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mRNA Expression of GPCRs in Rat Skeletal Muscle Tissues

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ABSTRACT

GPCRs are the largest family of proteins in the human genome and a target for huge numbers of therapeutic drugs. However, the highly expressed GPCRs in skeletal muscle is unclear. The purpose of this research paper was to identify genes encoding GPCRs highly expressed in skeletal muscle using microarray and real-time PCR techniques. Two male Wistar rats were used for this study. QRT-PCR (Taqman) and Agilent 4 x 44K DNA one color whole genome microarray experiments were used to measure the expression of 41091 genes in mixed fibre-type skeletal muscle from hindlimb. Expression of example members of the three major G protein coupling GPCR families was observed in rat skeletal muscle tissue. mRNA encoding G_s- (A_{2A} adenosine receptor, β_2-adrenoceptor), G_i- (A_1 adenosine receptor, α_{2A}-adrenoceptor), and G_q-coupled ($P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors) receptors were detected using gene microarray (Agilent, all ranked <10220 out of 41090). QRT-PCR (Taqman) identified α_{2A}-adrenoceptor and CB_1 cannabinoid receptor mRNA expression at low level similar across myoblasts, myotubes and skeletal muscle tissue. Differential expression of GPCRs was observed. Novel candidate GPCRs (Adrb2, $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors and Adra2a) of interest in terms of metabolism were found to be highly expressed in skeletal muscle. The functionality of these receptors needs further investigation as these receptors may play an important role in skeletal muscle functions.

Keywords: GPCRs, mRNA Expression, Skeletal Muscle and Therapeutic Targets

INTRODUCTION

G protein coupled receptors (GPCRs) are the largest family of proteins in the human genome. Indeed, GPCRs are the richest targets for pharmaceutical drugs on the market today; it is estimated that they are the targets of 30-50% of all medications due to their vast and varied roles in regulating the body processes, metabolism and signal transduction and their involvement in key biological functions [1, 2]. GPCRs are expressed in every tissue and play a major role in many diseases.

Skeletal muscle is a heterogeneous tissue since it contains a variety of fibres that differ in contractile, functional, metabolic and molecular characteristics [3, 4]. Moreover, skeletal muscle is the largest organ in the human body and represents ~40% of the human body mass and 35-40% of the total body weight in the rat [5, 6]. Indeed, skeletal muscle utilizes the majority (70-80%) of ingested glucose since it is the main site for insulin-dependent glucose uptake [7]. Therefore, it is generally considered a major site of peripheral insulin resistance.

By virtue of their large number, widespread expression and important mechanistic and regulatory roles in cell physiology and biochemistry, GPCRs play multiple well-recognized roles in clinical medicine. Therefore, GPCRs might be important in maintaining homeostasis in skeletal muscle though mediating responses to neurotransmitters and hormones. Finding the most highly expressed GPCRs will be hopefully helpful to define and characterize vital potential in terms of identifying novel targets related to clinical disorders. For most of those targets, it remains an open question whether the expression of these GPCRs in skeletal muscle is an important contributor to potential functional and metabolic roles in this tissue.

Identifying those receptors could be performed by assessing the binding of radioligands (i.e. radiolabelled agonists or antagonists), antisense approaches, expression studies (protein and mRNA level) and signalling pathways responses to such GPCRs. However, there are several challenges for some of these techniques. Indeed, the expression of receptor at protein level and radioligand binding assays can be difficult as limited validated antibodies to detect receptor protein and limited radioligands to bind to particular receptors are commercially available. Moreover, the availability of such agonists and antagonists to examine the signalling of GPCRs, in particular for orphan GPCRs, may be limited.

There are, however, very few studies about the expression of GPCRs and their

signalling and the diversity and roles of these receptors in skeletal muscle. There has yet been no comprehensive analysis carried out of GPCRs in skeletal muscle published in the literature. This study utilized microarray technology to identify the identity and relative levels of GPCRs expressed in skeletal muscle.

The purpose of the investigations in this paper was to characterize the mRNA expression of GPCRs in rat mixed fibre-type skeletal muscles using DNA microarray and QRT- PCR (Taqman) techniques.

Abbreviations

(GPCRs); G protein coupled receptors, (CB_1); cannabinoid receptor 1, (Adora2a); adenosine A2A receptor, (Adora2b); adenosine A2B receptor, (Adora1); adenosine A1 receptor, (Adrb2); adrenergic receptor, beta 2, (P2ry1); purinergic receptor P2Y, G-protein coupled 1, (Adra2a); adrenergic receptor, alpha 2a, FBS; (fetal bovine serum), (ANOVA); analysis of variance, (cDNA); Complementary DNA, (Ct); Cycle threshold, (DMSO); Dimethyl sulfoxide, (mRNA); messenger ribonucleic acid, (PBS); Phosphate buffered saline, (PCR); Polymerase chain reaction; (P-value); Probability, (RT-PCR); Reverse transcription polymerase chain reaction, (SEM); Standard error of the mean.

MATERIALS AND METHODS

Materials

TriReagent was purchased from Invitrogen. Whole genome rat 4*44K DNA microarrays were obtained from Agilent Technologies Inc. Agilent's One-Color Quick Amp Labeling kit, RNA Spike-In kit and Gene Expression Hybridization Kit were also purchased from Agilent Technologies Inc. RNeasy Mini Total RNA Purification kits were purchased from Qiagen (West Sussex, UK). Reagents for reverse transcription of RNA to cDNA were purchased from Invitrogen (Paisley, UK). Taqman reagents were purchased from Applied Biosystems (CA, USA). Primers and probes were purchased from Eurofins MWG GmBH (Ebersberg, Germany).

Tissue Collection

Two male adolescent Wistar rats (180-200 g, 4-6 weeks old) were killed by cervical dislocation without anesthesia. Liver, adipose (mixed from subcutaneous, epididymal and omental) and skeletal muscle were obtained. Skeletal muscle (mixed fibre-type from hindlimb) tissues were separated bilaterally: tissue was immediately frozen in liquid nitrogen and stored at -80 °C with liver and adipose tissue, muscle tissue was cultured after the isolation of satellite cells in gelatine-coated flasks.

Tissue Culture

Muscle culture was performed as Blau and Webster method with slight modification (Blau et al., 1981). Vastus lateralis muscles from Wistar rats were removed and immersed in phosphate buffered saline (PBS), washed to remove the remnants of blood, and minced finely with scissors and scalpel blades on a Petri dish. Then, the minced muscle was transferred to a 50 ml flask containing a "flea" and 5-10 ml of 0.25% (W/V) trypsin/EDTA (1X) for incubation at 37 Cº for 15 minutes. After that, the supernatant was transferred to a 50 ml flask and neutralised with an equal volume of medium (streptomycin, penicillin, foetal bovine serum and Ham's F10), then centrifuged at 1700 rpm for 5 minutes. The collected cells were filtered through 100 µm nylon mesh ("cell strainers") to purify the cells from the debris, and centrifuged for 10 minutes at 17,000 rpm (g=26) at room temperature. The supernatant layer was removed and the cell pellet (satellite cells) was re-suspended in Ham's F10 growth medium, pre-plated on uncoated flasks for 10 minutes at 37 C° to purify these satellite cells from fibroblasts present in the extract, and then transferred to culture flasks coated with 0.2% (W/V) gelatin. The satellite cells were then grown to confluent myoblasts and differentiated into myotubes in growth medium; 20% (V/V) fetal bovine serum (FBS) and 5 ml of penicillin and streptomycin (10,000 units penicillin and 10 mg streptomycin/ml in 0.9% NaCl) were added to Ham's F10. After one day, the cells were fed with fresh medium, cells require fresh medium every 48 hours. The cells were fed with 20% (V/V) FBS fresh medium for three weeks, then reduced to 10% (V/V) FBS fresh medium for two weeks and then changed to 6% horse serum and 10 mM glucose Ham's F10 for two to three days.

Microarray Procedure

Agilent 4*44K DNA one color whole genome microarrays were used to measure the expression of 41090 genes in liver, adipose tissue and mixed fibre-type of skeletal muscle from rat hindlimb. The microarray experiment was carried out according to manufacturing instructions. After assessing the quality control criteria (Appendix) generated from feature extraction software, extracted data were further processed with GeneSpring GX 11.

Normalization

Normalization is necessary in microarray experiments since the absolute amounts of RNA cannot be determined, due to variations in labelling and hybridization. Expression intensities resulting from the same amount of RNA can differ when comparing two microarrays. Therefore, comparison of data from multiple arrays or

multiple samples on a single array requires the data to be normalized. Data values below 1.0 were omitted and then set to 1.0. The raw data were then transformed by taking the log to base 2. Then, the 75^{th} percentile value was subtracted from each measurement in that array.

Reproducibility

The reproducibility of microarray was determined by calculating Pearson correlations using Graphpad Prism, version 5.03 (GraphPad Software Inc) on normalized data for all pair combinations of microarrays among biological replicate tissues (two animals, with 3 technical sites for skeletal muscle and one technical site for adipose and liver) (Table 1).

Concordance

The term entities "genes" were used throughout this study to designate the transcripts that are identified by these probes "features".

Entities were considered "present" only if the output was uniform, not saturated and above the background. Entities were also considered "marginal" only if the output was not above or equal to background. However, entities were considered "absent" only if the output was not uniform and saturated.

A feature is non-uniform if the pixel noise of the feature exceeds a threshold established for a "uniform" feature. A feature is saturated if 50% of the pixels in a feature are above the saturation threshold which equal 65000.

"Present", "marginal" or "absent" are terms defining the nature of the hybridization (binding of probe to gene) signals on each microarray. However, the terms "expressed transcripts", "weakly transcripts" and "not expressed transcripts" are different. The latter are terms defined by comparing the results obtained among different conditions (disease vs normal) and should not be confused with "present", "marginal" and "absent" [8]. Therefore, not all GPCR entities classified as "present" are expressed and some GPCR entities classified as "marginal" might be expressed.

The use of the threshold (present detection) will eliminate the entities that are likely to be unreliable and will keep the entities classified as "present". These entities, which are classified as "present", might be expressed or not. Consequently, the use of the threshold will increase true positive to false negative.

Filtering by signal will remove the entities with a signal close to background. However, the choice of how to determine the background is arbitrary (Background subtraction method was applied through identifying the position of the probe on the microarray and calculating the background signal and subtracting it from the

hybridization signal of the probe (Figure 1). However, many systematic sources may still remain to contribute to the background signal component, including any non-specifically-bound fluorescent signal or contaminants on the glass, fluorescent signal that is non- specifically associated with the DNA probes themselves and any artifacts from washing, hybridization and labeling (See Appendix- Figure 4 and Figure 5).

This method (Agilent microarray) was used in this study to eliminate the entities that are likely to be unreliable (entities are not classified as "present" in all replicates).

