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THE P2Y NUCLEOTIDE RECEPTORS IN THE HUMAN GENOME*

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Since the first identification of P2Y receptor sequences in 1993, it has quickly become apparent that this family of the G-protein coupled receptors is very diverse. Members of this receptor family are activated extra-cellularly by a wide variety of adenosine and uridine nucleotides including sugar-nucleotides. The recent decipherment of the Human Genome has enabled us to search for new, yet undiscovered P2Y receptor subtypes. In this article we examine the relationships of six orphan G-protein coupled receptor (GPCR) sequences which show considerable sequence homology to various P2Y receptors. The clustering at a few chromosomal loci of P2Y receptor genes and their related orphan genes further suggests that particular P2Y subsets were derived from the same ancestral gene during evolution.

Keywords: Human genome – database mining – orphan G-protein coupled receptors – ATP – P2Y nucleotide receptors

INTRODUCTION

Receptors for extracellular ATP ("purinergic receptors") were initially recognised pharmacologically in peripheral tissues by Burnstock and termed P2 receptors [11–13]. These receptors, recognising ATP and ADP, were distinguished from P1 receptors, at which adenosine and AMP are natural agonists. Based on pharmacological differences the P2 nucleotide receptors were deduced at an early stage to be of more than one type, initially designated as the P2X and P2Y [12, 14]. Later, evidence from patch-clamping studies in several laboratories (reviewed by Bean [7]) established that some of the P2 receptors are members of the transmitter-gated ion channels class and the term P2X was re-defined to designate specifically that type [3].

These P2X receptors mediating fast purinergic transmission were distinguished then from the others operating through second messengers in slow signalling. This denotes a fundamental division in molecular structure of the P2 nucleotide receptors:

* Dedicated to Professor Maria Wollemann on the occasion of her 80th birthday.

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P2X receptors are members of the 7-transmembrane domain (7-TM) G-protein coupled receptor (GPCR) super-family. This duality of signalling is known for most (but not all) of the transmitters which operate ion channels [4].

This division was validated in 1993 by the cloning of an ATP receptor cDNA encoding a functional 7-TM protein in our laboratory. This ATP/ADP-selective protein was assigned as the $P2Y_1$ receptor, i.e. the first member of the P2Y receptor family [32]. A cDNA encoding an ATP/UTP-selective 7-TM receptor was also cloned in 1993 [23] and this was designated as $P2Y_2$. The characteristics of the emerging P2Y family were defined in 1994 [5] and updated in [25, 26, 30]. The confirmation of the fundamentally different structure of the P2X family, with 2 TMs per subunit and an ion channel, also came from cDNA cloning of its first members [10, 29].

In this article we focus on the diverse family of P2Y receptors. On the basis of information derived from the recently assembled Human Genome database we have established the exact chromosomal localisation of all of the known P2Y genes. We also show evolutionary and structural similarity evidence that 4 of the orphan GPCR sequences previously deposited in the Genbank and EMBL genomic databases may be possible new members of the P2Y receptor family.

Subtypes of P2Y receptors: how many are there?

The total number of the subtypes of P2Y receptors is as yet unclear. To date at least 40 individual sequences proposed to encode P2Y receptors (based on sequences similarity), from species ranging from fish to man, have been deposited in various sequence databases such as Genbank, EMBL and DDJB. Some of the non-human genes included therein is species homologues of known human members, but in other cases this is not clear. The P2Y receptor nomenclature, adopted by the International Union of Pharmacology (IUPHAR) (for its last update, as far as $P2Y_{11}$, see [21]) confirmed the designation of P2Y receptor subtypes as $P2Y_1$ to $P2Y_n$, making the subscript allocation based on the chronology of cloning. To date, the numbered P2Y receptor family consist of 14 established or potential cloned members based on sequence similarity. Figure 1 shows the dendrogram of all 14. That analysis has been carried out using Align X (Vector NTI Suite 8 DNA analysis software, Informax Inc.) based on the Clustal W algorithm for multiple alignment [27]. Only 8 of the subtypes presented in the resulting dendrogram, namely $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$, are established mammalian members and demonstrated to be functionally active and to be expressed *in situ* (see [1] and [21] for review).

