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Sweet Regulation of Melanocortin Receptor (MC1R, Mc3r, and MC4R) Transcription Activity

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Abstract

Objective: This study is to understanding the transcription profile of nociception-related melanocortin receptor MC1R, obesity related neural melanocortin receptor Mc3r and MC4R.

Methods: For MC1R, Mc3r and MC4R, relative luciferase activity RLA and the distribution of phosphorylation [P], glycosylation sites [G] on transcription factor for promoter model were plotted on the same chart.

Results: Within the 3.2 kb upstream of the methionine ATG, for all three receptors, trend lines of RLA with that of [G] or ([P]-[G]), both tend to have negative reciprocal relationship. It brings up the question: for the neural receptors MC1R, Mc3r and MC4R, does the nutrition and obesity related glycosylation regulated the transcriptional activity in a negative reciprocal way?

Conclusion: The negative reciprocal relation between trend line of [G] or ([P]-[G]) and that of RLA, give a specific digit evidence for the first time to the theory, which the structure related glycosylation and the signal sensing phosphorylation, exhibit either independently or Interactively on regulation of transcription activity.

Keywords: Luciferase activity; Melanocortin receptor; Transcription factor

Abbrevations: RLA: Relative luciferase activity; DLA: Dual luciferase assay; MC1R: Human melanocortin 1 receptor; Mc3r: Mouse melanocortin 3 receptor; MC4R: Human melanocortin 4 receptor; Promoter model: A promoter model represents a framework of two or more conserved elements (e.g., transcription factor binding sites) with a defined distance (and strand orientation); TF: Transcription factor; P1: construct Plasmid 1; P7: construct Plasmid 7; P9: construct Plasmid 9; MC1R-P1: construct Plasmid 1 for human melancortin 1 receptor; MC1R-P9: construct Plasmid 9 for human melancortin 1 receptor; Mc3r-P1: construct Plasmid 1 for mouse melanocortin 3 receptor; Mc3r-P7: construct Plasmid 7 for mouse melanocortin 3 receptor; MC4R-P1: construct Plasmid 1 for human melanocortin 4 receptor; MC4R-P5: construct Plasmid 5 for human melanocortin 4 receptor; [P]: number of phosphorylation sites on TF for promoter models; [G]: number of glycosylation sites on TF for promoter models; [P]-[G]: number of difference between phosphorylation and glycosylation sites on TF for promoter models; [P]+[G]: number for sum of phosphoylation and glycosylation sites on TF for promoter models

Introduction

MC1R may be involved in the inflammatory aspects of pain signaling [1]. In the central nervous system (CNS), MC1R mRNA expression and protein levels are detected in the hypothalamus, amygdala, locus coeruleus and dorsal raphe nucleus, which are areas of the brain that are implicated in regulating emotion and stress. Therefore, MC1R not only functions in skin pigmentation, nociception and anti-inflammatory response, but also in melanocortin pathway involving motivation, reward and emotional states, and mood disorders such as anxiety and depression.

And Mc3r [2] is widely distributed in the Central Nerve System; it is involved in controlling ingestive behaviors and autonomic function. The central and peripheral Mc3rS are involved in the maintaining normal body weight.

Also, MC4R are distributed [1] in cortex, hypothalamus, brainstem, spinal cord, astrocytes, heart and lungs. Mutations of the MC4R gene that lead to reduced receptor functioning have been consistently associated with obesity. Obesity has been linked to MC4R signaling, suggesting that stimulation of this receptor promotes compulsive grooming and its blockade reverses the induction of this excessive behavior in rodents.

In addition, Mc3r and MC4R are in central melanocortin system [3,4], which involved in regulation of thermogenesis, body weight regulation through its role in appetite and energy expenditure, and dysregulation of the energy homeostasis, contributing to the pathogenesis of diseases such as cardiovascular, cerebrovascular diseases and pathophysiological process of cachexia, as well as to the type 2 diabetes and obesity.

On the other hand, primary gene induction or repression in eukaryotes does not require protein synthesis [5], suggesting the involvement of posttranslational modifications [6,7]. Since many different types of stimuli that affect gene expression also lead to the activation of protein kinases, analysis of transcription factor phosphorylation is essential for complete understanding of the signal pathways. The activity of transcription factors may be modulated by their signal-sensing domain including phosphorylation [8]. In addition, as nutrient sensitive sugar modification, glycosylation, interfere with the epigenetic control of gene expression.