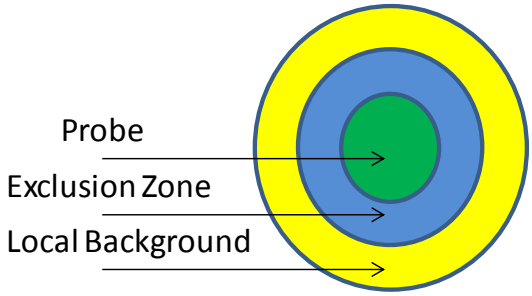

Figure 1: Background Substraction Method

RNA Extraction and QRT-PCR (Taqman)

Myoblast and myotube cells were grown and differentiated as described above. The cells were collected in TriReagent, and processed according to the manufacturer's directions. RNA was reverse transcribed into cDNA using Superscript III reverse transcriptase (Invitrogen). Then, QRT-PCR (Taqman) was performed as described according to the manufacturer's directions. Gene expression levels (in arbitrary units) were determined from the mean of triplicate determinants of each sample. Data from Taqman were only used if the slope of the standard curve for each plate was between -3.2 and -3.6 and R^2 values of more than 0.99. In addition, Ct values of triplicate readings for an individual sample, which were more than 0.5 Ct apart, were excluded. The Oligonucleotide sequences for probes, reverse primers and forward primers were represented in Table 2.

Statistical Analysis

Analysis was performed using GraphPad Prism, version 5.03 (GraphPad Software Inc). Data were represented as means ± standard error of mean (SEM).

RESULTS

Reproducibility

In order to examine the reproducibility between replicates, Pearson correlation was performed. Correlations on normalized data among skeletal muscles and among liver replicates were above 0.97 (Pearson correlation), indicating high reproducibility. Similarly, correlation between adipose replicates was slightly lower at ~ 0.96. Adipose tissue samples were taken from three different parts with different proportions of subcutaneous, epididymal and omental adipose tissue (Figure 2 and Table 3).

Confirmatory Expression

Glut4, fabp3, ppara and pparg were

selected as confirmatory genes to define skeletal muscle, liver and adipose tissue for the microarray experiments in order to reflect the microarray reliable data in a tissue specific manner.

The present study revealed that the *glut4*/muscle-fat glucose transporter was highly expressed in skeletal muscle and adipose tissue while low expression was observed in liver tissue (Figure 3A). Fatty acid binding protein 3 (*fabp3*) was also highly expressed in skeletal muscle while low expression was observed in liver and adipose tissues (Figure 3B). *Ppara* was highly expressed in liver and skeletal muscle tissue while low expression was observed in adipose tissue (Figure 3C). *Pparg* was highly expressed in adipose tissue while low expression was observed in liver and skeletal muscle tissues (Figure 3D).

Skeletal Muscle Type Definition

In order to determine the type of skeletal muscle fibres, the relative intensity values of the rat fast and slow fibre type specific structural subunits were determined (Figure 4); Troponin I (*tnni1*), troponin T 1 (*tnnt1*), tropomyosin 3 (*tpm3*), myosin light chain 2 (*myl2*), and, myosin heavy chain 7 (*myh7)* are markers for slow twitch muscle [9-13]. Troponin I 2 (*tnni2*), troponin T 3 (*tnnt3*), troponin C 2 (*tnnc2*) tropomyosin 1 (*tpm1*), myosin light chain 1 (*myl1*) and myosin light chain 3 (*myl3*) myosin heavy chain 1 (*myh1)* myosin heavy chain 2 (*myh2)* are markers for fast twitch muscle [9-11, 14, 15]. Slow twitch muscle structural subunits (*tnni1, tnnt1, tpm3, myl2* and *myh7*) and fast twitch muscle structural subunits (*tnni2, tnnt3, tnnc2, tpm1, myl1, myl3, myh1* and *myh2*) were detected (419 ranking out of 41090).

mRNA Expression of GPCRs

The mRNA relative intensity values of GPCR entities classified as "present" in all skeletal muscle replicates using microarray is shown in Table 6 in Appendix.

88 GPCRs out of 329 (taken from "The International Union of Basic and Clinical Pharmacology") in all skeletal muscle samples were classified as "present" using Agilent microarray.

38 GPCR entities were detected in skeletal muscle tissue (ranked higher (higher relative intensity values) than either nicotinic cholinergic alpha subunit (chrnα1) or reference gene (tbp) (12526 and 10220 ranking out of 41090, respectively)), see Table 6 in Appendix. From these 38 GPCR entities, β_2-adrenoceptors, $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors, A_1 and A_{2A}-adenosine receptors, NPY Y_1 receptor and α_2-adrenoceptors were selected as shown in Table 4. These GPCR entities include three main families that coupled to different G proteins (G_s, G_i and G_q) in this study.

Examples of G_s-GPCRs are β_2-adrenoceptor, A_{2A}-adenosine receptor, G_i-GPCRs are A_1 adenosine receptor and NPY Y_1 receptor, and G_q-GPCRs are $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors. Indeed, investigation of the different families (G_s, G_i and G_q) of GPCRs might help to confirm the expression and understand the expected signalling and functional role of these receptors and to investigate the possible cross-talk between GPCRs signalling and their signalling partners' genes.

mRNA Expression of GPCRs Using QRT-PCR (Taqman)

Some entities were selected to be examined by QRT-PCR (Taqman). The criteria was to cover different ranking areas from the microarray entities list and to select GPCR entities that possibly have some expected roles in skeletal muscle glucose metabolism [16-20].

Examining mRNA expression of these receptors (GPR119, α_2-adrenoceptors and GPR40) in skeletal muscle will be helpful in this study for further investigation. Moreover, a relationship between GPR40 or GPR119 and diabetes has been suggested. However, the mechanisms behind these issues are still unclear [16, 17]. In order to examine the expression of these receptors and validate the mRNA expression of GPCRs, QRT-PCR (Taqman) was performed.

mRNA of *actb* and *18S* were detected in QRT-PCR (Taqman) at Ct values of ~ 19 and 22, respectively (ranking in the microarray; 8 and 275, respectively, out of 41090). mRNA for *adra2a* and *cnr1* were detected in rat skeletal muscle tissues at Ct ~ 32 and 27, respectively (ranking in the microarray; 14208 and 34463/35034 out of 41090, respectively). There was no significant difference in the expression of *adra2a* and *cnr1* between skeletal muscle and adipose tissues using QRT-PCR (Taqman). *Gpr40* was not detected using QRT-PCR (Taqman) (ranking in the microarray; 40809 out of 41090). *Gpr119* was detected at Ct ~ 33 (ranking in the microarray; 25255 out of 41090- marginal in 4 skeletal muscle replicates out of 6 replicates). Overall, *actb*, *18S*, *cnr1*, *gpr119* and *adra2a* were detected in skeletal muscle tissues (Table 5).

Table 1: The two biological skeletal muscle, liver and adipose replicates. Site A is a mixture of extensor digitorum longus and tibialis anterior, site B is a mixture of soleus and plantaris, and site C is a mixture of red and white gastronemius muscle with roughly equal amounts of each muscle.

Rat A	Rat B
Skeletal muscle-Site A	Skeletal muscle-Site A
Skeletal muscle-Site B	Skeletal muscle-Site B
Skeletal muscle-Site C	Skeletal muscle-Site C
Liver	Liver
Adipose	Adipose

Table 2: Oligonucleotide sequences for probes and primers.

Gene	Sequences (5' - 3')	Amplicon size (bp)	Gene Bank Accession No.
18s	FWD CGGCTACCACATCCAAGGAA PROBE TGCTGGCACCAGACTTGCCCTC REV GCTGGAATTACCGCGGCT	188	M10098
Actb	FWD GAGCGTGGCTACTCCTTCGT PROBEACCACAGCTGAGCGCGAGATCGT REVGTAGCACAGCTTCTCCTTGATGTC	72	NM_001100
Cnr1	FWD CCAAAAGTGGAGAGCGACAAC PROBE ATCCAGATCACCATGCCGTTCACA REV CGTCTCGAAGGTCCCAATGT	68	NM_012784.4
Gpr40	FWD CCTGCCCGACTCAGTTTCTC REV CGGAGGCAGCCCACATAG PROBE TTCTGCTCTTCTTTCTGCCCTTGGTTATCA	80	NM_153304.1
Adra2a	FWD GGCCTCAGCGGACATCCT PROBE TGGCCACGCTGGTCATTCCCTT REV CATAACCTCGTTGGCCAAAGA	64	NM_012739.3
Gpr119	FWD TCCATATTCCAGCAGACCACCTA PROBE CATGGGCCCTGCACCTTCTTTGC REV GCACAAACCTTGGGTGAAACA	70	NM_181770.1

Table 3: Correlation between two biological replicates from mixed fibre-type hindlimb skeletal muscle (0.97) taken from two rats. Site A was a mixture of extensor digitorum longus and tibialis anterior, site B was a mixture of soleus and plantaris, and site C was a mixture of red and white gastrocnemius muscle with roughly equal amount of each muscle.

Correlation			Rat A			Rat B		
			Site			Site		
			A	B	C	A	B	C
Rat A	Site	A		0.98	0.95	0.97	0.98	0.97
		B	0.98		0.95	0.96	0.97	0.96
		C	0.95	0.95		0.96	0.96	0.95
Rat B	Site	A	0.97	0.96	0.96		0.98	0.98
		B	0.98	0.97	0.96	0.98		0.98
		C	0.97	0.96	0.96	0.98	0.98	

Table 4: Relative intensity values for selected detected GPCRs (comprising three major families of G protein signalling) in all skeletal muscle replicates. Site A is a mixture of extensor digitorum longus and tibialis anterior, site B is a mixture of soleus and plantaris, and site C is a mixture of red and white gastrocnemius muscle with roughly equal amounts of each muscle. The GPCRs was selected depending on known and available ligands local expertise, cost and above either cholinergic subunit (*chrnα1*) or *tbp* relative intensity values. NA: Not Applicable.