Two avian subtypes, plus one from amphibian and one from fish are also shown on the dendrogram (Fig. 1), namely the chicken $P2Y_3$ [33], the turkey $P2Y_{AC}$ [9], *Xenopus* $P2Y_8$ [8] and a *Raja* P2Y [18]. All of these have been cloned and shown to be functional P2Y receptors and to be expressed *in situ.* For the present they are shown as additional subtypes, since it is not yet determined whether those are homologues of known human subtypes or are further subtypes not yet identified in man. Thus, chicken $P2Y_3$ has 60% protein sequence identity to human $P2Y_6$ and has some

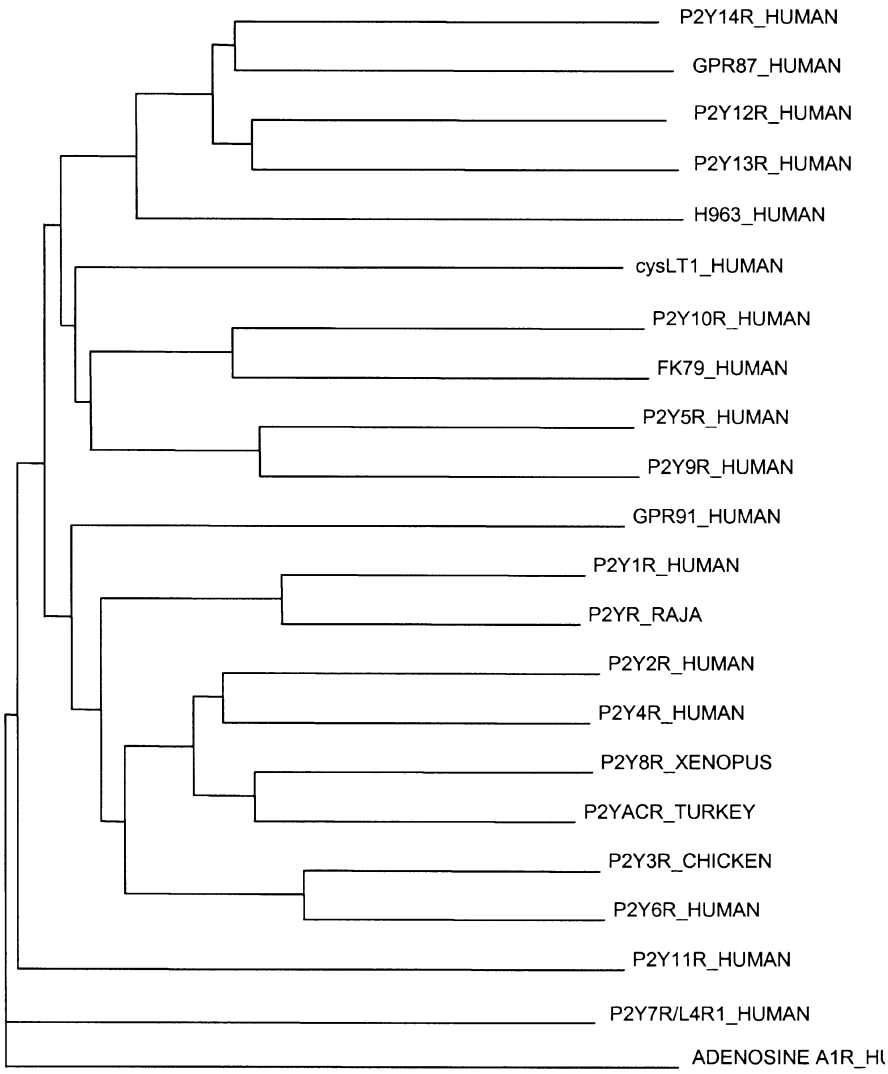

Fig. 1. Dendrogram depicting the relatedness of amino acid sequences among the cloned P2Y receptor subtypes. (Only the human sequences are shown except the chicken $P2Y_3$, turkey $P2Y_{AC}$, *Xenopus laevis* $P2Y_8$ and *Raja erinacea* P2Y.) The human adenosine A1 receptor sequence is used as an outgroup comparator. The sequences were aligned using Align X program (Vector NTI Suite 8 DNA analysis package, Informax Inc.) based on the Clustal W algorithm and the phylogenetic tree was built. The length of each pair of branches represents the sequence divergence between those receptors. The scale bar corresponds to 10% sequence divergence

pharmacological differences from it, which contrasts with 87% between the known orthologues, chicken $P2Y_1$ [32] and a human $P2Y_1$ or 82% between *Xenopus* $P2Y_1$ [16] and human $P2Y_1$. Skate (*Raja erinacea*) P2Y is closest to $P2Y_1$ sequences, but only at 61–64% to human or chicken $P2Y_1$, and skate P2Y and *Xenopus* $P2Y_8$ (closest to human $P2Y_4$, 62%) both have an unusual broad specificity for adenosine and uridine di-and tri-phosphates. The turkey receptor ($tP2Y_{AC}$) [9] is like $P2Y_{12}$–$P2Y_{14}$ in mediating adenylyl cyclase inhibition via a G_i protein [6], but in sequence it is very divergent from those, having the *Xenopus* $P2Y_8$ receptor as its closest relative (Fig. 1).

