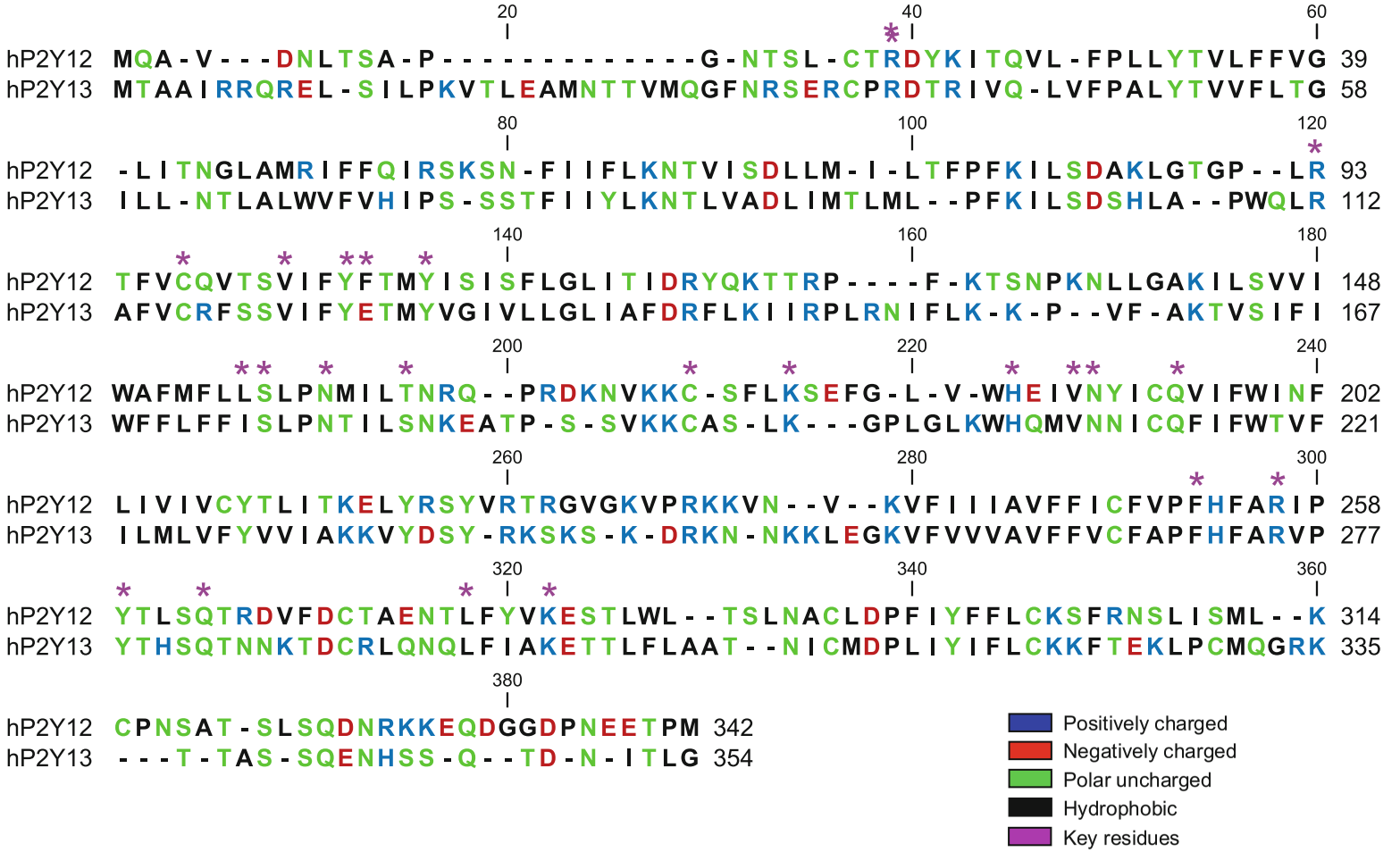

Fig. 5 Alignment of the amino acid sequences of human $P2Y_{12}$ and $P2Y_{13}$ receptors. Amino acids with similar chemical characteristics are colour-coded: R, H, K – positively charged side chains (*blue*); D, E – negatively charged chains (*red*); S, C, T, N, Q, Y – polar uncharged side chains (*green*); G, A, I, L, M, P, F, W, V – hydrophobic side chains (*black*). *Purple asterisks* indicate the location of key residues involved in agonist/antagonist binding to $P2Y_{12}$ receptor (Zhang et al. 2014a, b)

Table 3 Key amino acids that participate in 2MeSADP and AZD1283 binding to $P2Y_{12}$ receptors and their putative equivalents in $P2Y_{13}$ receptor

2MeSADP interaction		AZD1283 interaction	
hP2Y12	hP2Y13	hP2Y12	hP2Y13
R19	R38	V102	V121
R93	R112	Y105	Y124
C97	C116	***F106***	***E125***
Y105	Y124	Y109	Y128
F106	***E125***	***L155***	***I174***
L155	I174	N159	N178
S156	S175	V190	V209
N159	N178	N191	N210
T163	***S182***	Q195	Q214
C175	C194	F252	F271
K179	K198	R256	R275
H187	H206	Y259	Y278
N191	N210	L276	L295
R256	R275	K280	K299
Y259	Y278		
Q263	Q282		
K280	K299		

Non-conserved residues are in bold and italic

et al. 2015; Oestreich et al. 2014; Ou et al. 2016; Siasos et al. 2016; Zee et al. 2008). Moreover, several SNPs and haplotypes of the *P2RY12* gene are associated with lung function in patients with asthma (Bunyavanich et al. 2012). Polymorphisms in *P2RY2* gene have been related to cerebral and myocardial infarction, essential hypertension (Wang et al. 2009a, b, 2010) and decreased risk of osteoporosis (Wesselius et al. 2013). Recently, a gene-based analysis of regulatory variants identified *P2RY13* and *P2RY14* as putative asthma risk genes, and functional studies in mice demonstrated that selective agonists of both receptors can promote airway inflammation (Ferreira et al. 2016). However, the pathophysiological consequences of *P2RY13* polymorphisms in human populations remain unclear.

Over recent years, the availability of high-throughput DNA sequencing technologies has allowed whole genomes or exomes (protein-coding regions) of a large numbers of humans to be analysed. The recent generation of an extensive catalogue of human protein-coding genetic variation, the Exome Aggregation Consortium (ExAC), provides a powerful source of information about the patterns of low-frequency exome variants from nearly 70,000 individuals of diverse geographic ancestries, representing a public resource for the clinical interpretation of genetic variants observed in disease patients (Lek et al. 2016). Using the ExAC database (http://exac.broadinstitute.org; version 0.3, January 2015), we identified 184 candidate polymorphisms in the human *P2RY13* gene, 166 of which lie in exons. When only high-quality filtered variants were analysed, most (98.4%) had an allele frequency of less than 0.01% (very low-frequency variants) and 57% of them were singletons (only observed once in the data set). In terms of the mutational properties of exon variants, there are 48 synonymous (non--protein-altering) SNPs and 118 non-synonymous variants, the latter including 111 missense SNPs, 1 in-frame deletion, 1 stop lost and 3 protein-truncating variants (2 frame-shift and 1 stop gained mutations). In addition, 43 of the missense variants were predicted to be probable/possible damaging variants (Table 4).