		Rat A			Rat B		
		Site					
GPCR (gene name) [gene id]	Principal G protein	A	B	C	A	B	C
Purinergic receptor P2Y, G-protein coupled 2 (P2ry2), mRNA [NM_017255]	G_q	1.7	1.2	0.9	1.4	1.6	1.7
Adrenergic receptor, β 2 (Adrb2), mRNA [NM_012492]	G_s	1.2	0.8	1.2	1.1	1.1	0.9
Adenosine A2a receptor (Adora2a), mRNA [NM_053294]	G_s	0	-0.2	0.5	0.7	0.5	0.3
Pyrimidinergic receptor P2Y, G-protein coupled, 6 (P2ry6), mRNA [NM_057124]	G_q	-0.3	-0.7	0.1	0	1	0
Adenosine A1 receptor. [Source:Uniprot/SWISSPROT;Acc:P25099]	G_i	-0.6	-1.3	-0.7	-0.5	-0.4	-0.6
Adrenergic receptor, alpha 2a (Adra2a), mRNA [NM_012739]	G_i	0.2	0.1	-1.2	-0.5	-1.2	-1.5
Purinergic receptor P2Y, G-protein coupled 1 (P2ry1), mRNA [NM_012800]	G_q	-1.2	-1.2	-1.9	-1.8	-1.7	-0.9
Neuropeptide Y receptor Y1 (Npy1r), mRNA [NM_001013032]	G_i	-1.2	-1.1	-2.8	-1.8	-1	-1.7
TATA box binding protein (Tbp), mRNA [NM_001004198]	NA	-0.2	0	0.3	0.3	0.8	-0.2
Cholinergic receptor, nicotinic, alpha 1 (muscle) (Chrna1), mRNA [NM_024485]	NA	0.1	-0.6	-0.7	1.1	1.5	0.3

Table 5: Ct values for QRT-PCR (Taqman) for GPCR entities and reference genes for skeletal muscle cells and tissues and for liver and adipose tissues from two replicates from two rat repeats.

Gene/Ct value	Skeletal	Myotube	Myoblast	Liver	Adipose
Actb	19.5	16.5-17.5	16.5-17.9	20.0	17.6
18S	21.5-23.0	21.5	22.5	22.5	22.5
Gpr40	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined
Adra2a	31.0-32.0	30.0	28.0	37.0	30.0
Gpr119	32.0-33.0	35.0-36.0	35.0	31.0	31.5

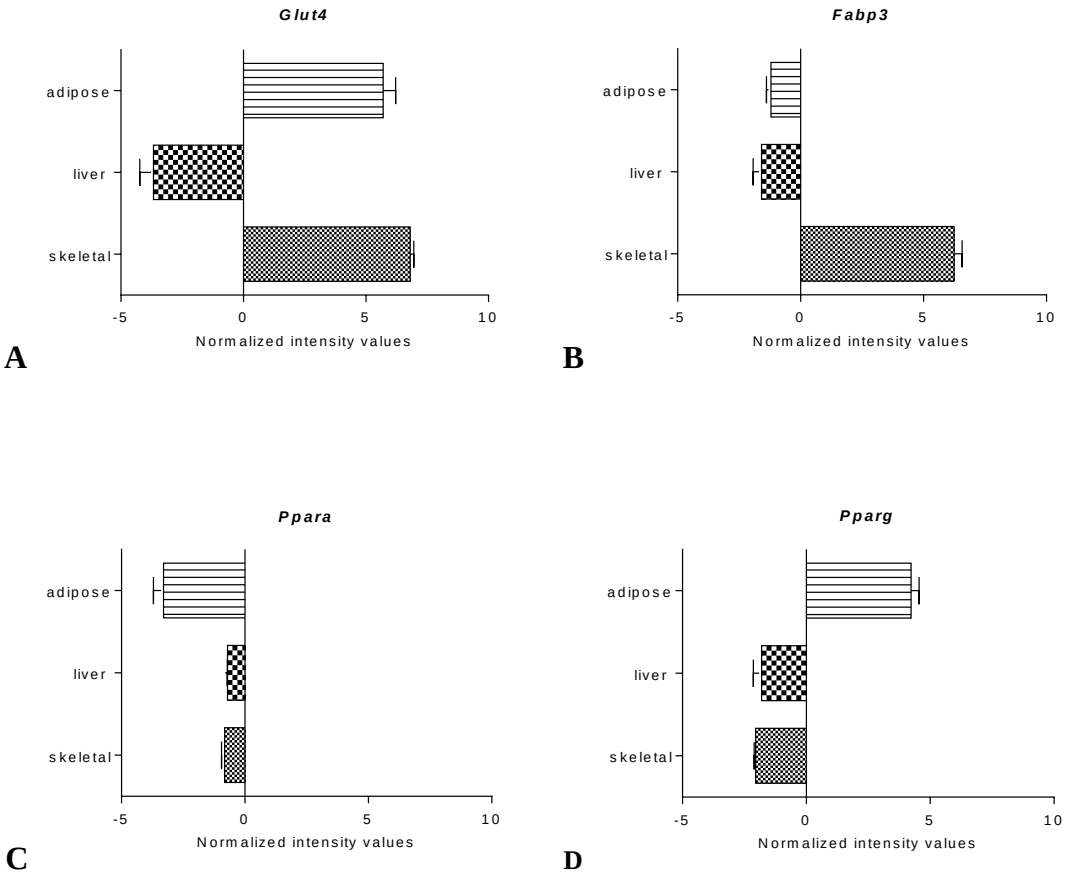

Figure 3: RNA transcript levels for (A) *glut4*, (B) *fabp3*, (C) *ppara* and (D) *pparg* in mixed skeletal muscle, liver and adipose from two male Wistar rats. (As number per group is not equal, statistics was not performed)

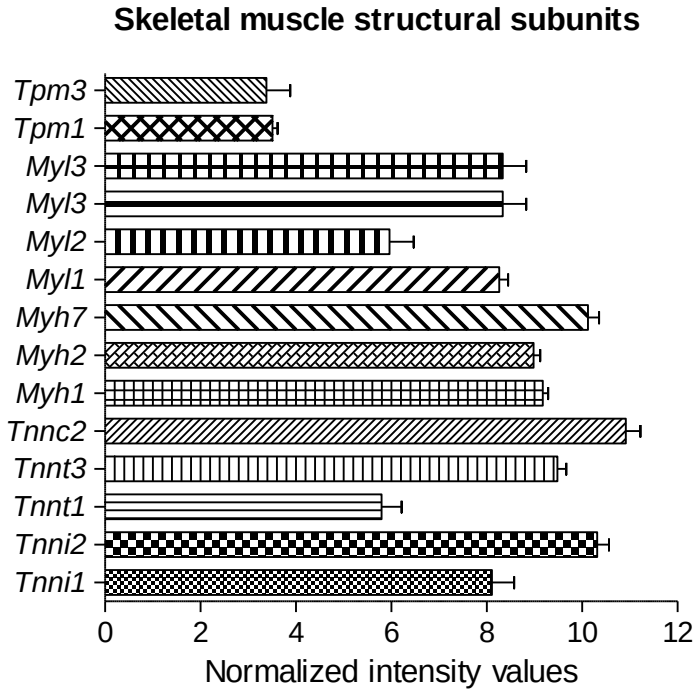

Figure 4: RNA transcript levels for muscle structural subunits classified as "present" in mixed skeletal muscle tissue for three replicates from two independent repeats (male Wistar rats).

DISCUSSION

Traditional methods for the quantification of gene expression, such as RT-PCR or Northern blot analysis, focus on a single gene at a time. Therefore, these techniques are not suitable to determine the relative mRNA expression of GPCRs, G proteins and their target enzymes in skeletal muscle. Microarrays can be used to study simultaneously the relative expression of many genes.

Validation of the Microarray

The two biological replicates were found to be reproducible since the Pearson correlations for normalized data for all pair combinations among biological replicate tissues were above 0.96.

Glut4, fabp3, ppara and pparg expressions were selected as confirmatory measurements since their relative expression levels in different tissues have been well documented in the literature. *Glut4* is highly expressed in skeletal muscle and adipose tissues [21], however, it is not detected in liver [22, 23]. *Fabp3* is not detected in liver and adipose but is highly expressed in skeletal muscle [24]. *Ppara* is highly expressed in liver and skeletal muscle with low expression in adipose tissue [25, 26]. *Pparg* is expressed predominantly in adipose tissue with low expression seen in liver and muscle [27, 28]. The findings of the present study confirmed that: a) *glut4* is highly expressed in skeletal muscle and adipose tissue relative to liver; b) *fabp3* is highly expressed in skeletal muscle and negligibly expressed in liver and adipose; c) *ppara* is highly expressed in liver and skeletal muscle; and d) *pparg* is highly expressed in adipose tissue with low expression seen in liver and skeletal muscle tissue. This gave the microarray data to be valid and reliable to detect relative mRNA expression of genes in a tissue specific manner.

Slow and fast twitch muscle structural subunit genes mRNA were expressed in skeletal muscle. This reflects that the dissection has been covered both fast and slow skeletal muscle fibres. This also indicates that the detected GPCR entities will cover different types of fibres.

GPCRs Expression in Skeletal Muscle

GPCRs expression was examined in this study using two different techniques, Agilent microarray and QRT-PCR (Taqman). Using Agilent microarray, 38 GPCR entities were found to be expressed in skeletal muscle in this study. These include LPA_1 lysophosphatidic acid receptors

(endothelial differentiation, lysophosphatidic acid G protein-coupled receptor, 2 (Edg2)), chemokine receptors 4 (CXCR4), glucagon receptor 2, platelet-activating factor receptors, $GABA_{B1}$ receptors, $S1P_2$ sphingosine-1-phosphate receptors (endothelial differentiation, sphingolipid G protein-coupled receptor, 5 (Edg5)), parathyroid hormone receptors, $mGlu_2$ and $mGlu_3$ metabotropic glutamate receptors, dopamine D_5 receptors, neurotensin receptor 2, opioid receptor delta 1, calcitonin receptors 1, arginine vasopressin receptors 1A, bradykinin B_2 receptors and $C5a_1$ complement peptide receptors. However, other GPCR entities can not be excluded for being expressed in skeletal muscle.

These GPCRs are somewhat less well-known in terms of their activity in the skeletal muscle system. Relatively little information in the literature is reported in both normal and disease state about the role of these GPCRs in functional activities and in signal transduction of skeletal muscle tissues.

The importance of such GPCR patterns detected in skeletal muscle tissue might be approved in the regulation of several intracellular functions. These functions in skeletal muscle might include contractile responses, glucose uptake, regulation of metabolism and skeletal muscle proliferation, differentiation and growth. To date, virtually no information is available regarding physiological functions, patho-physiological roles, regulation and gene expression patterns of such GPCRs in skeletal muscle tissues.