The pharmacologically established $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ (Fig. 1, lower part of the dendogram) show about 30–50% identity to each other. The $P2Y_{11}$ receptor sequence is more divergent with only about 25% identity. The upper part of the dendrogram in Fig. 1 contains 3 recently identified P2Y receptors. The $P2Y_{12}$ receptor is the long-postulated "P2T receptor" for the ADP activation of blood platelets, as reviewed by Barnard and Simon [6]. The $P2Y_{13}$ receptor is the latest addition to be found for the P2Y family [17, 37], being closest to $P2Y_{12}$ in sequence (Fig. 1, Table 1), and both are (unlike the aforementioned other five subtypes) G_i-linked. The human $P2Y_{14}$ receptor was formerly described as the KAA0001 or UDP-glucose receptor [15] and has no activity on any nucleoside di- or tri-phosphates. Although this receptor is only activated by the sugar-nucleotides, it is clearly on this second branch (together with $P2Y_{12}$ and $P2Y_{13}$) in the dendrogram [6, 17], and this has led now the IUPHAR Committee on P2Y receptor nomenclature to rename it as the $P2Y_{14}$ receptor [1]. These recent members of the P2Y receptor family display exceptionally low sequence identity to the other P2Y receptors, e.g. 18–19% to $P2Y_1$ (see Table 1).

The human $P2Y_5$ receptor together with $p2y_9$ and $p2y_{10}$ receptor lies on a third, separate branch in the middle of the tree (Fig. 1). These also have high sequence divergence from other P2Y members. $P2Y_5$ was first recognised from the chicken; when the protein (340-amino acid) is heterologously expressed in COS-7 cells it binds nucleotides and also, its mRNA is detectable in vivo, particularly in immune T-cells upon antigen activation [34]. For the human homologue of that protein [28]

Table 1
Percentage of identity of selected human P2Y receptors and P2Y receptor candidates

	$P2Y_1R$	GPR91	H963	$P2Y_{12}R$	$P2Y_{13}R$	GPR87	$P2Y_{14}R$
$P2Y_1R$	100	30	19	18	18	22	19
GPR91		100	28	28	29	24	27
H963			100	35	37	26	37
$P2Y_{12}R$				100	50	39	49
$P2Y_{13}R$					100	36	47
GPR87						100	44
$P2Y_{14}R$							100

Percentage of identity in amino acid sequence was calculated from the alignment of Fig. 1.

there is preliminary evidence that in oocyte expression it gives functional responses to ATP [20]. As for the $p2y_9$ and $p2y_{10}$ subtypes, related to $P2Y_5$, only their sequences were deposited into the Genbank database, and they have not yet been expressed and functionally characterised.