Although the consequences of loss-of-function mutations in the human *P2RY13* receptor remain unknown, the availability of a $P2Y_{13}$ knockout mice has proved to be an invaluable resource to assess both the physiological role of this receptor and its potential involvement in specific pathologies (Fabre et al. 2010). Mice lacking the $P2Y_{13}$ receptor display altered bone remodelling, with less complex bone structures. In addition, these mice suffer a significant reduction in the osteoclast and osteoblast populations on the surfaces of the cortical bone, leading to a reduced capacity for mineralization. These osteoblasts proliferate normally *in vitro*, although their mineralization capacity is dampened. Moreover, their gene expression is altered, with an upregulation of osteoprotegerin and a downregulation of the RhoA genes. Conversely, osteoclasts maintain their function, although the number of resorbing osteoclast is decreased. However, the bone phenotype of the $P2Y_{13}$ null mice protects against the loss of bone linked to oestrogen-deficiency (Orriss et al. 2011; Wang et al. 2012).

Table 4 Polymorphisms identified in human *P2RY13* gene using ExAC database (Lek et al. 2016)

Variant	Consequence	Mutational properties	Allele count	Allele frequency
3:151047352 T/C		Upstream gene	2	0.00009252
3:151047362 T/C		Upstream gene	1	0.00004633
3:151046811 AT/A		Intron	1	0.000008935
3:151046821 A/G		Intron	1	0.000009178
3:151046822 C/G		Intron	3	0.00002757
3:151046822 CAAAAG/C		Intron	1	0.000009189
3:151046823 A/G		Intron	1	0.000009182
3:151046842 T/G		Intron	1	0.000009522
3:151047240 C/T		Intron	1	0.00004658
3:151047252 C/T (rs112766831)		Intron	254	0.01179
3:151045729 T/C		3′ UTR	4	0.00003619
3:151045733 T/C		3′ UTR	5	0.00004524
3:151045735 C/T		3′ UTR	1	0.000009049
3:151045736 G/T		3′ UTR	16	0.0001447
3:151045736 G/A		3′ UTR	9	0.00008142
3:151045744 C/T		3′ UTR	7	0.00006332
3:151045755 A/G		3′ UTR	1	0.000009038
3:151045756 G/C		3′ UTR	14	0.0001265
3:151045782 G/T	p.Gly354Gly	Synonymous	1	0.000008995
3:151045812 A/G	p.His344His	Synonymous	4	0.00003530
3:151045821 T/C	p.Gln341Gln	Synonymous	1	0.000008766
3:151045862 G/A	p.Leu328Leu	Synonymous	1	0.000008340
3:151045863 C/T	p.Lys327Lys	Synonymous	1	0.000008334
3:151045914 A/G	p.Ile310Ile	Synonymous	1	0.000008264
3:151045931 A/G	p.Leu305Leu	Synonymous	2	0.00001653
3:151045959 C/G	p.Leu295Leu	Synonymous	1	0.000008272
3:151046064 G/A	p.Val260Val	Synonymous	2	0.00001656
3:151046067 A/C	p.Val259Val	Synonymous	1	0.000008283
3:151046094 G/A	p.Asn250Asn	Synonymous	1	0.000008292
3:151046124 T/C	p.Arg240Arg	Synonymous	1	0.000008296
3:151046127 A/G	p.Tyr239Tyr	Synonymous	3	0.00002489
3:151046142 T/C	p.Lys234Lys	Synonymous	1	0.000008307
3:151046166 C/T	p.Val226Val	Synonymous	1	0.000008287
3:151046178 G/T	p.Ile222Ile	Synonymous	1	0.000008282
3:151046280 T/C	p.Pro188Pro	Synonymous	7	0.00005796
3:151046303 A/G	p.Leu181Leu	Synonymous	2	0.00001656
3:151046307 C/T	p.Thr179Thr	Synonymous	3	0.00002484
3:151046310 A/G	p.Asn178Asn	Synonymous	1	0.000008301
3:151046322 G/A	p.Ile174Ile	Synonymous	1	0.000008310
3:151046325 G/A	p.Phe173Phe	Synonymous	1	0.000008312
3:151046328 G/A	p.Phe172Phe	Synonymous	1	0.000008314
3:151046331 C/T	p.Leu171Leu	Synonymous	10	0.00008314
3:151046346 G/A	p.Phe166Phe	Synonymous	1	0.000008320
3:151046358 C/T	p.Thr162Thr	Synonymous	3	0.00002498
3:151046382 T/A	p.Leu154Leu	Synonymous	1	0.000008345
3:151046405 T/G	p.Arg147Arg	Synonymous	1	0.000008334
3:151046409 G/A (rs142736005)	p.Ile145Ile	Synonymous	23	0.0001915
3:151046445 C/T	p.Leu133Leu	Synonymous	1	0.000008289
3:151046451 G/A	p.Ile131Ile	Synonymous	1	0.000008285

(continued)

Table 4 (continued)

Variant	Consequence	Mutational properties	Allele count	Allele frequency
3:151046484 C/T	p.Ser120Ser	Synonymous	8	0.00006611
3:151046493 A/C	p.Arg117Arg	Synonymous	1	0.000008258
3:151046502 A/G	p.Phe114Phe	Synonymous	1	0.000008255
3:151046508 T/C	p.Arg112Arg	Synonymous	8	0.00006602
3:151046511 G/C	p.Leu111Leu	Synonymous	2	0.00001650
3:151046556 A/C	p.Leu96Leu	Synonymous	1	0.000008245
3:151046576 A/G (rs150522091)	p.Leu90Leu	Synonymous	4	0.00003299
3:151046580 G/A	p.Ala88Ala	Synonymous	3	0.00002474
3:151046588 A/G (rs139399025)	p.Leu86Leu	Synonymous	19	0.0001567
3:151046604 G/T (rs3732757)	p.Ile80Ile	Synonymous	6992	0.05767
3:151046607 G/A	p.Ile79Ile	Synonymous	1	0.000008246
3:151046619 G/A	p.Ser75Ser	Synonymous	1	0.000008244
3:151046646 C/A	p.Leu66Leu	Synonymous	1	0.000008243
3:151046655 A/G	p.Thr63Thr	Synonymous	2	0.00001649
3:151046673 G/A	p.Thr57Thr	Synonymous	3	0.00002473
3:151046745 A/G	p.Ser33Ser	Synonymous	1	0.000008311
3:151047308 G/A	p.Ala3Ala	Synonymous	1	0.00004622
3:151046800 T/TA	c.49-6dupT	Splice region	1	0.000008749
3:151046803 C/A	c.49-8G > T	Splice region	1	0.000008797
3:151045784 C/T	p.Gly354Ser	Missense	1	0.000008986
3:151045807 C/A	p.Ser346Ile	Missense	2	0.00001775
3:151045810 C/T	p.Ser345Asn	Missense	1	0.000008845
3:151045820 C/G	p.Glu342Gln	Missense	3	0.00002635
3:151045829 A/G	p.Ser339Pro	Missense	7	0.00006076
3:151045832 C/A (rs145063671)	p.Ala338Ser	Missense	40	0.0003462
3:151045835 T/C	p.Thr337Ala	Missense	4	0.00003443
3:151045840 T/C	p.Lys335Arg	Missense	1	0.000008563
3:151045852 A/G	p.Met331Thr	Missense	2	0.00001686
3:151045856 A/G	p.Cys330Arg	Missense	15	0.0001258
3:151045867 T/G	p.Glu326Ala	Missense	1	0.000008312
3:151045873 A/T	p.Phe324Tyr	Missense	1	0.000008292
3:151045889 A/G	p.Phe319Leu	Missense	3	0.00002481
3:151045891 A/G	p.Ile318Thr	Missense	1	0.000008269
3:151045904 G/A	p.Pro314Ser	Missense	1	0.000008266
3:151045905 A/T	p.Asp313Glu	Missense	1	0.000008265
3:151045906 T/A	p.Asp313Val	Missense	1	0.000008266
3:151045915 A/G	p.Ile310Thr	Missense	1	0.000008265
3:151045939 G/A	p.Thr302Ile	Missense	1	0.000008267
3:151045951 G/A	p.Ala298Val	Missense	1	0.000008270
3:151045952 C/T (rs148292157)	p.Ala298Thr	Missense	1	0.000008270
3:151045954 A/G	p.Ile297Thr	Missense	1	0.000008270
3:151045976 T/C (rs61736003)	p.Arg290Gly	Missense	1131	0.009355
3:151046008 G/A	p.Thr279Ile	Missense	2	0.00001654
3:151046018 C/A	p.Val276Phe	Missense	1	0.000008272
3:151046063 C/T	p.Val261Met	Missense	16	0.0001325
3:151046068 A/G	p.Val259Ala	Missense	3	0.00002485
3:151046097 G/C	p.Asn249Lys	Missense	1	0.000008294
3:151046104 C/T	p.Arg247Lys	Missense	1	0.000008295
3:151046110 T/C	p.Lys245Arg	Missense	1	0.000008294