As a large number of GPCR entities were discussed in this study, these GPCR entities were divided in their groups (class A, class B and class C) as described in IUPHAR/BPS GUIDE TO PHARMACOLOGY:

G Protein-Coupled Receptors Class A

LPA_1 lysophosphatidic acid receptors gene mRNA was found to be expressed in a wide range of different human and mouse tissues including skeletal muscle, heart, brain, stomach, kidney, spleen, thymus, testis and lung [29-31]. LPA_1 lysophosphatidic acid receptors were also shown to couple to $G_{\alpha i}$, $G_{\alpha q}$, and $G_{\alpha 12/13}$ [32, 33]. As skeletal muscle expressed *lpar1* in this study, it is possible that the activation of LPA_1 lysophosphatidic acid receptors affect proliferation and differentiation in skeletal muscle. Therefore, LPA_1 lysophosphatidic acid receptor agonists might be considered as a therapeutic target to improve skeletal muscle mass. This is supported by the fact that 1). The

activation of LPA_1 lysophosphatidic acid receptors by LPA was shown to stimulate proliferation, migration, and invasion in human colon cancer cell lines (DLD1) which expressed *lpar1* [34]. 2) *Lpar1−/−* mice were also found to have a higher adiposity than wild-type mice [35]. 3) LPA_1 lysophosphatidic acid receptor activation reduced the differentiation of mouse 3T3F442A preadipocytes [35]. Apart from that potential role, LPA_1 lysophosphatidic acid receptors might have a role in glucose uptake. As skeletal muscle expressed *lpar1* in this study, it is also possible that LPA_1 lysophosphatidic acid receptors play a role in glucose uptake in skeletal muscle, and it might be, therefore, considered as a therapeutic option for diabetes. The implication of this is that LPA_1 lysophosphatidic acid receptor agonists should be recommended to be investigated as a therapy to treat diabetes. This is probably due to the fact that LPA was shown to stimulate GLUT4 translocation and glucose uptake in 3T3-L1 adipocytes and L6 GLUT4myc myotubes which expressed LPA_1 lysophosphatidic acid receptors [36], and acute administration of LPA in mice was also shown to cause a fall in blood glucose level [36].

Regarding another GPCR, GPCR activators such as chemokines, a large family of 8 to 10-kd cytokines or proteins, act as chemoattractants [37]. The activation of chemokine receptors by chemokines lead to activation of phospholipases through G proteins which yield to an increase in IP_3, the release of calcium and the activation of protein kinase C [37]. Moreover, chemokines were also shown to activate the Ras and Rho families [38]. Chemokines such as SDF1α, stromal cell-derived factor-1 alpha, can bind to specific GPCRs such as CXCR4 (G_i or G_q coupled). Indeed, *cxcr4* was detected in skeletal muscle in this study. This is consistent with *cxcr4* was expressed in skeletal muscle satellite cell lines [39]. As skeletal muscle expressed *cxcr4* and *sdf1a* (1277 ranking out of 41090) in this study, it is possible that CXCR4 in skeletal muscle plays a role in migration during myogenesis which is essential for skeletal muscle growth and regeneration. This is suggested due to the fact that it was shown that SDF1α enhances migration and proliferation of the immortalized C2C12 muscle cell line [40], and chemokines were also suggested to be important for muscle precursor cells in the migration during myogenesis [41]. Therefore, CXCR4

agonists might be recommended to be examined as a therapeutic option to improve skeletal muscle myogenesis, growth and to treat skeletal muscle regeneration disorders.

Another GPCR that needs attention is bradykinin B_2 receptor, bradykinin exerts its effect on two subtypes of G_q-GPCR, namely bradykinin B_1 and B_2 receptors [42]. Bradykinin B_2 receptor (*bdkrb2*) mRNA was detected in skeletal muscle in this study. This is consistent with the bradykinin B_2 receptor was found to be detected on the plasma membrane of skeletal muscle cells of the rat hindlimb [43], and it was also reported that bradykinin B_2 receptors were expressed in guinea pig skeletal muscle tissue [44].

As *bdkrb2* was detected in skeletal muscle in this study, it is possible that bradykinin B_2 receptors might play a role of insulin resistance and glucose uptake in skeletal muscle, and therefore bradykinin B_2 receptor agonists be recommended to be investigated as a therapeutic target for diabetic patients. This is supported by the fact that chronic *in vivo* administration of bradykinin was shown to significantly improve whole body glucose tolerance in the severely insulin resistant obese Zucker rat which was suggested to be a result of the enhanced insulin-stimulated skeletal muscle glucose uptake [45], and bradykinin B_2 receptor knockout mice was also shown to have impaired insulin-dependent glucose transport [46]. Apart from that potential role, bradykinin B_2 receptor might have an inflammatory role. Bradykinin, a nine amino acid polypeptide, is one of various inflammatory mediators released from inflamed tissues after tissue injury to mediate the inflammatory process [47]. As inflammatory muscle pain was shown to be associated with the up-regulation of both bradykinin B_1 and B_2 receptors which contributed to mechanical hyperalgesia in inflammatory muscle pain in male Swiss mice [48], and *bdkrb2* was also detected in skeletal muscle in this study, it is possible that bradykinin B_2 receptors might play a role in inflammatory skeletal muscle pain. Therefore, antagonizing bradykinin B_2 receptors in skeletal muscle might be considered as a therapeutic target for pain management.

With regard to another GPCR, $C5a_1$ complement peptide receptors gene mRNA was reported to be expressed in monocytes, neutrophils, esinophils and basophils, and it was also shown that c*5ar1* was expressed in human liver and HepG2, lung cells, astrocytes and

microglia cells [49, 50]. It was also shown that c*5ar1* was up-regulated in injured human skin and rat burn injury [51, 52] and up-regulated in casting and tenotomy-induced muscle atrophy in male mice [53]. Indeed, skeletal muscle expressed c*5ar1* in this study. Taken together, it is possible, therefore, that the $C5a_1$ complement peptide receptors play a role in skeletal muscle injury, wound pathophysiology and growth. However, little information in the literature was reported about the role of $C5a_1$ complement peptide receptors in skeletal muscle. Apart from that potential role, $C5a_1$ complement peptide receptors might have a role in obesity. It was also reported that c*5ar1* was up-regulated from omental adipose in obese human being compared to normal human being using gene expression microarray [54]. It is possible, therefore, that $C5a_1$ complement peptide receptors in skeletal muscle might be investigated as a therapeutic target for obesity. The implication of this is that $C5a_1$ complement peptide receptor antagonists should be investigated to decrease weight, to improve skeletal muscle growth and wound healing.

G Protein-Coupled Receptors Class B

Parathyroid hormone (PTH) receptors 1 (*pthr1*) gene mRNA was abundantly expressed in rat kidney and bone tissues, and it was also expressed in many other rat tissues including skeletal muscle, ovary, placenta, aorta, adrenal gland, bladder, brain, cerebellum, breast, heart, ileum, liver, lung, placenta, skin, spleen, stomach, uterus, and testes [55-57]. Moreover, PTH receptors 1 are thought to couple to G_s, G_q, G_i and G_{12} [58-60]. Indeed, skeletal muscle expressed *Pthr1* in this study. Overall, it is possible that PTH receptors 1 modulate cAMP and calcium release in skeletal muscle tissue which might be important for muscle growth and contraction as discussed previously. The implication of this is that PTH receptors 1 might be recommended to be investigated to improve contraction-stimulated glucose uptake, consequently the PTH receptors 1 agonist might be used to treat diabetes. Calcitonin receptor (*Calcr*) was shown to be linked to adenylyl cyclase and phospholipase C [61]. Calcitonin is released from the parathyroid glands and its inhibitory effect on bone resorption is caused by the activation of calcitonin receptors in mature osteoclasts [61]. *Calcr* was shown to be expressed in mature rat and human osteoclasts [62, 63]. However, this receptor was not detected in osteoclast progenitor cells, but the expression of this receptor

increased during mouse and rat osteoclast differentiation [64]. *Calcr* was also found to be expressed in mice and human satellite cells [65-67]. As *calcr* was expressed in skeletal muscle in this study, it is possible that calcitonin receptor might play a role in proliferation in skeletal muscle which might be important for skeletal muscle growth. This is supported by the fact that the activation of the calcitonin receptors was shown to increase ERK1/2 activity in HEK cells expressing calcitonin receptors [68], and it was also reported that calcitonin receptor protein expression was down-regulated on activated satellite cells [66]. Therefore, calcitonin receptor agonists should be recommended to be investigated as a therapeutic option to improve skeletal muscle growth.

G Protein-Coupled Receptors Class C

Metabotropic glutamate receptors are classified into group I ($mGlu_1$ and $mGlu_5$ metabotropic glutamate receptors), group II ($mGlu_2$ and $mGlu_3$ metabotropic glutamate receptors) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ metabotropic glutamate receptors) based on signal transduction and sequence homology. $mGlu_2$ and $mGlu_3$ metabotropic glutamate receptors (*grm2* and *grm3*) gene mRNA was expressed in rat neurons [69-71]. The action of glutamate can also exert through $mGlu_2$ and $mGlu_3$ metabotropic glutamate receptors. As skeletal muscle expressed *grm2* and *grm2* in this study, and $mGlu_2$ and $mGlu_3$ metabotropic glutamate receptors were also found to couple predominantly to G_i proteins [71, 72], and $mGlu_2$ metabotropic glutamate receptors was also found to inhibit adenylyl cyclase in CHO cells, it is possible that $mGlu_2$ and $mGlu_3$ metabotropic glutamate receptor decrease cAMP level in skeletal muscle which might be important, as discussed previously, for skeletal muscle growth.

Regarding 38 CPCRs detected in skeletal muscle in this study, three main families of GPCRs (G_s, G_i and G_q) were detected in skeletal muscle tissue using the microarray. Examples include G_s-GPCR (β-adrenoceptor, A_{2A} adenosine receptors), G_i-GPCR (A_1 adenosine receptor and NPY Y_1 receptor) and G_q-GPCR ($P2Y_1$ and $P2Y_2$ receptors).

Regarding the other GPCRs which were detected in skeletal muscle using QRT-PCR, *adra2a* mRNA were detected in QRT-PCR (Taqman) and these agreed with the microarray. GPR40 mRNA was not detected in QRT-PCR (Taqman) and this agreed with the microarray. CB_1 mRNA was also detected using QRT-

PCR (Taqman). However, it was classified as "marginal" in the microarray. This did not contradict with the reliability of the microarray data since it was reported that the validation did not often result in agreement between microarray and QRT-PCR data [73]. Moreover, GPR119 was detected using QRT-PCR (Taqman) at high Ct values (Ct~32-35, low expression). It also agreed with the microarray that detected GPR119 at ranking ~25000. So, in general, the intensity values from the microarray agreed with Ct values from QRT-PCR (Taqman) except with *cnr1*. Finally, *cnr1*, *gpr119* and *adra2a* were detected in skeletal muscle tissue and should be investigated later for further signalling and functional roles in skeletal muscle.

With the recognition of detected GPCRs mRNA relative expression, it is possible to understand the signalling of these GPCRs in skeletal muscle tissues. GPCRs, in general, can be linked to ubiquitous downstream effectors. Such receptor signalling systems can also offer alternative therapeutic approaches.

CONCLUSION

This study gave a picture and guide for the some GPCRs detected in skeletal muscle in skeletal muscle, which has not previously been reported, in order to be investigated later.

