FEBS 26131 FEBS Letters 520 (2002) 97–101

Identification of G protein-coupled receptor genes from the human genome sequence

Shigeki Takeda[a,b,1], Shiro Kadowaki[c], Tatsuya Haga[a,b,c,*], Hirotomo Takaesu[d], Shigeki Mitaku[d]

[a] *Department of Neurochemistry, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b] *Institute for Biomolecular Science, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan*
[c] *Japan Science and Technology Corporation (CREST), Tokyo, Japan*
[d] *Department of Biotechnology, Faculty of Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan*

Received 25 April 2002; accepted 25 April 2002

First published online 8 May 2002

Edited by Jacques Hanoune

Abstract **We have identified novel G protein-coupled receptors (GPCRs) with no introns in the coding region from the human genome sequence: 322 olfactory receptors; 22 taste receptors; 128 registered GPCRs for endogenous ligands; 50 novel GPCR candidates homologous to registered GPCRs for endogenous ligands; and 59 novel GPCR candidates not homologous to registered GPCRs. The total number of GPCRs with and without introns in the human genome was estimated to be approximately 950, of which 500 are odorant or taste receptors and 450 are receptors for endogenous ligands. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: G protein-coupled receptor; Genome; G protein; Proteomics; Ligand screening; Orphan receptor

1. Introduction

G protein-coupled receptors (GPCRs) are integral membrane proteins in the cell surface, and interact with and are activated by exogenous ligands, such as odorants and taste substances, or by endogenous ligands such as neurotransmitters and hormones in the extracellular space. Activated GPCRs interact with and activate the heterotrimeric form of G proteins in the intracellular space. GPCRs are also known to be targets of 30–60% of the present drugs. Identification of unknown GPCRs in the human genome and endogenous ligands for them should give a great impetus not only to our understanding of human physiology but also to the development of novel drugs.

GPCRs comprise one of the largest protein superfamilies. It is estimated that there are 1050 and 160 GPCR genes in the genomes of *Caenorhabditis elegans* [1] and *Drosophila melanogaster* [2], corresponding to 5.5% and 1% of their total genes, respectively. Recently, Venter et al. [3] reported the presence of 616 GPCR genes that belonged to the rhodopsin class, the secretin class and the metabotropic glutamate class in the human genome. It is estimated that in mice there are 500–1000 odorant receptors [4] and 40–50 taste receptors [5]. The lists on web sites enumerate over 200 non-odorant and non-taste human GPCRs (http://www.expasy.ch/cgi-bin/lists?7tmrlist.txt, http://www.gpcr.org/7tm/seq/dna.html) and over 300 odorant human GPCRs (http://bioinfo.weizmann.ac.il/HORDE/). Thus, many unregistered GPCR genes appear to exist in the human genome. There may be novel GPCRs that are not homologous to already registered GPCRs and that are difficult to find by using a simple homology search method. Although increasing attention is being paid to find novel genes in several genome databases, gene annotation is not sufficiently reliable yet. In fact, different methods of identifying genes from genome data do not give good agreement for predicting novel genes [6]. To classify novel GPCR genes in the human genome, we attempted to develop a new strategy for screening GPCR genes.

To identify GPCR genes, we took advantage of the fact that many mammalian GPCR genes do not have introns in their coding region [7] and attempted to screen GPCR genes with no introns in the coding regions. Thus, we could avoid the very difficult and risky task of predicting the exon–intron boundary in the genome sequences. We collected intronless open reading frames (ORFs), which were long enough to cover GPCRs from the human genome. Then we took advantage of the GPCRs' common characteristic that they have seven transmembrane segments, and adopted the program SOSUI which was developed to identify transmembrane segments in protein sequences and to discriminate membrane proteins. The reliability of SOSUI was 99% for the discrimination between soluble and membrane proteins and 96% for the prediction of transmembrane helices [8]. SOSUI has recently been used to analyze all proteins in whole genomes of 15 single-cell organisms [9].

Here, we report on the identification of novel GPCR genes and on our estimation of the total number of GPCRs in the human genome.

2. Materials and methods

We downloaded the human genome database dated August 8, 2000 from ftp://ncbi.nlm.nih.gov/genbank/genomes/H-sapiens/. The 'nr'

*Corresponding author. Fax: (81)-3-5992 1034.
E-mail address: tatsuya.haga@gakushuin.ac.jp (T. Haga).

[1] Present address: Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan.