The most disputed subtype is the $P2Y_7$, whose sequence is more divergent. Its cDNA was cloned from human erythroleukemia cells and it had been shown, when heterologously expressed in COS-7 cells, to bind [^{35}S]-dATPαS with high affinity, this binding being blocked by the general P2 antagonist suramin [2]. Furthermore, ATP increased the inositol phosphate (IP) levels in COS cells heterologously expressing this receptor. Later, this receptor was found to be the same in sequence as the leukotriene LTB_4 receptor [36]. Therefore, this receptor is listed here as a "$P2Y_7/LTB_4$" receptor (Fig. 1). However, it is interesting to note that the sequence of $P2Y_7/LTB_4$, unlike that of other known P2Y and P2X receptor subtypes, contains an extracellular consensus Walker-type ATP binding site [31], which suggests that ATP may act as a regulator of the LB_4 receptor at this site. Furthermore, an independent study (discussed further below) reported [24] that another well-established leukotriene receptor responds also to nucleotides, both in heterologous expression and in the native state. The exceptional possibility of the existence of certain receptors having dual leukotriene and P2Y type specificity now merits further investigation.

Mining of the Human Genome: P2Y genes, are there any more?

Mammalian orthologues of the 2 avian ($P2Y_3$ and $P2Y_{AC}$) receptors have not yet been found using conventional direct cloning techniques. The recent information on the human genome enabled us to search for their human orthologues (if they exist) and also, to see whether are there any, yet unidentified human P2Y receptor genes. After interrogation of the human genome database we showed that contains no sequence closer to chicken $P2Y_3$ than human $P2Y_6$ giving the conclusion that there is no human ortholoque of the chicken $P2Y_3$ receptor: either it is a non-mammalian subtype or $P2Y_3$ is the orthologue of the human $P2Y_6$, despite the greater-than-expected divergence of those two sequences. For the turkey $P2Y_{AC}$ and the *Xenopus* $P2Y_8$ pair (see Fig. 1), both functional receptors, this question cannot be resolved at present, due to their great divergence from any known human P2Y subtype.

However, we were able to find 4 human orphan GPCRs which were previously deposited into Genbank and now show considerable homology in sequence to known P2Y receptors. These are GPR87 (accession number: AF237763, [2]), GPR91 (accession number: AF348078, [35]), H963 (accession number: AF002986, [19, 22]) and FK79 (accession number: AF345567). GPR87 and H963 are clearly in the same branch with the newly discovered G_i-coupled $P2Y_{12}$–$P2Y_{14}$ subtypes (Fig. 1). GPR87 has 44% identity to the $P2Y_{14}$ receptor (Table 1). They show a high degree of conservation at the amino acid level in their TM3, TM6 and TM7 domains when aligned with other members of this branch (Fig. 2).

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                                TM3
                    ____________________________
P2Y12:      LRTFVCQVTSVIFYFTMYISISFLGLITIDRY
P2Y13:      LRAFVCRFSSVIFYETMYVGIVLLGLIAFDRF
P2Y14:      LNVFVCRVSAVLFYVNMYVSIVFFGLISFDRY
H963:       LKIFHCQVTACLIYINMYLSIIFLAFVSIDRC
GPR87:      FKFILCRYTSVLFYANMYTSIVFLGLISIDRY
Consensus:       C     L Y  MY  I     I  DR
                           I             V