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REFERENCES

1. Kobilka, B.K., *G protein coupled receptor structure and activation.* Biochim Biophys Acta, 2007. **1768**(4): p. 794-807.
2. Tilakaratne, N. and P.M. Sexton, *G-Protein-coupled receptor-protein interactions: basis for new concepts on receptor structure and function.* Clin Exp Pharmacol Physiol, 2005. **32**(11): p. 979-87.
3. Staron, R.S., et al., *Fiber type composition of four hindlimb muscles of adult Fisher 344 rats.* Histochem Cell Biol, 1999. **111**(2): p. 117-23.
4. Pette, D. and R.S. Staron, *Mammalian skeletal muscle fiber type transitions.* Int Rev Cytol, 1997. **170**: p. 143-223.
5. Delbono, O., et al., *Loss of skeletal muscle strength by ablation of the sarcoplasmic reticulum protein JP45.* Proc Natl Acad Sci U S A, 2007. **104**(50): p. 20108-13.
6. Pedersen, B.K., *Muscles and their myokines.* J Exp Biol, 2011. **214**(Pt 2): p. 337-46.
7. Toft, I., et al., *Insulin kinetics, insulin action, and muscle morphology in lean or slightly overweight persons with impaired glucose tolerance.* Metabolism, 1998. **47**(7): p. 848-54.
8. Rimbault, M., et al., *RNA profiles of rat olfactory epithelia: individual and age related variations.* BMC Genomics, 2009. **10**: p. 572.
9. Pieples, K. and D.F. Wieczorek,

Tropomyosin 3 increases striated muscle isoform diversity. Biochemistry, 2000. **39**(28): p. 8291-7.
10. Smerdu, V., et al., *Type IIx myosin heavy chain transcripts are expressed in type IIb fibers of human skeletal muscle.* Am J Physiol, 1994. **267**(6 Pt 1): p. C1723-8.
11. Tajsharghi, H., *Thick and thin filament gene mutations in striated muscle diseases.* Int J Mol Sci, 2008. **9**(7): p. 1259-75.
12. Gahlmann, R., et al., *Differential expression of slow and fast skeletal muscle troponin C. Slow skeletal muscle troponin C is expressed in human fibroblasts.* J Mol Biol, 1988. **201**(2): p. 379-91.
13. Barton, P.J., et al., *Close physical linkage of human troponin genes: organization, sequence, and expression of the locus encoding cardiac troponin I and slow skeletal troponin T.* Genomics, 1999. **57**(1): p. 102-9.
14. Periasamy, M., et al., *Fast skeletal muscle myosin light chains 1 and 3 are produced from a single gene by a combined process of differential RNA transcription and splicing.* J Biol Chem, 1984. **259**(21): p. 13595-604.
15. Schreier, T., L. Kedes, and R. Gahlmann, *Cloning, structural analysis, and expression of the human slow twitch skeletal muscle/cardiac troponin C gene.* J Biol Chem, 1990. **265**(34): p. 21247-53.
16. Swaminath, G., *Fatty acid binding receptors and their physiological role in type 2 diabetes.* Arch Pharm (Weinheim), 2008. **341**(12): p. 753-61.
17. Overton, H.A., M.C. Fyfe, and C. Reynet, *GPR119, a novel G protein-coupled receptor target for the treatment of type 2 diabetes and obesity.* Br J Pharmacol, 2008. **153 Suppl 1**: p. S76-81.
18. Ravinet Trillou, C., et al., *CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity.* Int J Obes Relat Metab Disord, 2004. **28**(4): p. 640-8.
19. Lindborg, K.A., et al., *Effects of in vitro antagonism of endocannabinoid-1 receptors on the glucose transport system in normal and insulin-resistant rat skeletal muscle.* Diabetes Obes Metab, 2011. **12**(8): p. 722-30.
20. Eckardt, K., et al., *Cannabinoid type 1 receptors in human skeletal muscle cells participate in the negative crosstalk between fat and muscle.* Diabetologia, 2008.
21. James, D.E., M. Strube, and M. Mueckler, *Molecular cloning and characterization of an insulin-regulatable glucose transporter.* Nature, 1989. **338**(6210): p. 83-7.
22. Rayner, D.V., M.E. Thomas, and P. Trayhurn, *Glucose transporters (GLUTs 1-4) and their mRNAs in regions of the rat brain: insulin-sensitive transporter expression in the cerebellum.* Can J Physiol Pharmacol, 1994. **72**(5): p. 476-9.
23. Liu, M.L., et al., *Expression and regulation of the human GLUT4/muscle-fat facilitative glucose transporter gene in transgenic mice.* J Biol Chem, 1992. **267**(17): p. 11673-6.
24. Heuckeroth, R.O., et al., *Analysis of the tissue-specific expression,*

developmental regulation, and linkage relationships of a rodent gene encoding heart fatty acid binding protein. J Biol Chem, 1987. **262**(20): p. 9709-17.
25. Lemberger, T., et al., *PPAR tissue distribution and interactions with other hormone-signaling pathways.* Ann N Y Acad Sci, 1996. **804**: p. 231-51.
26. Braissant, O., et al., *Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat.* Endocrinology, 1996. **137**(1): p. 354-66.
27. Kiec-Wilk, B., et al., *The selected pathophysiological aspects of PPARs activation.* J Physiol Pharmacol, 2005. **56**(2): p. 149-62.
28. Vidal-Puig, A.J., et al., *Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids.* J Clin Invest, 1997. **99**(10): p. 2416-22.
29. Choi, J.W., et al., *LPA receptors: subtypes and biological actions.* Annu Rev Pharmacol Toxicol, 2010. **50**: p. 157-86.
30. An, S., et al., *Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid.* J Biol Chem, 1998. **273**(14): p. 7906-10.
31. Ye, X., *Lysophospholipid signaling in the function and pathology of the reproductive system.* Hum Reprod Update, 2008. **14**(5): p. 519-36.
32. Fukushima, N., Y. Kimura, and J. Chun, *A single receptor encoded by vzg-1/lpA1/edg-2 couples to G proteins and mediates multiple cellular responses to lysophosphatidic acid.* Proc Natl Acad Sci U S A, 1998. **95**(11): p. 6151-6.
33. Ishii, I., et al., *Functional comparisons of the lysophosphatidic acid receptors, LP(A1)/VZG-1/EDG-2, LP(A2)/EDG-4, and LP(A3)/EDG-7 in neuronal cell lines using a retrovirus expression system.* Mol Pharmacol, 2000. **58**(5): p. 895-902.
34. Shida, D., et al., *Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1.* Cancer Res, 2003. **63**(7): p. 1706-11.
35. Simon, M.F., et al., *Lysophosphatidic acid inhibits adipocyte differentiation via lysophosphatidic acid 1 receptor-dependent down-regulation of peroxisome proliferator-activated receptor gamma2.* J Biol Chem, 2005. **280**(15): p. 14656-62.
36. Yea, K., et al., *Lysophosphatidic acid regulates blood glucose by stimulating myotube and adipocyte glucose uptake.* J Mol Med (Berl), 2008. **86**(2): p. 211-20.
37. Luster, A.D., *Chemokines--chemotactic cytokines that mediate inflammation.* N Engl J Med, 1998. **338**(7): p. 436-45.
38. Laudanna, C., J.J. Campbell, and E.C. Butcher, *Role of Rho in chemoattractant-activated leukocyte adhesion through integrins.* Science, 1996. **271**(5251): p. 981-3.
39. Ratajczak, M.Z., et al., *Expression of functional CXCR4 by muscle satellite cells and secretion of SDF-1 by muscle-derived fibroblasts is associated*

with the presence of both muscle progenitors in bone marrow and hematopoietic stem/progenitor cells in muscles. Stem Cells, 2003. **21**(3): p. 363-71.
40. Odemis, V., et al., *The chemokine SDF1 controls multiple steps of myogenesis through atypical PKCzeta.* J Cell Sci, 2007. **120**(Pt 22): p. 4050-9.
41. Vasyutina, E., et al., *CXCR4 and Gab1 cooperate to control the development of migrating muscle progenitor cells.* Genes Dev, 2005. **19**(18): p. 2187-98.
42. Leeb-Lundberg, L.M., et al., *International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences.* Pharmacol Rev, 2005. **57**(1): p. 27-77.
43. Figueroa, C.D., G. Dietze, and W. Muller-Esterl, *Immunolocalization of bradykinin B2 receptors on skeletal muscle cells.* Diabetes, 1996. **45 Suppl 1**: p. S24-8.
44. Rabito, S.F., et al., *Bradykinin B2 receptors on skeletal muscle are coupled to inositol 1,4,5-trisphosphate formation.* Diabetes, 1996. **45 Suppl 1**: p. S29-33.
45. Henriksen, E.J., et al., *Effect of chronic bradykinin administration on insulin action in an animal model of insulin resistance.* Am J Physiol, 1998. **275**(1 Pt 2): p. R40-5.
46. Duka, I., et al., *Role of the B(2) receptor of bradykinin in insulin sensitivity.* Hypertension, 2001. **38**(6): p. 1355-60.
47. Dray, A. and M. Perkins, *Bradykinin and inflammatory pain.* Trends Neurosci, 1993. **16**(3): p. 99-104.
48. Meotti, F., et al., *Inflammatory muscle pain is dependent on the activation of kinin B(1) and B(2) receptors and intracellular kinase pathways.* Br J Pharmacol, 2012. **166**(3): p. 1127-39.
49. Haviland, D.L., et al., *Cellular expression of the C5a anaphylatoxin receptor (C5aR): demonstration of C5aR on nonmyeloid cells of the liver and lung.* J Immunol, 1995. **154**(4): p. 1861-9.
50. Lacy, M., et al., *Expression of the receptors for the C5a anaphylatoxin, interleukin-8 and FMLP by human astrocytes and microglia.* J Neuroimmunol, 1995. **61**(1): p. 71-8.
51. Greco, J.A., 3rd, et al., *A microarray analysis of temporal gene expression profiles in thermally injured human skin.* Burns, 2010. **36**(2): p. 192-204.
52. Yang, E., et al., *Bioinformatics analysis of the early inflammatory response in a rat thermal injury model.* BMC Bioinformatics, 2007. **8**: p. 10.
53. Bialek, P., et al., *Distinct protein degradation profiles are induced by different disuse models of skeletal muscle atrophy.* Physiol Genomics, 2011. **43**(19): p. 1075-86.
54. Gomez-Ambrosi, J., et al., *Gene expression profile of omental adipose tissue in human obesity.* Faseb J, 2004. **18**(1): p. 215-7.
55. Lee, K., et al., *Localization of parathyroid hormone/parathyroid hormone-related peptide receptor mRNA in kidney.* Am J Physiol, 1996. **270**(1 Pt 2): p. F186-91.
56. Tian, J., et al., *Parathyroid hormone-parathyroid hormone related protein receptor messenger RNA is present in*