MC1R, Mc3r, and MC4R receptors are involved in anxiety, depression, cardiovascular and cachexia diseases as well as obesity, mutations and polymorphisms of melanocortin-1 receptor type 1 gene (MCR1) play a role in skin cancer [9]. Moreover, phosphorylation or glycosylation are perhaps required for the activation of transcription factors. However, the

regulatory mechanism and post translation modification in the epigenetic transcription regulation of these Melanocortin Receptors has yet to be fully investigated.

Methods

For Mc3r, determination of transcription starting site of Mc3r using 5'RACE was performed on total Mus musculus hypothalamus RNA using the 5'RLM-RACE kit [10] (nature protocol exchange), Series deletion fragments containing sequences extending from 3084 bp to 283 bp were subcloned into the upstream of the firefly luciferase gene in pGL3 vector, as confirmed by restriction enzyme digestion and sequencing.

And study of transcriptional activation and regulation of Mc3r was performed by using gene reporter assay [5], human HEK293 was transiently transfected using FuGENE's 6 Tranfection Reagent, 48 hours after transfection and over 65% cell confluency, Renila-Firefly Dual luciferase activity was measured. Assay was tested by triplicates on independent experiments. The activities of all constructs in pGL3 basic in HEK293 were measured. Afterwards, Promoter model [11] [A promoter model represents a framework of two or more conserved elements (e.g., transcription factor binding sites) with a defined distance (and strand orientation). Usually, promoter models are much more specific than single elements like transcription factor binding sites. Therefore, a promoter model can give higher evidence that the matching sites are functional] was inspected by Genomatix (http://www.genomatix.com/). The glycosylation (Table 1) and phosphorylation sites (Table 2) on the promoter model were searched by Protein Knowledge bases of Uniprot (http://www.uniprot.org/).

For MC1R, Relative Luciferase Activity (in SK-Mel-2 human melanoma cells) is from figure 1 in Osamu Moro's paper [12], and the sequence information for the corresponding constructs is from figure 2 in the same paper. Glycosylation (Table 3) and phosphorylation sites (Table 4) of promoter models were searched in the same way as for that of Mc3r.

For MC4R, Relative Luciferase Activity of HEK 293 is from figure 1 of Christian Vaisse's paper [13], and DNA sequence is from figure 3 in the same paper. Glycosylation (Table 5) and phosphorylation sites (Table 6) of promoter models were searched in the same way as for that of Mc3r and MC4R.

Results

Human Melanocortin 1 Receptor

For MC1R, as it shown in figure 2a, MC1R-P1 has RLA of 100.0, with length of 2918 bp, ranging from -3200 bp to -282 bp, there are 25 phosphorylation sites and 11 glysosylation sites on TF for promoter model between MC1R-P1 and MC1R-P2. MC1R-P2 has RLA of 130.0, with length of 2131 bp, ranging from -2413 bp to -282 bp; there are 33 phosphorylation sites and 8 glysosylation sites on TF for promoter model between MC1R-P2 and MC1R-P3. MC1R-P3 has RLA of 94.0, with length of 1100 bp, ranging from -1382 bp to -282 bp; there are 30 phosphorylation sites and 7 glysosylation sites on TF for promoter model between MC1R-P3 and MC1R-P4. MC1R-P4 has RLA of 131.0, with length of 648 bp, ranging from -930 bp to -282bp; there are 19 phosphorylation sites and 2 glysosylation sites on TF for promoter model between MC1R-P4 and MC1R-P5. MC1R-P5 has RLA of 116.0, with length of 284 bp, ranging from -566 bp to -282 bp; there are 2 phosphorylation sites and 1 glysosylation site on TF for promoter model between MC1R-P5 and MC1R-P6. MC1R-P6 has RLA of 88.4, with length of 235 bp, ranging from -517 bp to -282 bp; there are 5 phosphorylation sites and 3 glysosylation sites on TF for promoter model between MC1R-P6 and MC1R-P7. MC1R-P7 has RLA of 22.0, with length of 177 bp, ranging from -459 bp to -282 bp; there is no phosphorylation site and no glysosylation site on TF for promoter model between MC1R-P7 and MC1R-P8. MC1R-P8 has RLA of 3.0, with length of 165 bp, ranging from -447 bp to -282 bp; there is no phosphorylation site and no glysosylation site on TF for promoter model between MC1R-P8 and MC1R-P9. MC1R-P9 has RLA of 3.6, with length of 132 bp, ranging from -414 bp to -282bp; there are 22 phosphorylation sites and 6 glysosylation sites on TF for promoter model on MC1R-P9.