(continued)

Table 4 (continued)

Variant	Consequence	Mutational properties	Allele count	Allele frequency
3:151046112 A/C	p.Ser244Arg	Missense	1	0.000008295
3:151046119 G/A	p.Ser242Phe	Missense	1	0.000008296
3:151046121 C/G	p.Lys241Asn	Missense	1	0.000008296
3:151046123 T/G	p.Lys241Gln	Missense	1	0.000008295
3:151046129 A/G	p.Tyr239His	Missense	9	0.00007467
3:151046146 T/G	p.Lys233Thr	Missense	7	0.00005806
3:151046163 A/C (rs150366287)	p.Phe227Leu	Missense	5	0.00004144
3:151046167 A/G	p.Val226Ala	Missense	1	0.000008285
3:151046171 G/A	p.Leu225Phe	Missense	3	0.00002485
3:151046186 C/A	p.Val220Phe	Missense	1	0.000008279
3:151046207 A/T	p.Cys213Ser	Missense	2	0.00001655
3:151046207 A/G	p.Cys213Arg	Missense	1	0.000008274
3:151046222 T/C (rs149544268)	p.Met208Val	Missense	17	0.0001407
3:151046233 T/G	p.Lys204Thr	Missense	1	0.000008278
3:151046263 C/G (rs188633801)	p.Cys194Ser	Missense	4	0.00003312
3:151046263 C/A (rs188633801)	p.Cys194Phe	Missense	1	0.000008281
3:151046275 G/C	p.Ser190Cys	Missense	2	0.00001656
3:151046278 G/A	p.Ser189Leu	Missense	1	0.000008282
3:151046279 A/T	p.Ser189Thr	Missense	1	0.000008280
3:151046281 G/A	p.Pro188Leu	Missense	1	0.000008282
3:151046297 T/C	p.Asn183Asp	Missense	1	0.000008280
3:151046299 C/T	p.Ser182Asn	Missense	1	0.000008280
3:151046305 A/T	p.Ile180Asn	Missense	1	0.000008279
3:151046306 T/C	p.Ile180Val	Missense	1	0.000008280
3:151046308 G/A (rs1466684)	p.Thr179Met	Missense	104,481	0.8636
3:151046321 A/G	p.Ser175Pro	Missense	2	0.00001662
3:151046322 G/C	p.Ile174Met	Missense	5	0.00004155
3:151046323 A/T	p.Ile174Asn	Missense	2	0.00001662
3:151046341 C/G	p.Trp168Ser	Missense	1	0.000008318
3:151046359 G/A	p.Thr162Met	Missense	3	0.00002498
3:151046374 G/T	p.Pro157His	Missense	2	0.00001668
3:151046375 G/T (rs147188000)	p.Pro157Thr	Missense	3	0.00002503
3:151046378 T/G	p.Lys156Gln	Missense	11	0.00009175
3:151046398 A/G (rs138841969)	p.Leu149Ser	Missense	9	0.00007508
3:151046406 G/C	p.Ile146Met	Missense	1	0.000008330
3:151046413 T/C	p.Lys144Arg	Missense	1	0.000008322
3:151046418 G/T	p.Phe142Leu	Missense	2	0.00001663
3:151046441 C/G (rs144496684)	p.Gly135Arg	Missense	19	0.0001575
3:151046450 C/T (rs148391906)	p.Val132Met	Missense	2	0.00001657
3:151046458 A/C	p.Val129Gly	Missense	1	0.000008278
3:151046462 A/C	p.Tyr128Asp	Missense	2	0.00001655
3:151046469 C/G	p.Glu125Asp	Missense	1	0.000008269
3:151046477 A/G	p.Phe123Leu	Missense	9	0.00007438
3:151046486 A/T	p.Ser120Thr	Missense	1	0.000008261
3:151046490 A/T (rs184462683)	p.Phe118Leu	Missense	7	0.00005781
3:151046494 C/T	p.Arg117His	Missense	3	0.00002478
3:151046495 G/T	p.Arg117Ser	Missense	8	0.00006606
3:151046502 A/C	p.Phe114Leu	Missense	10	0.00008255
3:151046506 G/T (rs144128158)	p.Ala113Asp	Missense	510	0.004209

(continued)

Table 4 (continued)