Abbreviations: GPCR, G protein-coupled receptor; ORF, open reading frame

0014-5793/02/$22.00 © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(02)02775-8

protein database dated August 20, 2000 was obtained from ftp://ncbi.nlm.nih.gov/blast/db/. BLAST for the linux operating system was downloaded from ftp://ncbi.nlm.nih.gov/blast/executables/ and used for homology search. Registered GPCR sequences were obtained from web sites: 207 non-odorant and non-taste receptors from http://www.gpcr.org/7tm/seq/dna.html, and 43 odorant GPCRs from http://ycmi.med.yale.edu/senselab/ORDB. Selected ORFs were analyzed for homology using BLAST with the 'nr' protein database. Human tissue RNA fractions for RT-PCR were obtained from BioChain. RT-PCR was performed with Superscript II reverse transcriptase (Invitrogen) and KOD plus (Toyobo) polymerase.

3. Results and discussion

We translated the human genome data and extracted 335 570 ORFs which had 200–1500 amino acid residues. Over 97% of the 250 GPCRs registered on the web sites had amino acid residues in this range. We eliminated the ORFs in which any single amino acid residue comprised more than 20% of the whole sequence because most likely they are repeated sequences rather than coding regions. More than 99% of the registered GPCRs passed this procedure and 27 512 ORFs were removed.

The remaining ORFs were analyzed by SOSUI. Some of the registered GPCRs were recognized as having six or eight transmembrane segments by SOSUI when one of the transmembrane segments was not very hydrophobic or when a signal peptide was interpreted as a transmembrane segment. We selected as GPCR candidates the 7780 ORFs which were judged to have six to eight transmembrane segments. When multiple ORFs with different initiation codons were flanked by the same stop codon only the longest ORF was adopted. We also removed redundant ORFs when they were over 95% homologous to each other, by BLAST search. After selecting, we obtained 1714 non-redundant ORFs. These ORFs were analyzed for their homology with protein sequences in databases. One hundred and eighty were the same as GPCRs already registered and 342 were 20% or more homologous to GPCRs already registered (Table 1).

There were 322 putative odorant receptors, 41 of which had been registered as such, and 281 were 40–85% homologous to registered odorant receptors. Recently, Glusman et al. [10] also reported the presence of 322 odorant receptor genes in the human genome. Putative taste receptors were also detected, including 11 registered ones and 11 novel ones with homology of 30–70% to registered bitter receptors of mouse or rat. These bitter receptor candidates were encoded by genes with no intron in the coding region and had a short N-terminal extracellular segment in accord with bitter receptors [5,11,12], whereas sweet receptors are known to have introns and a long extracellular segment [13–16].

We also found non-odorant and non-taste GPCRs: 128 of the GPCRs for endogenous ligands had already been registered on the 'nr' database as of August 20, 2000 and 50 of the GPCR candidates had not been registered on the database. The homology search indicated that out of the 50 GPCR candidates, eight were homologous to amine receptors, four to peptide receptors, six to nucleotide receptors, four to lipid receptors, six to putative pheromone receptors, nine to various kinds of GPCRs, and 13 to orphan receptors (Table 2). After completion of our computational screening, it turned out that 50 GPCR candidates contained an ADP receptor (hGPCR17) [17,18] and pheromone receptor candidate, *V1RL1* (hGPCR25) [19]. Eventually, 37 of the 50 GPCR candidates were registered as new GPCRs on a public database between August 2000 and April 2002. These results indicate the potential of our strategy to screen novel GPCRs. Rhodopsin and other pigment opsins, sweet receptors, and opioid receptors were not detected, because they have introns in their coding regions. On the other hand, we could identify several GPCR genes with introns outside their seven transmembrane segment region, such as glutamate receptors 1 and 2.

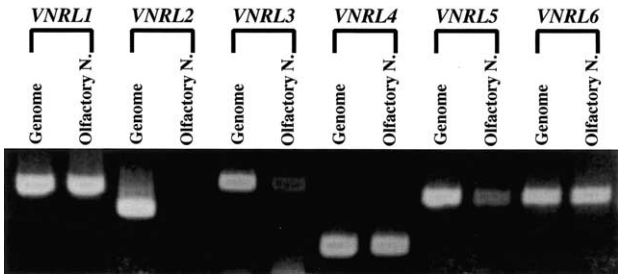

Fig. 1. RT-PCR was performed on mRNA from olfactory nerve tissue of a 36 year old man for putative pheromone receptors. *VNRL1* is the same gene as *V1RL1* reported previously [19]. The PCR products, amplified from the genome and the olfactory nerve mRNA, have the same size, indicating that these mRNA sequences were not divided by an intron on the genome.