For MC1R, If plot the RLA data and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model on the same chart, got figure 3, the polynomial behavior of MC1R is: trend line of RLA is ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$); trend line of [G] is ($y=1E-11x^4+8E-08x^3+0.000x^2+0.120x+29.89$); trend line of [P]-[G] is ($y=3E-11x^4+2E-07x^3+0.000x^2+0.272x+63.35$); trend line of [P] is ($y=5E-11x^4+3E-07x^3+0.000x^2+0.393x+93.25$); trend line of [P]+[G] is ($y=6E-11x^4+4E-07x^3+0.000x^2+0.513x+123.1$). Trend line RLA and trend line of ([G], [P], [P] ± [G]) give an inverted image to each other. In detail, the sign of leading coefficient is opposite to each other, and sign of coefficient of corresponding degree are also opposite to each other. Furthermore, trend line of RLA ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$) and trend line of [G] ($y=1E-11x^4+8E-08x^3+0.000x^2+0.120x+29.89$) tend to have a relation of negative reciprocal (Figure 3b). At the same time, trend line of RLA ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$) and trend line of [P]-[G] ($y=3E-11x^4+2E-07x^3+0.000x^2+0.272x+63.35$) tend to have a relation of negative reciprocal as well (Figure 3a).

Mouse Melanocortin 3 receptor

For Mc3r, as it indicated in figure 2b, Mc3r-P1 has RLA of 0.964, with length of 2998 bp, ranging from -3114 bp to -116 bp, there are 29 phosphorylation sites and 6 glysosylation sites on TF for promoter model between Mc3r-P1 and Mc3r-P2. Mc3r-P2 has RLA of 1.342, with length of 2054 bp, ranging from -2170 bp to -116 bp; there are 15 phosphorylation sites and 8 glysosylation sites on TF for promoter model between Mc3r-P2 and Mc3r-P3. Mc3r-P3 has RLA of 2.596, with length of 1062 bp, ranging from -1178 bp to -116 bp; there are 4 phosphorylation sites and no glysosylation site on TF for promoter model between Mc3r-P3 and Mc3r-P4. Mc3r-P4 has RLA of 2.844, with length of 862 bp, ranging from -978 bp to -116 bp; there are 11 phosphorylation sites and 1 glysosylation site on TF for promoter model between Mc3r-P4 and Mc3r-P5. Mc3r-P5 has RLA of 3.010, with length of 649 bp, ranging from -765 bp to -116 bp; there are 13 phosphorylation sites and 4 glysosylation sites on TF for promoter model between Mc3r-P5 and Mc3r-P6. Mc3r-P6 has RLA of 3.098, with length of 488 bp, ranging from -604 bp to -116 bp; there are 8 phosphorylation sites and 2 glysosylation sites on TF for promoter model between Mc3r-P6 and Mc3r-P7. Mc3r-P7 has RLA of 1.845, with length of 257 bp, ranging from -373 bp to -116 bp; there are 10 phosphorylation sites and no glysosylation site on TF for promoter model on Mc3r-P7.