many tissues besides the kidney. Am J Nephrol, 1993. **13**(3): p. 210-3.
57. Urena, P., et al., *Parathyroid hormone (PTH)/PTH-related peptide receptor messenger ribonucleic acids are widely distributed in rat tissues.* Endocrinology, 1993. **133**(2): p. 617-23.
58. Singh, A.T., et al., *G alpha12/G alpha13 subunits of heterotrimeric G proteins mediate parathyroid hormone activation of phospholipase D in UMR-106 osteoblastic cells.* Endocrinology, 2005. **146**(5): p. 2171-5.
59. Mahon, M.J., et al., *A docking site for G protein betagamma subunits on the parathyroid hormone 1 receptor supports signaling through multiple pathways.* Mol Endocrinol, 2006. **20**(1): p. 136-46.
60. Plati, J., et al., *Structural features of parathyroid hormone receptor coupled to Galpha(s)-protein.* Biophys J, 2007. **92**(2): p. 535-40.
61. Poyner, D.R., et al., *International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors.* Pharmacol Rev, 2002. **54**(2): p. 233-46.
62. Chen, M., et al., *[Investigation of the expression of calcitonin receptor mRNA in human osteoclasts on deciduous teeth].* Hua Xi Kou Qiang Yi Xue Za Zhi, 2004. **22**(3): p. 235-7.
63. Nicholson, G.C., et al., *Abundant calcitonin receptors in isolated rat osteoclasts. Biochemical and autoradiographic characterization.* J Clin Invest, 1986. **78**(2): p. 355-60.
64. Quinn, J.M., et al., *Calcitonin receptor antibodies in the identification of osteoclasts.* Bone, 1999. **25**(1): p. 1-8.
65. Yamaguchi, M., et al., *Calcitonin receptor and Odz4 are differently expressed in Pax7-positive cells during skeletal muscle regeneration.* J Mol Histol, 2012.
66. Fukada, S., et al., *Molecular signature of quiescent satellite cells in adult skeletal muscle.* Stem Cells, 2007. **25**(10): p. 2448-59.
67. Gnocchi, V.F., et al., *Further characterisation of the molecular signature of quiescent and activated mouse muscle satellite cells.* PLoS One, 2009. **4**(4): p. e5205.
68. Chen, Y., et al., *The calcitonin receptor stimulates Shc tyrosine phosphorylation and Erk1/2 activation. Involvement of Gi, protein kinase C, and calcium.* J Biol Chem, 1998. **273**(31): p. 19809-16.
69. Tanabe, Y., et al., *A family of metabotropic glutamate receptors.* Neuron, 1992. **8**(1): p. 169-79.
70. Tanabe, Y., et al., *Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4.* J Neurosci, 1993. **13**(4): p. 1372-8.
71. Parmentier, M.L., et al., *Cloning and functional expression of a Drosophila metabotropic glutamate receptor expressed in the embryonic CNS.* J Neurosci, 1996. **16**(21): p. 6687-94.
72. Niswender, C.M. and P.J. Conn, *Metabotropic glutamate receptors: physiology, pharmacology, and disease.* Annu Rev Pharmacol Toxicol,

2010. **50**: p. 295-322.
73. Morey, J.S., J.C. Ryan, and F.M. Van Dolah, *Microarray validation: factors influencing correlation between oligonucleotide microarrays and real-time PCR.* Biol Proced Online, 2006. **8**: p. 175-93.

APPENDIX

Table 6: Relative intensity values for GPCRs classified as "present" in all skeletal muscle samples from two rats. Site A is a mixture of extensor digitorum longus and tibialis anterior, site B is a mixture of soleus and plantaris, and site C is a mixture of red and white gastronemius muscle with roughly equal amount of each muscle. Bold text indicated 38 GPCR entities detected in skeletal muscle.

GPCRs	Rat A			Rat B		
	Site					
	A	B	C	A	B	C
Rattus norvegicus EGF, latrophilin and seven transmembrane domain containing 1 (Eltd1), mRNA [NM_022294]	**4.6**	**4.2**	**4.5**	**4.4**	**4.1**	**4.8**
PREDICTED: Rattus norvegicus G protein-coupled receptor, family C, group 5, member C (Gprc5c), mRNA [XM_213518]	**3.5**	**3.8**	**3.4**	**4.4**	**3.8**	**4**
Rattus norvegicus latrophilin 1 (Lphn1), mRNA [NM_022962]	**3.9**	**3.5**	**4.3**	**3.5**	**3.6**	**3.6**
Rattus norvegicus endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2 (Edg2), mRNA [NM_053936]	**2.9**	**2.1**	**2.2**	**2.8**	**4.1**	**4**
Rattus norvegicus G protein-coupled receptor 116 (Gpr116), mRNA [NM_139110]	**2.3**	**2.1**	**1.8**	**2.4**	**2.2**	**2.1**
Rattus norvegicus chemokine (C-X-C motif) receptor 4 (Cxcr4), mRNA [NM_022205]	**1.8**	**1.6**	**2.4**	**2.3**	**2**	**2.1**
Rattus norvegicus chemokine (C-X-C motif) receptor 4 (Cxcr4), mRNA [NM_022205]	**1.8**	**1.6**	**2.3**	**2.2**	**2.1**	**2.1**
Rattus norvegicus purinergic receptor P2Y, G-protein coupled 2 (P2ry2), mRNA [NM_017255]	**1.7**	**1.2**	**0.9**	**1.4**	**1.6**	**1.7**
PREDICTED: Rattus norvegicus G protein-coupled receptor, family C, group 5, member B (predicted) (Gprc5b_predicted), mRNA [XM_215095]	**1**	**1.7**	**1.4**	**1.1**	**1.2**	**0.7**
Rattus norvegicus leucine-rich repeat-containing G protein-coupled receptor 4 (Lgr4), mRNA [NM_173328]	**1**	**1.2**	**0.9**	**1.2**	**1**	**1.2**
Rattus norvegicus chemokine orphan receptor 1 (Cmkor1), mRNA [NM_053352]	**0.9**	**1.1**	**-0.2**	**1.3**	**1.6**	**1.8**
PREDICTED: Rattus norvegicus brain-specific angiogenesis inhibitor 2 (predicted) (Bai2_predicted), mRNA [XM_232778]	**0.1**	**1.9**	**2.6**	**0.8**	**0.3**	**0.6**
Rattus norvegicus adrenergic receptor, beta 2 (Adrb2), mRNA [NM_012492]	**1.2**	**0.8**	**1.2**	**1.1**	**1.1**	**0.9**
Rattus norvegicus angiotensin receptor-like 1 (Agtrl1), mRNA [NM_031349]	**0.6**	**1.1**	**1.2**	**1.4**	**1.1**	**0.2**

PREDICTED: Rattus norvegicus G protein-coupled receptor 125 (predicted) (Gpr125_predicted), mRNA [XM_223485]	**1**	**1.3**	**0.6**	**0.7**	**0.8**	**0.6**
PREDICTED: Rattus norvegicus G protein-coupled receptor 68 (predicted) (Gpr68_predicted), mRNA [XM_001065526]	**0.2**	**0**	**0.4**	**0.8**	**1.2**	**0.8**
Rattus norvegicus glucagon receptor (Gcgr), transcript variant 2, mRNA [NM_172091]	**1**	**1.6**	**0.8**	**0.4**	**-1.4**	**0.4**
Rattus norvegicus platelet-activating factor receptor (Ptafr), mRNA [NM_053321]	**0.3**	**0.2**	**0.3**	**0.2**	**1.1**	**0.6**
Rattus norvegicus adenosine A2a receptor (Adora2a), mRNA [NM_053294]	**0**	**-0.2**	**0.5**	**0.7**	**0.5**	**0.3**
Rattus norvegicus adrenergic receptor, alpha 1d (Adra1d), mRNA [NM_024483]	**-0.7**	**-1.1**	**0.2**	**0.9**	**0.6**	**0.8**
Rattus norvegicus pyrimidinergic receptor P2Y, G-protein coupled, 6 (P2ry6), mRNA [NM_057124]	**-0.3**	**-0.7**	**0.1**	**0**	**1**	**0**
Rattus norvegicus gamma-aminobutyric acid (GABA) B receptor 1 (Gabbr1), mRNA [NM_031028]	**-0.1**	**0.3**	**0.4**	**-0.3**	**-0.2**	**-0.6**
Rattus norvegicus G protein-coupled receptor 4 (Gpr4), mRNA [NM_001025680]	**-0.3**	**-0.7**	**-0.6**	**0.4**	**0**	**0**
Rattus norvegicus G protein-coupled receptor 56 (Gpr56), mRNA [NM_152242]	**-0.4**	**0.1**	**-0.6**	**0.6**	**0.1**	**-1.4**
Rattus norvegicus endothelial differentiation, sphingolipid G-protein-coupled receptor, 5 (Edg5), mRNA [NM_017192]	**-0.4**	**-0.7**	**-0.4**	**-0.9**	**0.2**	**-0.1**
Rattus norvegicus latrophilin 2 (Lphn2), mRNA [NM_134408]	**-0.2**	**-0.3**	**-0.3**	**-0.5**	**-0.4**	**-0.5**
Rattus norvegicus parathyroid hormone receptor 1 (Pthr1), mRNA [NM_020073]	**-0.1**	**-0.6**	**-0.7**	**-0.6**	**0.1**	**-0.6**
Rat metabotropic glutamate receptor 2 mRNA, primary transcript. [M92075]	**-1**	**-0.6**	**0.4**	**-0.6**	**-1.2**	**-0.1**
Rattus norvegicus similar to purinergic receptor P2Y, G-protein coupled, 5 (MGC112684), mRNA [NM_001045843]	**-0.3**	**-0.2**	**-1.1**	**-0.9**	**-0.8**	**-0.1**
Rat metabotropic glutamate receptor 3 mRNA, primary transcript. [M92076]	**-0.1**	**-0.4**	**-0.5**	**-0.5**	**-1.2**	**-0.9**
Rattus norvegicus endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2 (Edg2), mRNA [NM_053936]	**-1.1**	**-1.1**	**-1**	**-1.1**	**0.4**	**0**
Cadherin EGF LAG seven-pass G-type receptor 2 (Multiple epidermal growth factor-like domains 3) (Fragment). [Source:Uniprot/SWISSPROT;Acc:Q9QYP2] [ENSRNOT00000027263]	**-1.4**	**-0.7**	**0.2**	**-0.3**	**-1.1**	**-0.8**
Adenosine A1 receptor. [Source:Uniprot/SWISSPROT;Acc:P25099] [ENSRNOT00000004602]	**-0.6**	**-1.3**	**-0.7**	**-0.5**	**-0.4**	**-0.6**
Rattus norvegicus adrenergic receptor, alpha 2a (Adra2a), mRNA [NM_012739]	**0.2**	**0.1**	**-1.2**	**-0.5**	**-1.2**	**-1.5**
Rattus norvegicus endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2 (Edg2), mRNA [NM_053936]	**-0.5**	**-0.4**	**-3**	**-0.9**	**0.5**	**0.1**