Variant	Consequence	Mutational properties	Allele count	Allele frequency
3:151046559 C/T	p.Met95Ile	Missense	1	0.000008246
3:151046573 T/C	p.Ile91Val	Missense	1	0.000008246
3:151046581 G/A	p.Ala88Val	Missense	2	0.00001649
3:151046587 A/G	p.Leu86Ser	Missense	2	0.00001650
3:151046602 T/C	p.Tyr81Cys	Missense	1	0.000008248
3:151046627 G/A	p.Pro73Ser	Missense	1	0.000008244
3:151046630 T/C	p.Ile72Val	Missense	1	0.000008244
3:151046636 C/T	p.Val70Ile	Missense	1	0.000008244
3:151046637 A/C	p.Phe69Leu	Missense	1	0.000008244
3:151046648 G/C	p.Leu66Val	Missense	1	0.000008242
3:151046690 T/C	p.Thr52Ala	Missense	1	0.000008244
3:151046696 G/C	p.Leu50Val	Missense	1	0.000008244
3:151046716 A/G	p.Val43Ala	Missense	1	0.000008254
3:151046723 G/A	p.Arg41Trp	Missense	1	0.000008260
3:151046725 G/A	p.Thr40Ile	Missense	1	0.000008263
3:151046732 T/C (rs146597143)	p.Arg38Gly	Missense	1	0.000008273
3:151046740 C/T	p.Arg35Gln	Missense	2	0.00001658
3:151046741 G/A	p.Arg35Trp	Missense	2	0.00001660
3:151046752 T/C	p.Asn31Ser	Missense	1	0.000008342
3:151046763 C/T	p.Met27Ile	Missense	3	0.00002519
3:151046768 C/T	p.Val26Met	Missense	6	0.00005059
3:151046776 T/G	p.Asn23Thr	Missense	4	0.00003395
3:151046778 C/T (rs141361811)	p.Met22Ile	Missense	1	0.000008517
3:151046783 C/T	p.Ala21Thr	Missense	2	0.00001713
3:151046785 T/C	p.Glu20Gly	Missense	1	0.000008567
3:151046785 T/A	p.Glu20Val	Missense	1	0.000008567
3:151046795 C/T	p.Val17Met	Missense	3	0.00002607
3:151047289 C/G	p.Glu10Gln	Missense	1	0.00004619
3:151047304 T/C	p.Ile5Val	Missense	1	0.00004617
3:151047307 C/T (rs139632884)	p.Ala4Thr	Missense	1	0.00004622
3:151047309 G/A	p.Ala3Val	Missense	2	0.00009235
3:151046309 T/C	p.Thr179Ala	Missense	1	0.000008302
3:151045796 TGTC/T	p.Asp349del	Inframe deletion	1	0.000008937
3:151045781 A/G	p.Ter355ArgextTer11	Stop lost	4	0.00003599
3:151045931 A/AG	p.Ala306GlyfsTer4	Frameshift	3	0.00002480
3:151046097 G/GT	p.Asn249LysfsTer38	Frameshift	1	0.000008294
3:151046230 C/T	p.Trp205Ter	Stop gained	1	0.000008277

Another major feature of the $P2Y_{13}$ knockout mice is related to lipoprotein metabolism. Even though HDL levels in plasma are normal (Fabre et al. 2010) or slightly decreased (Blom et al. 2010), hepatic uptake of HDL holo-particles is impaired in the absence of the receptor. Furthermore, mice exhibit deficient biliary cholesterol excretion and a reduced hepatic cholesterol content. Remarkably, the reverse cholesterol transport (RCT) that is crucial for the atheroprotective role of HDL proteins is also affected, with a striking reduction in macrophage-to-faeces RCT, making them more sensitive to a high cholesterol diet (Fabre et al. 2010; Lichtenstein et al. 2013). Despite the strong reduction in RCT, $P2Y_{13}$ null mice do not exhibit enhanced atherosclerosis, possibly to the notable differences between human and mouse lipoprotein metabolism. However, dual $P2Y_{13}$/apoE knockout mice develop enhanced aortic sinus lesions with more

infiltrated macrophages (Lichtenstein et al. 2015). Importantly, the atherosclerotic pathology is independent of the $P2Y_{13}$ blood cell receptors. Moreover, a high fat diet is also responsible of neuropathies that affect the enteric nervous system, crucial for the maintenance and regulation of gastrointestinal activity. Experiments performed on $P2Y_{13}$ null mice fed with a high fat diet reveal that the purinergic receptor is responsible for the myenteric neuronal loss induced by the high fat diet or palmitic acid (Voss et al. 2014).

In summary, there is strong evidence that the $P2Y_{13}$ receptor constitutes a promising therapeutic target for pathologies like osteoporosis, atherosclerosis and intestinal neuropathies. However, the role of the receptor in distinct tissues in the organism implies that its involvement in other pathologies should not be ruled out, encouraging further research into this receptor.

5 $P2Y_{13}$ Receptor Signaling

The range of signals transmitted by $P2Y_{13}$ receptors extend beyond their versatility in G protein coupling, and several intracellular pathways can be activated independently of the canonical inhibition of adenylate cyclase.

5.1 G Protein Signalling

Like the $P2Y_{12}$ receptor, the $P2Y_{13}$ receptor is an ADP receptor mainly coupled to Gi proteins. Activation of the $P2Y_{13}$ receptor with ADP or the analogue 2MeSADP leads to the inhibition of adenylate cyclase and a decrease in cAMP production triggered by forskolin, a direct adenylate cyclase activator or following Gs-coupled GPCR stimulation. The EC_{50} values for these events are in the nanomolar range (Communi et al. 2001; Zhang et al. 2002; Marteau et al. 2003; Carrasquero et al. 2005), yet such inhibition was reversed at higher agonist concentrations. From the very first studies, biphasic dose-response curves for the effect of ADP on forskolin-cAMP accumulation were described, with inhibition at nanomolar agonist concentrations and potentiation at micromolar levels, clearly demonstrating the versatility of $P2Y_{13}$ receptor coupling to different G proteins (Gi/Gs) (Communi et al. 2001; Marteau et al. 2003).

Studies with Gα16/Gαq in heterologous expression systems (Communi et al. 2001; Zhang et al. 2002; Marteau et al. 2003) and native tissues, such as immature human dendritic cells and rat cerebellar astrocytes (Carrasquero et al. 2005; Marteau et al. 2004), revealed that $P2Y_{13}$ can also couple to PLC (Fig. 6). Functional $P2Y_{13}$ receptors are present in cerebellar astrocytes as ADP elicits calcium transients that are not sensitive to the $P2Y_1$ specific antagonist, MRS2179. The presence of Gq-coupled $P2Y_{13}$ receptors is restricted to astrocytic populations other than those expressing $P2Y_1$ receptors, indicating some degree of specialization (Carrasquero et al. 2005; Jimenez et al. 1999, 2000). A link to the canonical pathway of adenylate cyclase inhibition further demonstrated the identity of the $P2Y_{13}$ receptor in this glial model. Moreover, when $P2Y_{13}$ and $P2X_7$ purinergic receptors are functionally expressed in rat cerebellar astrocytes they mediate the increase in intracellular calcium elicited by BzATP in these cells (Carrasquero et al. 2009). Moreover, some populations of rat cerebellar granule neurons exhibit 2MeSADP-mediated calcium mobilization with the pharmacological profile of a $P2Y_{13}$ receptor (Hervas et al. 2003).