For Mc3r, If plot the RLA data and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model on the same chart, got figure 1, the polynomial behavior of Mc3r is: trend line of RLA is ($y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$); trend line of [P]+[G] is ($y=1E-13x^5+7E-10x^4+1E-06x^3+0.001x^2+0.404x+55.37$); trend line of [G] is ($y=2E-14x^5+1E-10x^4+2E-07x^3+0.000x^2+0.033x+1.873$); trend line of [P] is ($y=9E-14x^5+5E-10x^4+1E-06x^3+0.001x^2+0.371x+53.50$); trend line of [P]-[G] is ($y=7E-14x^5+4E-10x^4+9E-07x^3+0.000x^2+0.337x+51.63$). Trend line RLA and trend line of ([G], [P], [P] ± [G]) give an inverted image to each other. In detail, the sign of leading coefficient is opposite to each other, and sign of coefficient of corresponding degree are also opposite to each other. Furthermore, trend line of RLA ($y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$) and trend line of [G] ($y=2E-14x^5+1E-10x^4+2E-07x^3+0.000x^2+0.033x+1.873$) tend to have a relation of negative reciprocal (Figure 1b). At the same time, trend line of RLA ($y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$) and trend line

Promoter Model	Sequence	bp	G	TF
P1		-3114		
E2FF_SP1F_01	GCTCAGGGTGGGTTTGG	-3054	G	V$SP1F
CEBP_CREB_01	CCCAGCTGAGGAAATTATTTT	-2812	G	V$CREB
NFKB_SP1F_04	GCCCTGGGCAGAGGCCC	-2438	G	V$SP1F
EGRF_SP1F_01	GATGTGGGCGGGACCAA	-2383	G	V$SP1F
KLFS_SP1F_01	GATGTGGGCGGGACCAA	-2383	G	V$SP1F
NR2F_SF1F_01	TTGCCAAGGACCCGT	-2278	G	V$SP1F
P2		-2170		
CEBP_EBOX_01	TAGACACGTGCTC	-1982	G	V$EBOX
CREB_EBOX_02	GGTAGAGCACGTGTCTAATAA	-1982	G	V$CREB
CREB_EBOX_02	TAGACACGTGCTC	-1982	G	V$EBOX
EBOX_SREB_01	TAGACACGTGCTC	-1982	G	V$EBOX
EBOX_SREB_01	TAGACACGTGCTC	-1982	G	V$EBOX
SMAD_EBOX_02	TAGACACGTGCTC	-1982	G	V$EBOX
EBOX_SREB_01	AGAGCACGTGTCT	-1981	G	V$EBOX
EBOX_SREB_01	AGAGCACGTGTCT	-1981	G	V$EBOX
P3		-1178		
P4		-978		
SORY_ETSF_01	CATTGACAATATTGCAGCTGTCACC	-773	G	V$SORY
P5		-765		
ETSF_SP1F_05	GGCGGTGGCGGGGGTCG	-704	G	V$SP1F
SP1F_YY1F_01	GGCGGTGGCGGGGGTCG	-704	G	V$SP1F
E2FF_SP1F_01	GGGAAGGGCTGGGCCAG	-686	G	V$SP1F
SP1F_ETSF_01	GGGAAGGGCTGGGCCAG	-686	G	V$SP1F
P6		-604		
SP1F_ETSF_04	GCAGGGGGCGCCAATCT	-517	G	V$SP1F
SP1F_MYOD_01	GCAGGGGGCGCCAATCT	-517	G	V$SP1F
P7		-373		