Rattus norvegicus dopamine receptor D5 (Drd5), mRNA [NM_012768]	-1.4	-1	-0.5	-0.8	-1.8	-0.7
Rattus norvegicus neurotensin receptor 2 (Ntsr2), mRNA [NM_022695]	-1.1	-1	-1	-0.6	-1	-1.8
Rattus norvegicus opioid receptor, delta 1 (Oprd1), mRNA [NM_012617]	-1.1	-1.7	-0.1	-0.9	-1.8	-1
Rattus norvegicus EGF-like module containing, mucin-like, hormone receptor-like sequence 1 (Emr1), mRNA [NM_001007557]	-1.8	-1.5	-1.3	-1.3	-0.1	-1.2
Rattus norvegicus calcitonin receptor (Calcr), transcript variant 1, mRNA [NM_053816]	-0.5	-0.9	-0.9	-1.8	-1.4	-1.7
Cardiac sphingosine-1-phosphate specific receptor (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q9QZG4] [ENSRNOT00000019473]	-0.6	-0.8	-1.4	-0.8	-1.5	-2.1
Rattus norvegicus arginine vasopressin receptor 1A (Avpr1a), mRNA [NM_053019]	-2.2	-2	-1.3	-1.2	-1	-0.2
Rattus norvegicus MAS-related GPR, member F (Mrgprf), mRNA [NM_153722]	-1.2	-1.4	-1.7	-1.4	-0.6	-1.8
PREDICTED: Rattus norvegicus G protein-coupled receptor 114 (predicted) (Gpr114_predicted), mRNA [XM_240979]	-0.6	-0.6	-0.8	-3.1	-2.2	-1.1
PREDICTED: Rattus norvegicus chemokine (C-C motif) receptor-like 2 (predicted) (Ccrl2_predicted), mRNA [XM_236658]	-1.6	-2.2	-1.2	-1.4	-1.3	-1
Rattus norvegicus purinergic receptor P2Y, G-protein coupled 1 (P2ry1), mRNA [NM_012800]	-1.2	-1.2	-1.9	-1.8	-1.7	-0.9
Rattus norvegicus bradykinin receptor, beta 2 (Bdkrb2), mRNA [NM_173100]	0.3	-0.9	-2.1	-2.1	-2.2	-2
Rattus norvegicus complement component 5, receptor 1 (C5r1), mRNA [NM_053619]	-2	-2.6	-2.7	-1.7	-0.1	-0.5
Rattus norvegicus neuropeptide Y receptor Y1 (Npy1r), mRNA [NM_001013032]	-1.2	-1.1	-2.8	-1.8	-1	-1.7
Rattus norvegicus purinergic receptor P2Y, G-protein coupled 12 (P2ry12), mRNA [NM_022800]	-1.6	-2.1	-2	-2.1	-0.4	-1.2
Rattus norvegicus adrenergic receptor, alpha 2b (Adra2b), mRNA [NM_138505]	-1.9	-1.2	-1.5	-1.9	-2.6	-1.6
Rattus norvegicus corticotropin releasing hormone receptor 2 (Crhr2), mRNA [NM_022714]	-1.2	-1.2	-2.2	-1.2	-1.4	-4.1
Rattus norvegicus cholinergic receptor, muscarinic 3 (Chrm3), mRNA [NM_012527]	-2.6	-2.6	-1	-1.9	-1.7	-1.3
Rattus norvegicus adrenomedullin receptor (Admr), mRNA [NM_053302]	-1.4	-1.3	-3	-1.8	-1.9	-2
Rattus norvegicus G protein-coupled receptor 19 (Gpr19), mRNA [NM_080579]	-1.9	-2.1	-1.7	-1.8	-1.9	-2.1
Rattus norvegicus G protein-coupled receptor 153 (Gpr153), mRNA [NM_001034855]	-1.6	-2.3	-2.1	-2	-1.6	-2.2
Rattus norvegicus thromboxane A2 receptor (Tbxa2r), mRNA [NM_017054]	-2	-2	-1.8	-2.1	-2.3	-2.4
Rattus norvegicus G protein-coupled receptor 153 (Gpr153), mRNA [NM_001034855]	-2.3	-2.7	-2.5	-2.4	-1.2	-1.7

Rattus norvegicus mRNA for GABAB receptor 1d, complete cds. [AB016161]	**-2**	**-2**	**-1.3**	**-3.1**	**-3.2**	**-1.7**
Rattus norvegicus purinergic receptor P2Y, G-protein coupled, 14 (P2ry14), mRNA [NM_133577]	**-2.9**	**-3.3**	**-2.4**	**-2.1**	**-1**	**-2.4**
Rattus norvegicus 5-hydroxytryptamine (serotonin) receptor 2B (Htr2b), mRNA [NM_017250]	**-2.1**	**-2.6**	**-2.8**	**-2.5**	**-1.9**	**-2**
PREDICTED: Rattus norvegicus endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor 6 (predicted) (Edg6_predicted), mRNA [XM_234930]	**-2.3**	**-1.5**	**-2.9**	**-2.3**	**-2.7**	**-2.3**
Rattus norvegicus MAS-related GPR, member D (Mrgprd), mRNA [NM_001001506]	**-2**	**-2**	**-2**	**-2.5**	**-3**	**-2.7**
Rattus norvegicus G protein-coupled receptor 157 (Gpr157), mRNA [NM_001012107]	**-2.2**	**-2.4**	**-1.6**	**-2.4**	**-3**	**-3**
Rattus norvegicus vasoactive intestinal peptide receptor 2 (Vipr2), mRNA [NM_017238]	**-2.3**	**-2.5**	**-3**	**-2.5**	**-1.5**	**-2.7**
Rattus norvegicus adenosine A2B receptor (Adora2b), mRNA [NM_017161]	**-2.2**	**-2.9**	**-3.5**	**-2.4**	**-1.6**	**-2.1**
Rattus norvegicus endothelin receptor type B (Ednrb), mRNA [NM_017333]	**-2.8**	**-3.4**	**-3.6**	**-2.3**	**-2.1**	**-1.7**
Rattus norvegicus tachykinin receptor 2 (Tacr2), mRNA [NM_080768]	**-2.6**	**-2.4**	**-1.7**	**-2.8**	**-3.9**	**-2.8**
Rattus norvegicus G protein-coupled receptor 20 (Gpr20), mRNA [NM_022216]	**-1.8**	**-2.2**	**-3.5**	**-2.7**	**-2.5**	**-3.6**
Rattus norvegicus galanin receptor 2 (Galr2), mRNA [NM_019172]	**-3.1**	**-3.2**	**-3**	**-2.6**	**-2.3**	**-2.8**
Rattus norvegicus chemokine (C-C motif) receptor 1 (Ccr1), mRNA [NM_020542]	**-3.5**	**-2.7**	**-3.2**	**-3.5**	**-1.8**	**-2.2**
Rattus norvegicus neuropeptides B/W receptor 1 (Nbpwr1), mRNA [NM_001014784]	**-2.2**	**-2.4**	**-2.2**	**-3**	**-3.4**	**-3.7**
PREDICTED: Rattus norvegicus G protein-coupled receptor 45 (predicted) (Gpr45_predicted), mRNA [XM_237112]	**-4.2**	**-2.1**	**-1.8**	**-2.8**	**-3.2**	**-3**
Rattus norvegicus somatostatin receptor 5 (Sstr5), mRNA [NM_012882]	**-2.3**	**-2.3**	**-2.4**	**-3.2**	**-3.7**	**-3.3**
Rattus norvegicus gamma-aminobutyric acid (GABA) B receptor 2 (Gabbr2), mRNA [NM_031802]	**-2.7**	**-3**	**-3.2**	**-3.4**	**-2.7**	**-2.5**
PREDICTED: Rattus norvegicus G protein-coupled receptor 126 (predicted) (Gpr126_predicted), mRNA [XM_218313]	**-3.2**	**-3.5**	**-3.1**	**-3.3**	**-2.8**	**-2.5**
Rattus norvegicus G protein-coupled receptor 3 (Gpr3), mRNA [NM_153727]	**-2.8**	**-3.5**	**-3**	**-2.7**	**-3.8**	**-2.8**
Rattus norvegicus G protein-coupled receptor 149 (Gpr149), mRNA [NM_138891]	**-3**	**-4.2**	**-3.4**	**-2.8**	**-3.6**	**-1.9**
Rattus norvegicus relaxin 3 receptor 1 (Rln3r1), mRNA [NM_001008310]	**-2.5**	**-2.1**	**-3.6**	**-3**	**-4.3**	**-3.4**
Rattus norvegicus somatostatin receptor 3 (Sstr3), mRNA [NM_133522]	**-2.6**	**-3.4**	**-3.3**	**-3.8**	**-3**	**-3.2**
Rattus norvegicus 5-hydroxytryptamine (serotonin) receptor 7 (Htr7), mRNA [NM_022938]	**-3.6**	**-2.9**	**-3.6**	**-2.9**	**-3.3**	**-3**
Rattus norvegicus cadherin EGF LAG seven-pass G-type receptor 3 (Celsr3), mRNA [NM_031320]	**-2.6**	**-2.8**	**-2.7**	**-3.7**	**-4.2**	**-3.3**

Cadherin EGF LAG seven-pass G-type receptor 2 (Multiple epidermal growth factor-like domains 3) (Fragment). [Source:Uniprot/SWISSPROT;Acc:Q9QYP2] [ENSRNOT00000027263]	**-3.2**	**-2.9**	**-2.6**	**-3.3**	**-4**	**-3.5**
Rattus norvegicus adrenergic receptor, alpha 2c (Adra2c), mRNA [NM_138506]	**-3.5**	**-3.9**	**-3.2**	**-2.7**	**-2.6**	**-3.6**
Rattus norvegicus histamine H4 receptor (Hrh4), mRNA [NM_131909]	**-3.1**	**-2.8**	**-2.8**	**-3.7**	**-4.1**	**-3.6**
Rattus norvegicus arginine vasopressin receptor 2 (Avpr2), mRNA [NM_019136]	**-3.6**	**-3**	**-2.5**	**-3.5**	**-4**	**-3.8**
Rattus norvegicus adrenergic receptor, beta 1 (Adrb1), mRNA [NM_012701]	**-3.7**	**-3.2**	**-2.5**	**-3.6**	**-3.8**	**-3.7**
Rattus norvegicus B2 bradykinin receptor mRNA, complete cds. [M59967]	**-3.4**	**-3.1**	**-3.1**	**-3.5**	**-3.4**	**-4.1**
Rattus norvegicus adenosine A2a receptor (Adora2a), mRNA [NM_053294]	**-3.8**	**-3.7**	**-3.8**	**-3.3**	**-3.5**	**-3.3**
PREDICTED: Rattus norvegicus G protein-coupled receptor 162 (predicted) (Gpr162_predicted), mRNA [XM_342757]	**-3.9**	**-3.3**	**-3.5**	**-3.9**	**-3.3**	**-4.3**
Rattus norvegicus prostaglandin E receptor 1 (Ptger1), mRNA [NM_013100]	**-4.2**	**-4.2**	**-2.7**	**-3.7**	**-3.9**	**-4.2**
Cadherin EGF LAG seven-pass G-type receptor 2 (Multiple epidermal growth factor-like domains 3) (Fragment). [Source:Uniprot/SWISSPROT;Acc:Q9QYP2] [ENSRNOT00000027263]	**-3.8**	**-4.3**	**-3**	**-3.5**	**-4.8**	**-4**
Rattus norvegicus dopamine receptor D3 (Drd3), mRNA [NM_017140]	**-3.9**	**-4.1**	**-4.1**	**-3.8**	**-4.1**	**-3.4**
Rattus norvegicus adenosine A1 receptor (Adora1), mRNA [NM_017155]	**-4.2**	**-4.2**	**-4**	**-4**	**-3.8**	**-4.3**