5.2 MAP Kinase Activation

Downstream of the first membrane effectors, $P2Y_{13}$ receptor stimulation induces Gi-dependent Mitogen Activated Protein Kinase (MAPK) activation (Communi et al. 2001). The $P2Y_{13}$ receptor was first seen to couple to the ERK1/2 MAPK in native human dendritic cells (Marteau et al. 2004). ERK activation triggered by $P2Y_{13}$ receptor agonists was sensitive to ARC-699931MX and intracellular calcium chelation by BAPTA-AM. Again, rat cerebellar

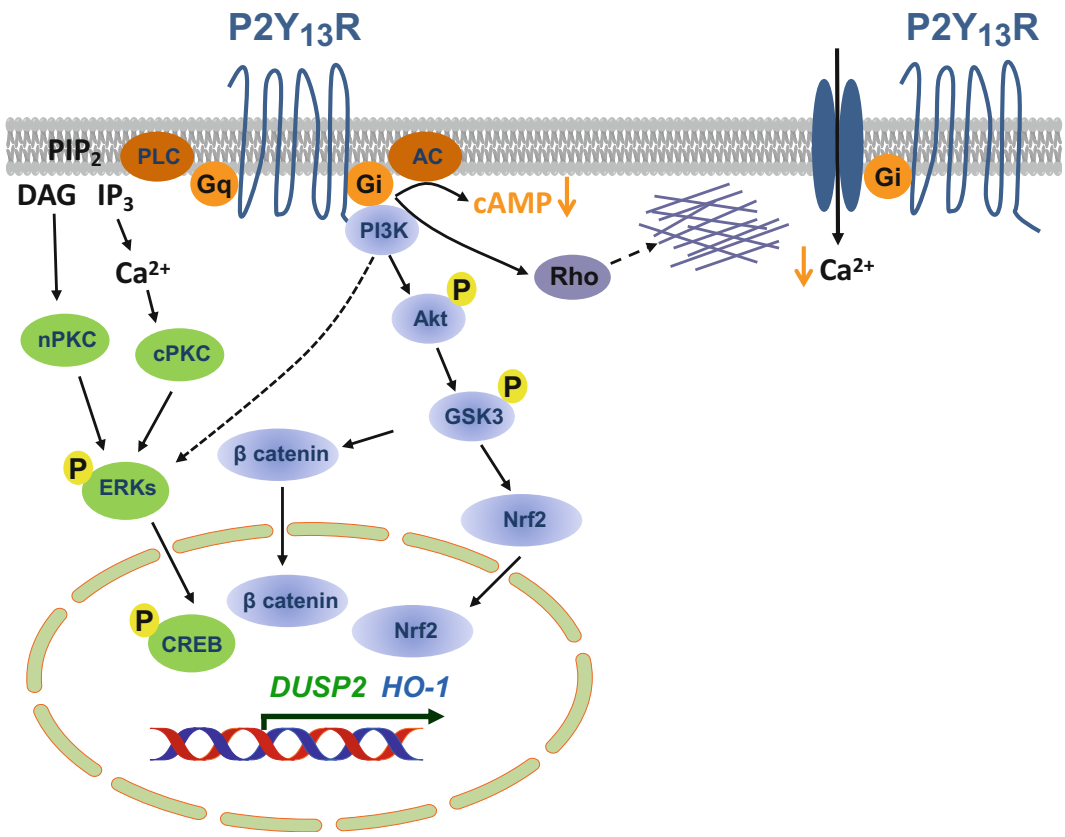

Fig. 6 Schematic representation of the intracellular signalling cascades activated by P2Y$_{13}$ receptor stimulation. P2Y$_{13}$ couples to adenylate cyclase inhibition through the canonical mechanism described for Gi-coupled receptors. P2Y$_{13}$ receptor stimulation *via* the Gi protein also triggers the PI3K/Akt/GSK3 axis. Phosphorylation of GSK3 causes enzyme inactivation and releases two key proteins, β-catenin and Nrf2, which act as transcription factors. The activation of the antioxidant Nfr2/HO-1 axis promotes cell survival in the face of oxidative stress. The P2Y$_{13}$ receptor also couples to Gq proteins in some cell types, promoting phospholipase C activation that in turn stimulates intracellular calcium mobilization and DAG production. These second messengers activate different PKC isoforms and induce ERK activation, which is required for *Dusp2* phosphatase gene transcription. DUSP2 could be responsible for the neuroprotection displayed by the P2Y$_{13}$ receptor to counteract the neurotoxic actions of cisplatin. In some cell models, ERK activation can also be achieved *via* PI3K (broken lines). The P2Y$_{13}$ receptor also couples to RhoA activation and cytoskeleton reorganization, and it may inhibit Ca^{2+} channel activity *via* the βγ subunits of the activated Gi protein and modulate neurotransmitter release

astrocytes and granule neurons provided examples of Gi-dependent 2MeSADP-mediated ERK activation, which was completely inhibited by Pertussis toxin (Carrasquero et al. 2005; Ortega et al. 2011; Perez-Sen et al. 2015). In addition, the sensitivity to the specific antagonist MRS2211, together with lack of effect of MRS2179, a P2Y$_1$ antagonist, confirmed the participation of P2Y$_{13}$ receptors in this signalling.

ERK activation by P2Y$_{13}$ receptors in cerebellar astrocytes and granule neurons resembles that of tyrosine kinase receptors, with maximal activation reached after 10–15 min. In astrocytes, the EC_{50} value of ERK activation correlated with that obtained in experiments where cAMP production was inhibited (around 40 nM). Interestingly, the activation of ERKs was dependent on nProtein Kinase C and src-like kinase activation (Carrasquero et al. 2005). Alternatively, PI3 kinase (PI3K) seemed to lie upstream of ERK activation in granule neurons, as its activity was completely abolished by the PI3K inhibitors

wortmannin and LY294002. In this neuronal model the CREB (cAMP response element-binding) transcription factor was activated in an ERK-dependent manner, conferring neuroprotection against apoptotic stimuli (Fig. 6) (Ortega et al. 2011; Perez-Sen et al. 2015).

In recent studies, new targets of ERK signalling activated by $P2Y_{13}$ receptors have been identified (Morente et al. 2014). The stimulation of granule neurons by 2MeSADP induced the $P2Y_{13}$ dependent expression of an early gene, *Dusp2*, which was sensitive to the $P2Y_{13}$ antagonist, MRS2211. DUSP2 is a dual specificity protein phosphatase that is involved in regulating MAPK activity. As described below, DUSP2 may be responsible for some neuroprotection associated to $P2Y_{13}$ receptor stimulation. Opposite effects were found in pancreatic β cells, where the $P2Y_{13}$ receptor inhibition activated ERK/Akt/CREB signalling (Tan et al. 2010).

5.3 PI3K/Akt/GSK3 Activation

Another interesting feature of the $P2Y_{13}$ receptor is its specific coupling to the PI3K/Akt/GSK3 axis in granule neurons. Accordingly, 2MeSADP induces Thr^{308} phosphorylation and activation of Akt in these cells, which is sensitive to Pertussis toxin and PI3K inhibition. This mechanism connects $P2Y_{13}$ receptors to important intracellular pathways in granule neurons. As such, 2MeSADP induces the rapid and transient phosphorylation of one of the main Akt targets, GSK3 (Ser^{21} and Ser^{9} residues of the α and β isoforms, respectively), which inhibits its catalytic activity. The EC_{50} value for 2MeSADP is close to 20 nM, and both the $P2Y_{13}$ antagonist MRS2211 and the PI3K inhibitors wortmaninn and LY294002, prevent this effect (Ortega et al. 2008).