Table 1: Glycosylation site on promoter model of mMC3r

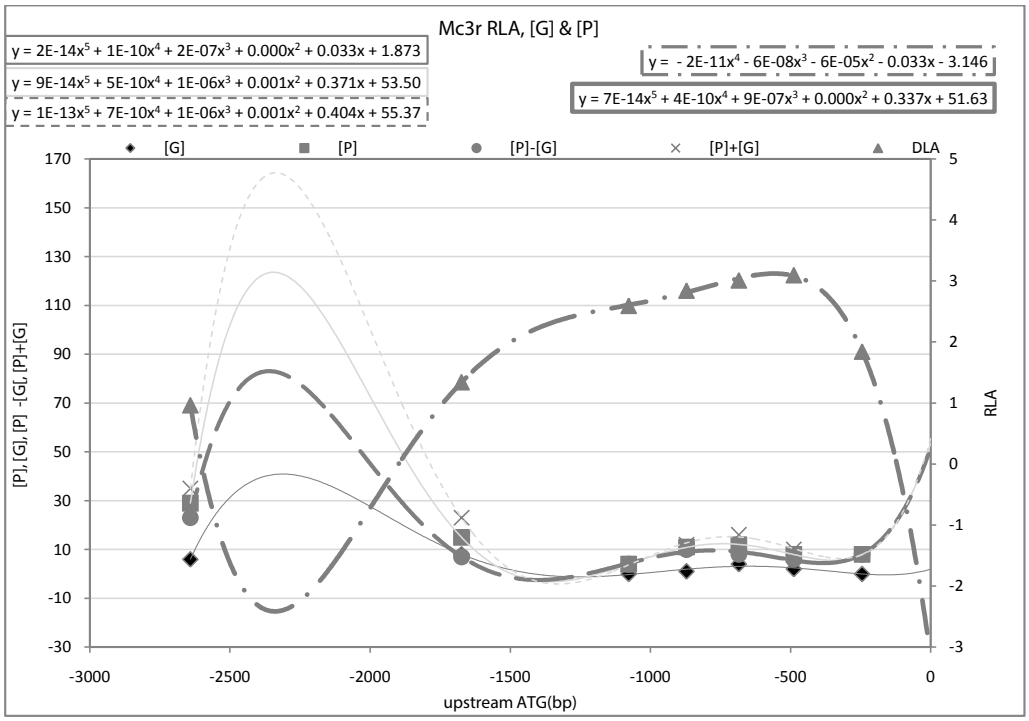
of [P]-[G] ($y=7E-14x^5+4E-10x^4+9E-07x^3+0.000x^2+0.337x+51.63$) tend to have a relation of negative reciprocal as well (Figure 1a).

Human Melanocortin 4 Receptor

For MC4R, as shown in figure 2c, MC4R-P1 has RLA of 14.7, with length of 2510 bp, ranging from -2926 bp to -416 bp; there are 12 phosphorylation sites and no glysosylation site on TF for promoter model between MC4R-P1 and MC4R-P2. MC4R-P2 has RLA of 19.8, with length of 1510 bp, ranging from -1926 bp to -416 bp; there are 14 phosphorylation sites and 1 glysosylation site on TF for promoter model between MC4R-P2 and MC4R-P3. MC4R-P3 has RLA of 22.3, with length of 760 bp, ranging from -1176 bp to -416 bp; there are 7 phosphorylation sites and 1 glysosylation site on TF for promoter model between MC4R-P3 and MC4R-P4. MC4R-P4 has RLA of 20.8, with length of 140 bp, ranging from -556 bp to -416 bp; there are 4 phosphorylation sites and 1 glysosylation site on TF for promoter model between MC4R-P4 and MC4R-P5. MC4R-P5 has RLA of 9.0, with length of 60 bp, ranging from -476 bp to -416 bp; there are 10 phosphorylation sites and 2 glysosylation sites on TF for promoter model on MC4R-P5.

For MC4R, If plot the RLA data and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model on the same chart, got figure 4, the polynomial behavior of MC4R is: trend line of RLA is ($y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$); trend line of [G] is ($y=3E-11x^4+1E-07x^3+0.000x^2+0.117x+28.39$); trend line of [P]-[G] is ($y=3E-14x^5+3E-10x^4+9E-07x^3+0.001x^2+0.753x+169.0$); trend line of [P] is ($y=4E-14x^5+3E-10x^4+1E-06x^3+0.001x^2+0.870x+197.4$); trend line of [P]+[G] is ($y=4E-14x^5+3E-10x^4+1E-06x^3+0.001x^2+0.988x+225.8$). Trend line RLA and trend line of ([G], [P], [P] ± [G]) give an inverted image to each other. In detail, the sign of leading coefficient is opposite to each other, and sign of coefficient of corresponding degree are also opposite to each other. Furthermore, trend line of RLA ($y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$) and trend line of [G] ($y=3E-11x^4+1E-07x^3+0.000x^2+0.117x+28.39$) tend to have a relation of negative reciprocal (Figure 4b). At the same time, trend line of RLA ($y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$) and trend line of [P]-[G] ($y=3E-14x^5+3E-10x^4+9E-07x^3+0.001x^2+0.753x+169.0$) tend to have a relation of negative reciprocal as well (Figure 4a).

a)



b)