The pheromone receptor homologues, vomeronasal receptor-like 1–6 (*VNRL1–6*), were divided to two categories according to their homology. *VNRL1–5* were similar to the pheromone receptors of rodents, with a short extracellular sequence at the N-terminus. Only *VNRL6* was homologous to *V2R2* of rodents, with a long N-terminal extracellular domain. We could not, however, detect the long N-terminal domain in *VNRL6*. It is possible that the N-terminal domain is encoded in exons other than those encoding the seven transmembrane regions. RT-PCR revealed that, except *VNRL2*, these pheromone receptor-likes were expressed in the human olfactory nerve of a 36 year old man (Fig. 1). *VNRL2* might be expressed at a specific age or only in females. In the genome, *VNRL2* and *3* were located very close to each other,

Table 1
Number of identified GPCRs and estimation of total number of GPCRs in the human genome

	Number of identified receptors[a] (already registered receptors)	Estimated number of total receptors[b]
Odorant receptors	322 (41)	481
Taste receptors	22 (11)	28
Receptors for endogenous ligands	178 (128)	330
ORFs without homology to registered GPCRs[c]	59 (0)	109
Total	581 (180)	948

[a]Numbers in parentheses are those for already registered GPCRs in the 'nr' protein database.
[b]The total numbers of GPCRs in each category were estimated using performance of the present strategy, which is shown in Table 3.
[c]See text for criteria to discriminate GPCR candidates from non-candidates.

Table 2
Summary of 50 novel GPCR candidates

Tentative identification number	Homologous receptors[a]	Homology (%)	Identical residues/ homologous region[b]	Tissue specificity[c]	Accession number[d]	Corresponding mRNA[e]
Amine receptors						
hGPCR1	serotonin receptor 4	21	64/295	brain	AB083583	NM014373
hGPCR2	dopamine receptor 1	27	95/350	ubiquitous	AB083584	XM066873
hGPCR3	histamine H2	27	87/312	brain	AB083585	NM031936
hGPCR4	melatonin receptor type 1A	26	98/364	ubiquitous	AB083586	NM020370
hGPCR5	β3-adrenergic receptor	32	39/120		AB083587	
hGPCR6	serotonin receptor 1A	22	101/455	brain	AB083588	NM054021
hGPCR7	serotonin receptor 1D	26	72/272		AB083589	
hGPCR8	serotonin receptor 6	28	99/344	brain	AB083590	
Peptide receptors						
hGPCR9	allatostatin receptor	27	43/154	small intestine	AB083591	XM069498
hGPCR10	galanin receptor type 3	23	79/330		AB083592	XM068829
hGPCR11	somatostatin- and angiotensin-like SALPR	43	141/322	small intestine	AB083593	XM060898
hGPCR12	thyrotropin-releasing hormone receptor	23	76/326		AB083594	XM064062
Nucleotide receptors						
hGPCR13	GPR34	23	77/323	ubiquitous	AB083595	XM080817
hGPCR14	KIAA0001	47	149/314	ubiquitous	AB083596	NM022788
hGPCR15	KIAA0001	47	140/295	ubiquitous	AB083597	NM023914
hGPCR16	putative purinergic receptor P2Y1	36	109/299	colon	AB083598	XM062888
hGPCR17	putative purinergic receptor P2Y10	50	148/292	ubiquitous	AB083599	NM032553
hGPCR18	putative purinergic receptor P2Y5	39	113/284	ubiquitous	AB083600	NM020400
Lipid receptors						
hGPCR19	EDG-6	28	76/268	ubiquitous	AB083601	
hGPCR20	EDG-8	86	343/397	ubiquitous	AB083602	XM085864
hGPCR21	leukotriene D4 receptor	38	114/298	ubiquitous	AB083603	NM020377
hGPCR22	leukotriene B4 receptor	33	92/276	ubiquitous	AB083604	NM019839
Pheromone receptors						
hGPCR23 (*VNRL4*)	pheromone receptor 1	43	90/206	olfactory nerve	AB083605	
hGPCR24 (*VNRL1*)	pheromone receptor M21	28	86/300	olfactory nerve	AB083606	NM020633
hGPCR25 (*VNRL3*)	pheromone receptor VN3	30	84/277	olfactory nerve	AB083607	XM064908
hGPCR26 (*VNRL5*)	pheromone receptor VN6	31	99/310	olfactory nerve	AB083608	
hGPCR27 (*VNRL2*)	pheromone receptor VN6	33	79/236		AB083609	XM064909
hGPCR28 (*VNRL6*)	pheromone receptor V2R2	76	250/327	olfactory nerve	AB083610	XM067401
Others						
hGPCR29	Frizzled-1	32	27/82		AB083611	
hGPCR30	Frizzled-3	70	197/278		AB083612	
hGPCR31	Frizzled-4	53	235/438		AB083613	NM012193
hGPCR32	Frizzled-10	23	23/96		AB083614	
hGPCR33	calcium ion sensing receptor	45	132/288		AB083615	XM069224
hGPCR34	G protein-coupled receptor LGR5	44	120/269	ubiquitous	AB083616	XM086283
hGPCR35	KIAA0758	44	177/398		AB083617	XM069343
hGPCR36	KIAA0758	38	187/480		AB083618	
hGPCR37	KIAA0758	25	114/444	ubiquitous	AB083619	XM092364
Orphan receptors						
hGPCR38	FMLP-related receptor I	27	90/324		AB083620	XM061833
hGPCR39	GPR32	84	199/236		AB083621	XM064958
hGPCR40	GPR34	23	77/323	ubiquitous	AB083622	
hGPCR41	hypothetical protein similar to GPCR (*C. elegans*)	24	55/226		AB083623	
hGPCR42	hypothetical protein similar to GPCR (*C. elegans*)	23	69/288	ubiquitous	AB083624	NM017986
hGPCR43	hypothetical protein similar to GPCR (*D. melanogaster*)	23	59/253	ubiquitous	AB083625	
hGPCR44	Mas proto-oncogene	37	111/294	small intestine, colon	AB083626	NM054030
hGPCR45	Mas proto-oncogene	34	113/327		AB083627	XM089955
hGPCR46	Mas proto-oncogene	38	108/283		AB083628	XM089843
hGPCR47	retinoic acid-induced putative GPCR	47	138/290		AB083629	NM018654
hGPCR48	probable GPCR HM74	41	122/294		AB083630	XM092406
hGPCR49	probable GPCR HM74	52	178/341		AB083631	NM032554
hGPCR50	probable GPCR HM74	92	335/362		AB083632	XM090326