The signalling triggered by $P2Y_{13}$ receptors that inhibits GSK3 deserves some attention, particularly as some key GSK3 substrates normally retained in the cytosol escape from GSK3-mediated phosphorylation and their subsequent proteasomal degradation (Fig. 6). The nuclear translocation of these GSK3 substrates occurs very rapidly in granule neurons, after a 10–30 min stimulation with 1 μM 2MeSADP, such as for the transcriptional regulator β-catenin (Ortega et al. 2008). Likewise, the nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which regulates the expression of antioxidant genes, is also regulated by GSK3, and a new mechanism by which GSK3 can phosphorylate Nrf2 to promote its further degradation has been demonstrated (Cuadrado 2015; Rada et al. 2012; Rojo et al. 2008). The inhibition of GSK3 induced by $P2Y_{13}$ receptors lies upstream of the increase in Nrf2, and it accumulates in the nucleus of granule neurons after a 6 h stimulation with 2MeSADP, which also turned out to provide neuroprotection (see below). Furthermore, GSK3 has a key role in the formation of amyloid plaques and neurofibrillary tangles, being one of the most relevant therapeutic targets for Alzheimer's disease (Diaz-Hernandez et al. 2012; Maqbool et al. 2016).

The activation of the PI3K/Akt signalling pathway is not exclusive to $P2Y_{13}$ receptors, as it is also induced by the Gi-coupled $P2Y_{12}$ receptor. In C6 glioma cells, the intracellular cascade triggered by 2MeSADP to induce proliferation involves Rap1 activation by Gβγ subunits, which leads to PI3K-dependent Akt activation. In this pathway, Ca^{2+} mobilization and assembly of the PyK2/Src/PLD2 complex is required to achieve the final effect, and this signalling contributes to the proliferation elicited by $P2Y_{12}$ receptors in glioma cells (Van Kolen et al. 2006; Van Kolen and Slegers 2004).

As described for other GPCRs, including the $P2Y_{12}$ receptor, $P2Y_{13}$ stimulation also couples to Rho signalling and subsequent cytoskeleton reorganization. The $P2Y_{13}$ receptor activates RhoA and ROCK I in hepatocytes and osteoblasts, inducing relevant physiological effects (as discussed below). The mechanism of action for RhoA activation is still not completely understood, although it could involve Gi subunits binding to Rho specific guanine exchange factors (Rho-GEF) (Wang et al. 2012; Malaval et al. 2009).

5.4 Molecular Mechanisms Regulating $P2Y_{13}$ Receptor Expression

It is well known that basal ubiquitination and deubiquitination are important to control the cell surface expression of several GPCRs (Dores and Trejo 2012). In terms of the $P2Y_{13}$ receptor, constitutive ubiquitination of its C- terminus tail is directly modulated in the endoplasmic reticulum. Consequently, the $P2Y_{13}$ receptor is degraded through the proteasome pathway, thereby controlling the density of functional receptors at the cell surface and cellular responsiveness (Pons et al. 2014). In this context, the discovery of deubiquitinating enzymes that specifically regulate $P2Y_{13}$ receptor ubiquitination might provide the basis for a novel therapeutic approach to improve hepatic HDL uptake and bile acid secretion, for instance preventing or impairing the development of atherosclerosis (Serhan et al. 2013).

6 Physiological Relevance of $P2Y_{13}$ Receptors

Given the widespread distribution of the purinergic $P2Y_{13}$ receptor, its signalling could be involved in regulating multiple activities in different tissues and organs. The $P2Y_{13}$ receptor is mainly expressed in the spleen, bone marrow cells, peripheral leukocytes, brain, liver, pancreas and heart. Here, we will summarize the direct evidence for the physiological roles of the $P2Y_{13}$ receptor.

6.1 Metabolic Disorders: Atherosclerosis and Diabetes

One of the most promising activities of $P2Y_{13}$ receptor is related to its influence on atherosclerosis. The $P2Y_{13}$ receptor plays a pivotal role in HDL metabolism, which transports cholesterol from peripheral tissues to the liver for elimination. The identification of a new pathway in the liver involving the F1-ATPase and the $P2Y_{13}$ receptor, which regulates the removal of HDL-cholesterol (HDL-c), has enhanced our understanding of HDL metabolism (Jacquet et al. 2005). HDL endocytosis triggered by the $P2Y_{13}$ receptor is dependent on activation of the small GTPase RhoA and ROCK1, producing the cytoskeletal arrangements that drive endocytosis. This step is followed by an increase in biliary lipid secretion (Malaval et al. 2009). The participation of the $P2Y_{13}$ receptor is clearly evident when the effects of its agonists are studied, such as cangrelor and the new available drug, CT1007900 (6-[1-(2-Dimethylaminopyrimidin-5-ylmethyl)-piperidin-4-yl]-2-morpholin-4-yl-pyrimidin-4-ol monohydrate). Acute administration of these compounds increases RCT in the same way as ADP, and in the case of cangrelor, its effect was greater than that found with the physiological agonist. This was unexpected for a compound initially designed as a $P2Y_{12}$ and $P2Y_{13}$ antagonist, and that turned out to act as a partial agonist for $P2Y_{13}$ receptors (Jacquet et al. 2005; Serhan et al. 2013; Goffinet et al. 2014). The fact that impaired HDL clearance occurred in $P2Y_{13}$ null mouse as well as in-loss-of function experiments (using $P2Y_{13}$-shRNA) favours a central role of the $P2Y_{13}$ receptor in RCT (Fabre et al. 2010; Lichtenstein et al. 2013).

The therapeutic potential of $P2Y_{13}$ agonists was also tested in long-term studies carried out through their continuous delivery, which efficiently induced the clearance of circulating cholesterol in the form of HDL particles and its elimination in the form of bile acid secreted by the liver. Moreover, after 1 month of oral treatment with the new agonist CT1007900, HDL particle size was reduced and fewer atherosclerotic plaques were deposited in a mouse model of atherosclerotic pathology (Lichtenstein et al. 2015; Goffinet et al. 2014). These studies provided clues that the $P2Y_{13}$ receptor is a promising therapeutic target for the treatment of atherosclerosis, in mice at least.

Therefore, in clinical trials to assess cangrelor as an anti-aggregating therapy, it should be borne in mind that it may act on hepatic $P2Y_{13}$ receptors and reduce the size of HDL particles. Indeed, it

seems that the same effects as those observed in mice will take place in humans. However, while treatment with cangrelor and the $P2Y_{13}$ agonist CT1007900 exerts a significant effect on circulating HDL, it does not substantially affect plasma HDL-C levels (Martinez et al. 2015). In addition, the $P2Y_{13}$ receptor may also be involved in insulin secretion as $P2Y_1$ and $P2Y_{13}$ receptors modulate insulin release from pancreatic β cells. The activation of $P2Y_1$ Gq-coupled receptors increases intracellular calcium levels and induces insulin release from isolated pancreatic β cells, whereas $P2Y_{13}$ receptor activation in these cells had the opposite effect. Administration of MRS2211, a $P2Y_{13}$ antagonist during glucose injection in mice results in both increased insulin secretion and reduced glucose levels (Amisten et al. 2010), providing a therapeutic opportunity for $P2Y_{13}$ receptor antagonists in the treatment of diabetes. Similarly, blocking $P2Y_{13}$ receptors protects pancreatic beta cells from apoptosis (Tan et al. 2013).