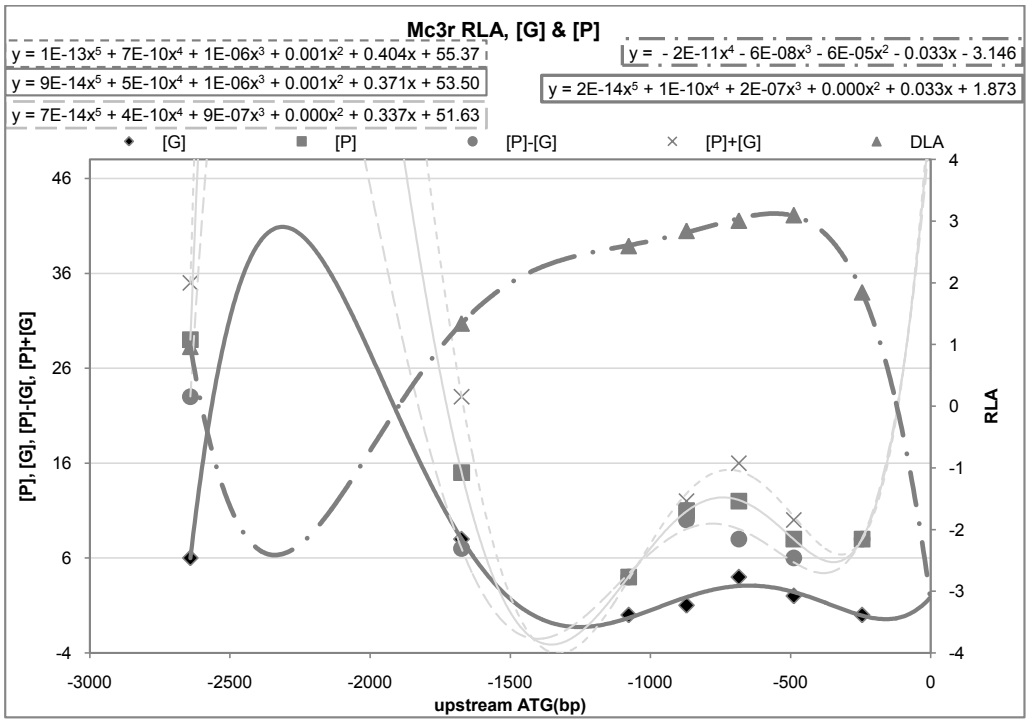


Figure 1: RLA data of Mc3r and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model.

For Mc3r, plot the RLA data and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model on the same chart, the polynomial behavior of Mc3r is: trend line of RLA is ($y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$); trend line of [P]+[G] is ($y=1E-13x^5+7E-10x^4+1E-06x^3+0.001x^2+0.404x+55.37$); trend line of [G] is ($y=2E-14x^5+1E-10x^4+2E-07x^3+0.000x^2+0.033x+1.873$); trend line of [P] is ($y=9E-14x^5+5E-10x^4+1E-06x^3+0.001x^2+0.371x+53.50$); trend line of [P]-[G] is ($y=7E-14x^5+4E-10x^4+9E-07x^3+0.000x^2+0.337x+51.63$). Trend line RLA and trend line of ([G], [P], [P] ± [G]) give an inverted image to each other. In detail, the sign of leading coefficient is opposite to each other, and sign of coefficient of corresponding degree are also opposite to each other. Furthermore, trend line of RLA and trend line of [P]-[G] tend to have a relation of negative reciprocal (a). Concurrently, trend line of RLA and trend line of [G] tend to have a relation of negative reciprocal as well (b).