[a]The homology to registered GPCRs in the 'nr' protein database dated August 20, 2000 was estimated by BLAST.
[b]Number of identical amino acid residues/length of amino acid residues for homologous region.
[c]Expression profiles were determined by RT-PCR. Only high expression tissues were noted.
[d]Nucleotide sequence data are available in the DDBJ databases under these accession numbers.
[e]Accession number for public database is shown in the case that an ORF was registered after August 2000.

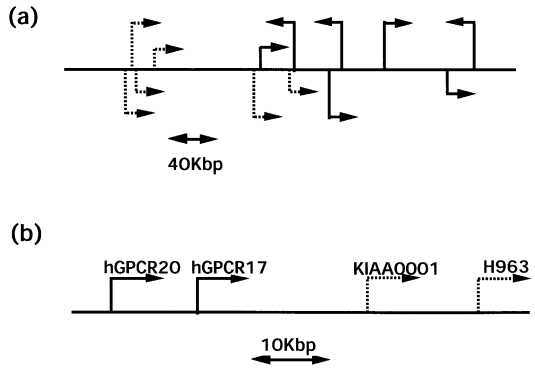

Fig. 2. Gene clusters of putative GPCRs were found on the human genome. a: Taste receptors formed a cluster on chromosome 12. The dotted lines correspond to registered taste receptors [11], and the solid lines to novel taste receptor candidates. b: Nucleotide receptors and their homologues formed a tandem structure on chromosome 3. KIAA0001 and P2RY12 were receptors for UDP-glucose [20] and ADP [17,18], respectively. The dotted lines correspond to registered receptors and the solid lines to novel receptors in this study.

within 60 000 bases, on chromosome 19. In contrast, *VNRL1* was isolated from *VNRL2* and *3* on chromosome 19. *VNRL4* and *5* were within 20 000 bases of each other on chromosome 1.

Similar analyses were also performed for other novel GPCR gene candidates. RT-PCR revealed at least 27 GPCR candidates expressed in some tissues (Table 2). We identified several gene clusters for odorant and taste receptors as described recently [10,12] (Fig. 2a). Moreover, we found a tandem structure of orphan receptor genes. Four homologues of purinergic receptors were within 60 000 bases of each other on chromosome 3 (Fig. 2b) and two homologues of Mas proto-oncogenes were within 350 000 bases of each other on chromosome 11. Registered 'retinoic acid-induced putative GPCR' and its novel homologue, hGPCR48, were within 40 000 bases of each other on chromosome 12.