                               TM6
                    ____________________
P2Y12:      VFIIIAVFFICFVPFHFARIPYTLS
P2Y13:      VFVVVAVFFVCFAPFHFARVPYTHS
P2Y14:      IFSIVFVFFVCFVPYHIARIPYTKS
H963:       ILLVTTGYIICFVPYHIVRIPYTLS
GPR87:      IRVVVAVFFTCFLPYHLCRIPFTFS
Consensus:           CF P H  R P T S

                               TM7
                    ____________________
P2Y12:      STLWLTSLNACLDPFIYFFLC
P2Y13:      TTLFLAATNICMDPLIYIFLC
P2Y14:      FTLLLSAANVCLDPIIYFFLC
H963:       ATLLLAVSNLCFDPILYYHLS
GPR87:      ITLFLSACNVCLDPIIYFFMC
Consensus: TL L   N C DP IY
                          L
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Fig. 2. Alignment (as for Fig. 1) of amino acid sequences of the human $P2Y_{12}$-related receptors and orphans in their TM3, TM6 and TM7 domains. They show a high degree of conservation in these regions, as shown in bold type. The consensus sequence motifs for each of the 3 TMs are also shown. Note that these motifs are characteristic only of this branch of the P2Y family

A consensus sequence for the receptor proteins in this $P2Y_{12}$ subfamily can be drawn within each of these TMs: C-X-X-X-X-X-I/L-X-Y-X-X-M-Y-X-X-I-X-X-X-X-X-I/V-X-X-D-R (for TM3), C-F-X-P-X-H-X-X-R-X-P-X-T-X-S (for TM6) and T-L-X-L-X-X-X-N-X-C-X-D-P-X-I/L-Y (for TM7) (Fig. 2).

The orphan GPR91 is only distantly related to these sequences and, in fact, it is closer to the $G_{q/11}$-coupled $P2Y_1$–$P2Y_6$ branch, having 30% identity to the $P2Y_1$ receptor (Table 1).

Another branch of the family contains the $P2Y_5$ receptor (discussed above) and three orphans, $p2y_9$ (accession number: U90322), $p2y_{10}$ (accession number: AF000545) and FK79 (accession number: AF345567) (Fig. 1). Interestingly, this branch also embraces the cysteinyl leukotriene-1 (cysLT1) receptor as shown. The latter receptor is present in the immune system and has recently been suggested to be a unique member of the P2Y family since it can also be activated by UDP to mobilise intracellular Ca^{2+} from stores, and since UDP can also cross-desensitise it to the action of a cysLT1 agonist [24].

Chromosomal localisation of P2Y receptors

A remarkable clustering of 4 known P2Y genes occurs on chromosome 3 at the q25 band. Three of these genes, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, are branched together in the dendrogram and they are in close proximity at the 3q25.1 sub-band on 2 contiguous cos-