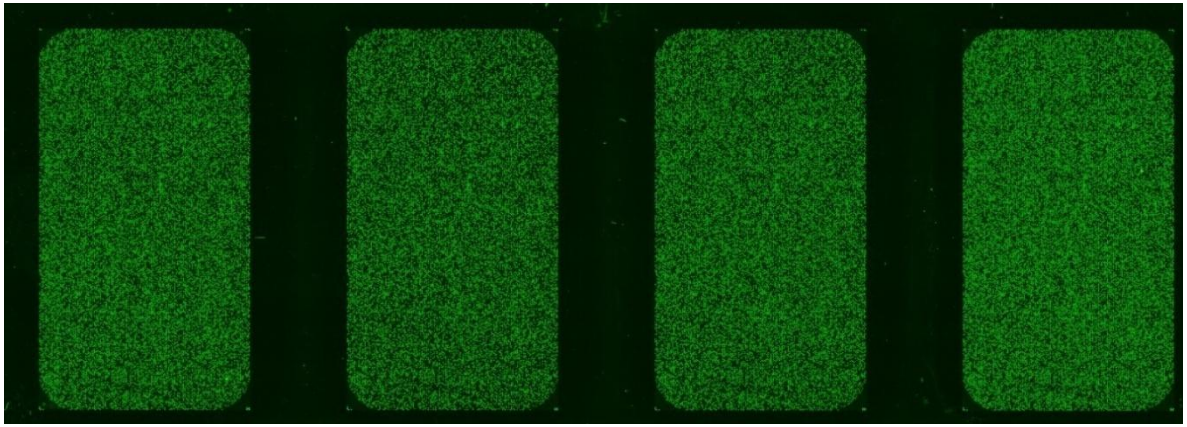
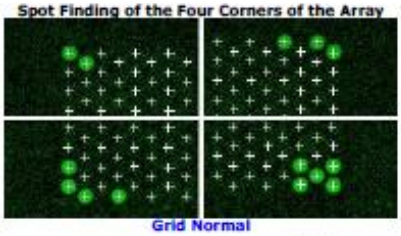


Figure 4: Representative TIFF image from the microarray

QC Report - Agilent Technologies : 1 Color Gene Expression — QCMetrics InRange (9 of 9)

Date	Wednesday, August 05, 2009 - 10:46	Grid	014879_D_20070207
Image	US90803647_251487918216_S01_H [1_2]	BG Method	No Background
Protocol	GE1_105_Dec08 (Read Only)	Background Detrend	On(FeatNCRange, LoPass)
User Name	Administrator	Multiplicative Detrend	True
FE Version	10.5.1.1	Additive Error	1(Green)
Sample(red/green)		Saturation Value	557281 (g)

Spot Finding of the Four Corners of the Array



Grid Normal

	Feature	Local Background
	Green	Green
Non Uniform	143	1
Population	158	643

Spatial Distribution of All Outliers on the Array

532 rows x 85 columns

FeatureNonUnif (Green) = 143(0.32%)

GeneNonUnif (Green) = 121 (0.295 %)

BG NonUniform BG Population

Green FeaturePopulation Green Feature NonUniform

Net Signal Statistics

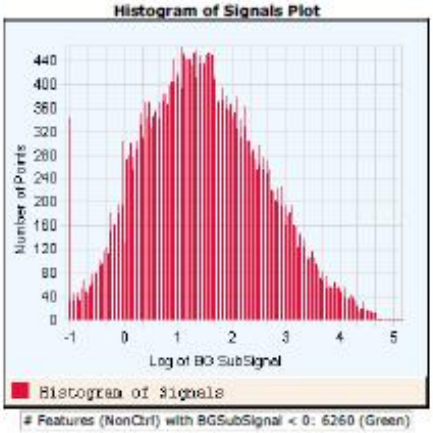
Agilent SpikeIns:

	Green
# Saturated Features	0
99% of Sig. Distrib.	20541
50% of Sig. Distrib.	106
1% of Sig. Distrib.	16

Non-Control probes:

	Green
# Saturated Features	0
99% of Sig. Distrib.	12523
50% of Sig. Distrib.	33
1% of Sig. Distrib.	17

Histogram of Signals Plot



Features (NonCtrl) with BGSubSignal < 0: 6260 (Green)

Negative Control Stats

	Green
Average Net Signals	18.69
StdDev Net Signals	1.34
Average BG Sub Signal	-1.32
StdDev BG Sub Signal	1.02

Local Bkg (inliers)

	Green
Number	44375
Avg	21.94
SD	1.26

Foreground Surface Fit

	Green
RMS_Fit	0.95
RMS_Resid	1.14
Avg_Fit	27.30

Multiplicative Surface Fit

	Green
RMS_Fit	0.14

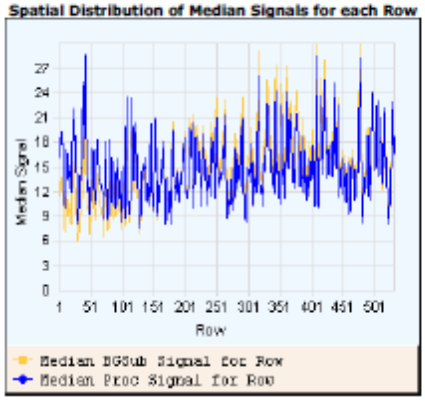
Reproducibility: %CV for Replicated Probes

	Median %CV Signal (inliers)	
	Non-Control probes	Agilent SpikeIns
	Green	Green
BGSubSignal	15.71	15.50
ProcessedSignal	6.96	6.89

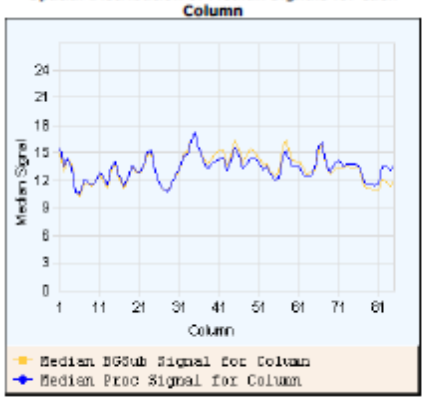
Agilent SpikeIns Signal Statistics

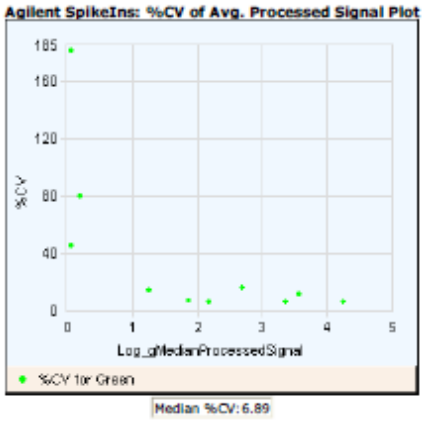
Probe Name	Log (Relative Conc.)	Median (Log Proc. Sig.)	% CV	StdDev
(+)E1A_r60_3	0.30	0.06	45.33	0.12
(+)E1A_r60_a104	1.30	0.06	181.55	0.23
(+)E1A_r60_a107	2.30	0.20	80.03	0.21
(+)E1A_r60_a135	3.30	1.27	13.86	0.07
(+)E1A_r60_a20	3.83	1.86	6.89	0.03
(+)E1A_r60_a22	4.30	2.18	6.37	0.03
(+)E1A_r60_a97	4.82	2.69	15.95	0.10
(+)E1A_r60_n11	5.30	3.37	6.66	0.03
(+)E1A_r60_n9	5.82	3.56	11.46	0.06
(+)E1A_r60_1	6.30	4.25	6.54	0.03

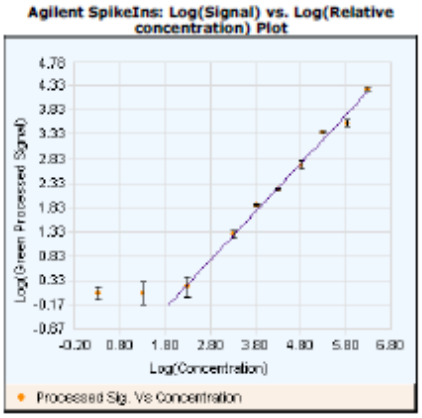
Spatial Distribution of Median Signals for each Row



Spatial Distribution of Median Signals for each Column







Evaluation Metrics for GE1_QCMT_Dec08

Metric Name	Value	UpLim	LowLim	IsMandatory
AnyColorPrcntFeatNonUnif...	0.32	1.00	NA	False
DetectionLimit	0.59	2.00	0.01	False
absGE1E1aSlope	0.99	1.20	0.90	False
gE1aMedCVProcSignal	6.89	8.00	NA	False
gNegCtrlAveBGSubSig	-1.32	5.00	-10.00	False
gNegCtrlAveNetSig	18.69	40.00	NA	False
gNegCtrlSDevBGSubSig	1.02	10.00	NA	False
gNonCntrlMedCVProcSignal	6.96	8.00	NA	False
gSpatialDetrendRMSFilter...	1.14	15.00	NA	False

♦ In Normal Range ♦ Evaluate

Agilent Spike-In Concentration-Response Statistics

Linear Range Statistics:

Low Signal	-0.31
High Signal	5.87
Low Relative Concentration	1.73
High Relative Concentration	7.99
Slope	0.99
R^2 Value	0.99

Signal Detection Limit Statistics

Saturation Point	5.75
Low Threshold	-0.35
Low Threshold Error	0.33
Spike-In Detection Limit	0.59

Figure 5: The Quality Control From the Feature Extraction Software After Scanning