6.2 Bone Homeostasis

The $P2Y_{13}$ receptor also contributes to bone formation and remodelling, a new and important function for this receptor. The first clue regarding this activity of $P2Y_{13}$ came from the striking bone phenotype and altered bone turnover in $P2Y_{13}$ null mice (Wang et al. 2012). The lack of the $P2Y_{13}$ receptor mainly affected the osteogenic response, since activation of this receptor was crucial to obtain adequate differentiation of bone marrow cells into osteoblasts (Biver et al. 2013) due to the activation of RhoA/ROCK (see below) (Wang et al. 2012). These changes in bone phenotype were age-dependent and while $P2Y_{13}$ receptors modulate bone remodelling in mature animals, at younger ages this receptor affects hormonal regulators of phosphate homeostasis. Indeed, in young mice, the increase in trabecular bone formation is correlated to higher serum phosphate levels and increased FGF23 production (Wang et al. 2014).

Another important aspect of $P2Y_{13}$ receptor activity in bone homeostasis is associated to its coordinated activity with other nucleotide receptors. In red blood cells, the $P2Y_{13}$ receptor provides negative feedback modulation of ATP release and thus, increased ATP production in $P2Y_{13}$ null mice may facilitate an osteogenic response of osteoblasts upon mechanical stimulation. The enhanced osteogenic response together with the protective role of the $P2Y_{13}$ receptor in conditions of oestrogen deprivation, suggest that specific inactivation of this receptor could have therapeutic applications in preventing bone loss in diseases like osteoporosis (Wang et al. 2013).

6.3 The Nervous System and Neurological Implications

Prior to the cloning of Gi coupled ADP receptors, several studies reported the inhibitory effect of P2Y-like receptors in modulating neurotransmitter release in both the peripheral sympathetic and central nervous system (Koch et al. 1997; von Kugelgen et al. 1994). Later on, the identification of the $P2Y_{12}$ and $P2Y_{13}$ receptors, and specific antagonists, helped to identify $P2Y_{13}$ as the receptor responsible for inhibiting noradrenaline release in the rat vas deferens and in rat brain hippocampal slices (Csolle et al. 2008; Queiroz et al. 2003). Moreover, $P2Y_{13}$ receptors were also involved in fine-tuning cholinergic transmission at mammalian neuromuscular junctions, where a specific $P2Y_{13}$ antagonist abolishes the effect of 2MeSADP, inhibiting spontaneous and evoked presynaptic acetylcholine release (Guarracino et al. 2016). Indeed, direct negative modulation of N-type Ca^{2+} channels by Gi protein subunits may underlie these effects (Wirkner et al. 2004).

6.3.1 Pain Transmission

$P2Y_{13}$ receptors have also been implicated in pain transmission, and both pro-nociceptive and anti-nociceptive actions have been described in different experimental pain models. In peripheral sensory neurons, the expression of several types of P2Y receptors responding to ADP following

peripheral nerve injury and they modulate nociceptive signalling in different ways. While $P2Y_1$ is pro-nociceptive and facilitates pain transmission, the stimulation of a Gi-coupled ADP receptor with the pharmacological profile of $P2Y_{13}$ produces analgesic effects. Indeed, ADP and other $P2Y_{13}$ agonists reduce the magnitude of depolarization-evoked Ca^{2+} transients in dorsal root ganglia (DRG) sensory neurons, and this effect was also reproduced in $P2Y_1$ knockout mice (Malin and Molliver 2010). Notably, $P2Y_1$ was required for the full expression of inflammatory hyperalgesia after peripheral nerve injury, and when antagonized it occluded the action of Gi-coupled ADP receptors. Only after $P2Y_1$ blockade was the anti-nociceptive action of $P2Y_{13}$ fully revealed. The integration of these opposing signals adjusts nociceptor sensitivity. However, $P2Y_1$ receptors may exert anti-nociceptive effects in other pain models (Ando et al. 2010; Selden et al. 2007) and the selective $P2Y_1$ receptor agonist MRS2365 has potent analgesic activity against neuropathic and acute pain.

In line with the anti-nociceptive effects of ADP, new roles for $P2Y_1$ and $P2Y_{13}$ receptors have been described in the regulation of inhibitory glycinergic neurotransmission in the spinal cord. Interestingly, $P2Y_{13}$ acts in conjunction with $P2Y_1$ receptors to regulate glycine transporters in primary neuronal cultures of the spinal cord and in brainstem preparations (Jimenez et al. 2011). They reduced the activity of the neuronal glycine transporter, GLYT2, to increase the levels of inhibitory glycine neurotransmitter in the synaptic cleft. Conversely, both receptors, along with the $P2Y_{12}$ receptor, activate the glial GLYT1 transporter, reducing glycine levels at glycinergic and glutamatergic synapses, and thereby decreasing glycine concentrations in the NMDA receptor milieu. This produced a net increase in the inhibitory over the excitatory pathways that may contribute to anti-nociception.

The intracellular mechanisms triggered by $P2Y_1$/$P2Y_{13}$ receptor stimulation involved PLC/PKC activation, nitric oxide (NO) release and paracrine PKG-I activation. Regulation of GLYT activity by this pathway was corroborated in heterologous COS cell systems expressing recombinant glycine transporters and the $P2Y_1$ receptor. In fact, the regulatory activity of $P2Y_1$ receptors was lost after siRNA knockdown of NO synthase activity. This common mechanism of action reflects how PKG mediates the phosphorylation of key residues in either glycine transporters or adaptor proteins, which explains the contrasting changes in transport activity reported. These studies reveal a paracrine regulation of GLYT1 and GLYT2 by ADP-P2Y receptors in the spinal cord that contributes to the processing of nociceptive information. This paracrine regulatory mechanism extends the role of ADP receptors to the regulation of nociceptive signalling, an activity involving the modulation of the glycine levels that influences both excitatory and inhibitory neurotransmission at spinal cord synapses. Indeed, GLYT2 pharmacological blockade in the spinal cord produces pain relief in models of acute pain (Dohi et al. 2009).

In comparison to the anti-nociceptive action of $P2Y_{13}$ receptors in sensory neurons, $P2Y_{12}$ receptors expressed in spinal cord microglia also participate in the establishment of neuropathic pain and mechanical allodynia following peripheral nerve injury. Activation of p38 signalling by $P2Y_{12}$ receptors contributes to the generation and maintenance of hyperalgesia (Kobayashi et al. 2008; Tatsumi et al. 2015; Tozaki-Saitoh et al. 2008). Although $P2Y_{13}$ receptors may be expressed sporadically in microglial cells, several P2Y receptors are up-regulated in response to nerve injury, including $P2Y_{13}$, which can then contribute to the development of neuropathic pain (Kobayashi et al. 2012). In this respect, the increases in calcium promoted by Gq-coupling of $P2Y_{13}$ receptors in dorsal spinal cord microglia contribute to the early phase of pain hyper-sensitization and to the changes in size of microglia (Kobayashi et al. 2013; Zheng et al. 2014).