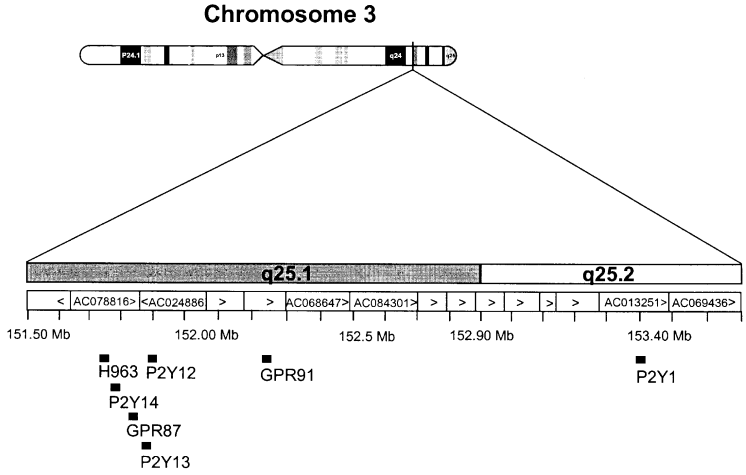

Fig. 3. The cluster of P2Y receptor and related genes on chromosome 3. The schematic representation was reproduced from an Ensembl database search using Human ContigView (http://www.ensembl.org/Homo_sapiens/ contigview). The exact locations of the 4 known P2Y as well as 3 orphan receptor genes located are shown. $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, as well as the two orphan receptor genes, H963 and GPR87 are at the 3q25.1 chromosomal sub-band in 2 contiguous cosmids. The GPR91 gene is nearby at this locus. The $P2Y_1$ receptor gene is on the neighbouring 3q25.2 chromosomal sub-band approximately 1 Mb away from the $P2Y_{12}$ gene

Table 2
Localisation of P2Y receptor and P2Y-like orphan receptor genes on human chromosomes

Chromosome bands/sub-bands	P2YR or P2Y-like orphan receptor genes	Relative distance Mb
3q25.1	H963	0.020
	$P2Y_{14}$	0.080
	GPR87	0.040
	$P2Y_{13}$	0.010
	$P2Y_{12}$	0.500
	GPR91	1.000
3q25.2	$P2Y_1$	
11q13.4	$P2Y_2$	0.030
	$P2Y_6$	
13q14.2	$P2Y_5$	
	cysLT2	
19q13.2	$P2Y_{11}$	
Xq13.1	$P2Y_4$	
Xq21.1	cysLT1	0.550
	$P2Y_9$	0.125
	$P2Y_{10}$	0.250
	FK79	

Genes were localised by searching the Human Genome Database (http://www.ensembl.org).

mids (Fig. 3, Table 2). The fourth gene, that of the distantly related $P2Y_1$ receptor, is also present in this cluster, although approximately 1 Mb away on the neighbouring 3q25.2 sub-band (Fig. 3, Table 2). Genes for three of the four orphan GPCRs identified in this work as P2Y receptor candidates are also present in this cluster on 3q25 (Fig. 3). Genes for GPR87 and H963, structurally most similar to the $P2Y_{12}$–$P2Y_{14}$ series, are also at the 3q25.1 chromosomal sub-band, neighbouring the $P2Y_{13}$ and $P2Y_{14}$ genes. This strongly suggests that they diverged in evolution from the same ancestral gene. The third orphan GPR91 gene is also at this chromosomal sub-band but some 500 kb away from this 5-gene cluster, toward the $P2Y_1$ gene (Fig. 3, Table 2). Indeed it is structurally more related to the $P2Y_1$ receptor then the $P2Y_{12}$–$P2Y_{14}$ sub-group.

Another clustering of two P2Y receptor genes ($P2Y_2$, and $P2Y_6$), occurs on chromosome 11 at the 11q13.4 sub-band. Again these receptors are in very close proximity to each other (Table 2).

Chromosome X also contains a number of P2Y genes, including the $P2Y_4$ gene at the Xq13.1 (Table 2) chromosomal sub-band. However, this gene is far from the P2Y cluster present on this chromosome at the Xq21.1 sub-band. This cluster contains genes for the $p2y_9$ and $p2y_{10}$ receptors as well as genes for the cysLT1 and the orphan FK79 receptors. These receptors structurally are also similar; they branch together on the dendrogram (Fig. 1). $p2y_9$ and $p2y_{10}$, although deposited as P2Y sequences, are at present orphan receptors (and therefore, following the IUPHAR rules for unexpressed receptors, are left in lower-case lettering).

Since the cysLT1 receptor may be functionally P2Y-related (see above), it is possible that all of these four genes in this Xq21.1 cluster encode P2Y receptors of related pharmacology.

In conclusion, we have found by mining the human genome suggestive evidence that the P2Y receptor series (even in man, where the largest number is known) may not be complete. We identified 4 candidates for possible inclusion in the P2Y series. They are structurally and evolutionarily related to known members of the P2Y receptor family. Functional studies on these receptors are warranted to explore this possibility.

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