Many ORFs had six to eight transmembrane segments according to SOSUI but were not significantly homologous to the registered GPCRs. We have introduced two criteria to discriminate between the GPCR candidates and the non-candidates. Firstly, we eliminated ORFs homologous to membrane-bound proteins including enzymes, ion channels, other kinds of receptors, transporters and virus proteins. Secondly, we chose ORFs with intra- and extracellular loops with lengths comparable to those of registered GPCRs. In general, extracellular loops and the first and second intracellular loops of GPCRs consist of 10–30 amino acid residues. The third intercellular loops of some GPCRs consist of more than 100 residues. Considering these facts, we eliminated ORFs with a long loop of more than 70 residues except for the third intercellular loop or a short loop of fewer than five residues. ORFs with only six transmembrane segments and fewer than 30 residues at both the N-terminus and the C-terminus were also eliminated. Finally, we chose 59 ORFs as GPCR candidates. They may represent a new class of GPCRs, although this remains to be confirmed. It should be noted that they could not be identified by homology search alone.

We have screened GPCRs with no intron in the coding region from the human genome. In order to estimate the total number of GPCRs, we first checked the proportions of intronless GPCRs for 152 of the registered GPCRs of which both cDNA and genomic clones had been sequenced (Table 3a). We did not find introns for human odorant receptor genes in the public database. In taste receptors, 11 bitter receptors were intronless [5,11] and three sweet receptors contained introns [13–15]. There were 63 intronless ORFs out of 95 known GPCR genes that had endogenous ligands. We also tested if the registered GPCRs could be predicted correctly as having six to eight transmembrane segments by SOSUI analysis (Table 3b). Correct prediction was obtained for 77% of the 43 odorant receptors, 100% of the 11 taste receptors and 95% of the 207 receptors for endogenous ligands. It remains unknown why the performance for odorant receptors was much less than for the other receptors. In addition, we checked which proportion of registered GPCR genes could be found in the database that we used in the present screening (Table 3c). We found 79% of the 43 odorant receptors, 100% of the 11 taste receptors and 90% of the 207 receptors for endogenous ligands.

The efficiency of the present screening method was independently checked by subjecting registered GPCR genes to the present system. We obtained recoveries of 67% of the odorant receptors, 79% of the taste receptors and 54% of the receptors for endogenous ligands. The recovery value of 54% for receptors for endogenous ligands was essentially the same as the products (56%) of three factors – the proportion of intronless (66%) GPCRs, the performance of SOSUI (95%), and the completeness of the genome (90%). Similar correspondence was also observed for odorant receptors (67% vs. 61%) and taste receptors (79% vs. 79%) (Table 3). These results indicate that the efficiency of the present screening strategy depends solely on the above three factors.

On the assumption that the efficiency to screen novel GPCRs is the same between registered and non-registered GPCRs, we estimated the total number of GPCRs in the human genome to be 839 (Table 1). This consists of 481 odorant receptors, 28 taste receptors, and 330 receptors for endogenous ligands. In addition, approximately 100 ORFs might represent a new class of GPCRs as they were not homologous to registered GPCRs.

Finally, we summarize some shortcomings in the present strategy. Firstly, some of the novel GPCR gene candidates could be pseudo-genes. We could exclude this possibility for

Table 3
Performance of the present screening applied for already registered GPCRs

	Odorant receptors	Taste receptors[a]	Receptors for endogenous ligand
(a) Proportion of intronless GPCR genes	100% (43/43)	79% (11/14)	66% (63/95)
(b) Performance of SOSUI	77% (33/43)	100% (11/11)	95% (197/207)
(c) Completeness of the genome data	79% (34/43)	100% (11/11)	90% (186/207)
(a)×(b)×(c)	61%	79%	56%
Performance of the present screening	67% (29/43)	79% (11/14)	54% (111/207)

[a]For taste receptors, 11 bitter receptors and three sweet receptors were employed. Umami receptor, truncated mGluR4 [18], was categorized in 'receptors for endogenous ligand'.

several GPCR candidates, by RT-PCR, but not for all. Secondly, there is a possibility that the ORFs identified here may contain an intron–exon boundary and therefore they may not correspond to proteins with seven successive transmembrane segments or may be expressed with the extra N- or C-terminal tails. Cloning of corresponding cDNA should solve these problems. The final goal of the present project should be attained when endogenous ligands for these GPCR candidates are identified.

Acknowledgements: This study was supported in part by grants from the Japan Science and Technology Corporation (CREST), the Japan Society for Promotion of Science (Research for Future Program) and the Ministry of Education, Culture, Sports, Science and Technology (the Special Coordination Fund).

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