All these findings indicate that purinergic receptors fine tune different populations of sensory neurons and glial cells in the spinal cord and dorsal horn to modulate nociceptive sensitivity. Before ADP formation and activation of P2Y receptors, ATP can exert a direct and acute

stimulation of nociceptive signalling through the activation of ionotropic P2X receptors, mainly P2X2/3 receptors present in DRG neurons (Lewis et al. 1995). In addition, P2X4 receptors participate in the maintenance of neuropathic pain by acting on microglial cells (Inoue and Tsuda 2012). A similar interaction between $P2Y_1$ and $P2Y_{13}$ receptors occurs at the axonal growth cone, where both receptors modulate intracellular signalling triggered by P2X7 receptors to control axonal elongation and sprouting (del Puerto et al. 2012). Moreover, the $P2Y_{13}$ receptor antagonist MRS2211 accelerates neurite outgrowth in PC12, Neuro2a and MEB5 cells (Yano et al. 2012).

6.3.2 Cell Survival and Neuroprotection

In contrast to the pro-apoptotic role of $P2Y_{13}$ receptors in enteric neurons and pancreatic β cells, these receptors play a predominant survival role in the central nervous system. $P2Y_{13}$ receptors promoted the survival of cerebellar astrocytes and granule neurons, both neural models in which $P2Y_{13}$ is co-expressed with $P2Y_1$ receptors, and where specialized functions are elicited by their coupling to different signalling targets. In granule neurons, $P2Y_{13}$ receptors provide neuroprotection against different types of apoptotic stimuli and their activation protects granule neurons from oxidative stress induced by hydrogen peroxide. Indeed, both the production of reactive oxygen species and cell death induced by H_2O_2 treatment diminish after a 2 h pre-treatment with the $P2Y_{13}$ agonist, 2MeSADP. The intracellular mechanism responsible for this neuroprotective effect involves activation of the antioxidant axis Nrf2/heme oxygenase-1 (HO-1). Nrf2 transcriptional activity induces the expression of HO-1, whose levels increase 6 h after $P2Y_{13}$ receptor activation. Both HO-1 expression and survival in response to oxidative stress are prevented in cultured neurons obtained from Nrf2 knockout mice, demonstrating that the antioxidant Nrf2/HO-1 axis is functional in granule neurons and that it is regulated by $P2Y_{13}$ receptors (Perez-Sen et al. 2015; Espada et al. 2010). Similar neuroprotection against oxidative stress is elicited by ADP-P2Y receptors in astrocytes (unpublished results).

Along similar lines, $P2Y_{13}$ receptors also prevent cell death induced by toxic extracellular concentrations of glutamate. The neuroprotection elicited by 2MeSADP is not as potent as that exerted by well-known trophic factors in granule neurons, such as the neurotrophin BDNF (brain derived neurotrophic factor), although it shares a similar mechanism of action. Indeed, a 2 h pre-treatment with both 2MeSADP and BDNF prevents caspase-3 activation in a manner dependent on the activation of the ERK/CREB pathway. The increase in neuron survival promoted by 2MeSADP is also abolished by the antagonist MRS2211 and the PI3K inhibitor wortmannin, again validating the contribution of a Gi-coupled PI3K activity triggered by the $P2Y_{13}$ receptor (Ortega et al. 2011).

$P2Y_{13}$ receptors also protect against other kinds of stress. Genotoxic stress induced by both UV exposure and cisplatin, the cytotoxic drug employed in chemotherapy, also produces apoptotic cell death of granule neurons. Again, $P2Y_{13}$ receptor activation enhances cell survival in these conditions and this protection was abolished by MRS2211 (Morente et al. 2014). In addition, to the PI3K and ERK signalling activated by $P2Y_{13}$ receptors, other signalling mechanisms appear to contribute to the increase in cell survival. Interestingly, these stress conditions promote MAPK p38 over-activation and long-term accumulation of phosphorylated p38 in the nucleus. 2MeSADP pre-treatment before exposure to the cytotoxic stimuli decreases nuclear p38 phosphorylation towards basal levels, indicative of an activation of protein phosphatase activity. In particular, the induction of the expression of an immediate early gene, *Dusp2*, is promoted by 2MeSADP-mediated ERK1,2 signalling in granule neurons (Morente et al. 2014). DUSP2 is a dual specificity phosphatase with a nuclear localization that is selective for p38 and JNK MAPK. Therefore, $P2Y_{13}$ receptors participate in homeostatic mechanisms

in granule neurons contributing to bidirectional regulation of MAPK signalling cascades (Perez-Sen et al. 2015; Marin-Garcia et al. 2009).

7 Concluding Remarks

The data available regarding the $P2Y_{13}$ receptor reflects the great efforts and advances made by scientists working in this area. It is currently accepted that the protein sequence of the $P2Y_{13}$ receptor is very similar to that of the $P2Y_{12}$ receptor, for which a crystallographic structure is available. Thus, plausible explanations for the similarities in their pharmacological properties can be drawn, which in turn make their clear and unequivocal characterization more difficult. However, the presence of two pockets in the receptor structure could explain the agonistic effect of the Ap_3A diadenosine triphosphate, and the possible existence of an allosteric modulatory site near to the orthosteric site where the endogenous agonistic ligand, ADP, binds. Hence, the development of so-called biotopic orthosteric/allosteric ligands could define a functionally selective ligand able to specifically discriminate the members of this receptor subfamily, mainly $P2Y_{12}$, although this field remains unexplored. Nevertheless, such biotopic GPCR agonists and antagonists have recently been reported for the muscarinic receptor M2 and A1 adenosine receptor (Keov et al. 2011). Beyond the similarities between the $P2Y_{13}$ and $P2Y_{12}$ receptor, their expression does not seem to overlap in different tissues, which favours their specialized functions. In addition, while they share some signalling properties, $P2Y_{13}$ is distinct in its ability to switch to Gq proteins and calcium mobilization pathways, as well as to activate signalling cascades that seem to be exclusive to them.

On the other hand, there are no data available concerning the formation of homo- or heterodimers of $P2Y_{13}$, even though this receptor coexists in most cells with other P2Y receptors and GPCRs. Indeed, the complexity of the signalling cascades highlights the possibility of a broad crosstalk among these receptors. The co-expression of $P2Y_{13}$ together with $P2Y_1$ receptors in many cellular models seems to be a general rule of particular interest. The best-known example of interactions between two P2Y ADP-responding receptors is that previously described in platelets for $P2Y_1$ and $P2Y_{12}$ receptors. Both these receptors can act in a coordinated way to regulate complementary functions, or even to behave in an antagonistic fashion. Thus, $P2Y_{13}$ appears to interact with the $P2Y_1$ receptor in the axonal growth cone, modulating the signalling of the ionotropic P2X7 receptor and resulting in axonal elongation, just one such example among many (del Puerto et al. 2012). These interactions testify to the complexity and broad possibilities of the signalling cascades that regulate metabolism. It is noteworthy that beyond the classical pathways regulated by protein kinases, $P2Y_{13}$ can also induce the expression of different genes involved in protection against oxidative stress. Likewise, it can drive the expression of protein phosphatases like the dual phosphatases family member, DUSP, which can restore the steady cell state and allow the cell to respond to new agonist challenges.

In the near future, more precise and specific pharmacological agonists and antagonists may become available, as well as molecular tools. Thus, the pharmacological potential of $P2Y_{13}$ receptors as a target in pathophysiological situations will be revealed in full, advancing the biomedical horizons for this purinergic receptor.

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