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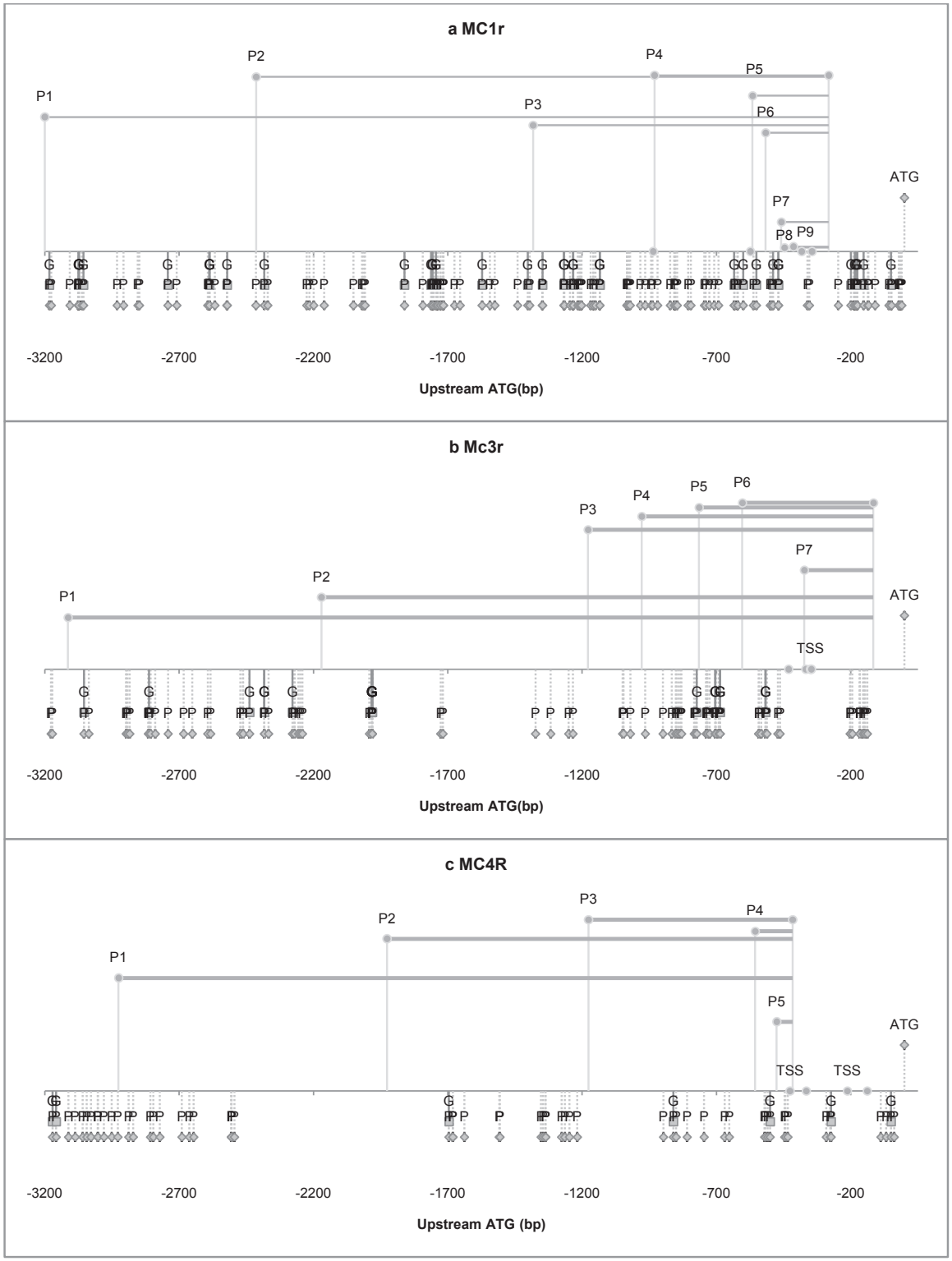

Figure 2: RLA of corresponding plasmids for (a) MC1R (b) Mc3r (c) MC4R and distribution of phosphorylation site and glycosylation site on TF for promoter model.

The RLA of the corresponding plasmids is plotted on upper Y axis (Y>0) with the corresponding sequence of sense primer and that of antisense primer, concurrently, a horizontal line is drawn to link the starting point and ending point of each constructed plasmid respectively. On the other hand, within the 3200 base pair (bp) upstream the methionine-ATG (0 bp), on the lower Y axis area (Y<0), the phosphorylation (P) sites and glycosylation (G) sites is also pinned on the corresponding sequence (bp) for transcription factors promoter models. It shows the distribution of P and G. And Transcription Starting Site (TSS) is plotted on X axis (Y=0). (a) MC1R, plasmids (P1, P2, P3, P4, P5, P6, P7, P8, P9). (b) Mc3r, plasmids (P1, P2, P3, P4, P5, P6, P7). (c) MC4R, plasmids (P1, P2, P3, P4, P5).

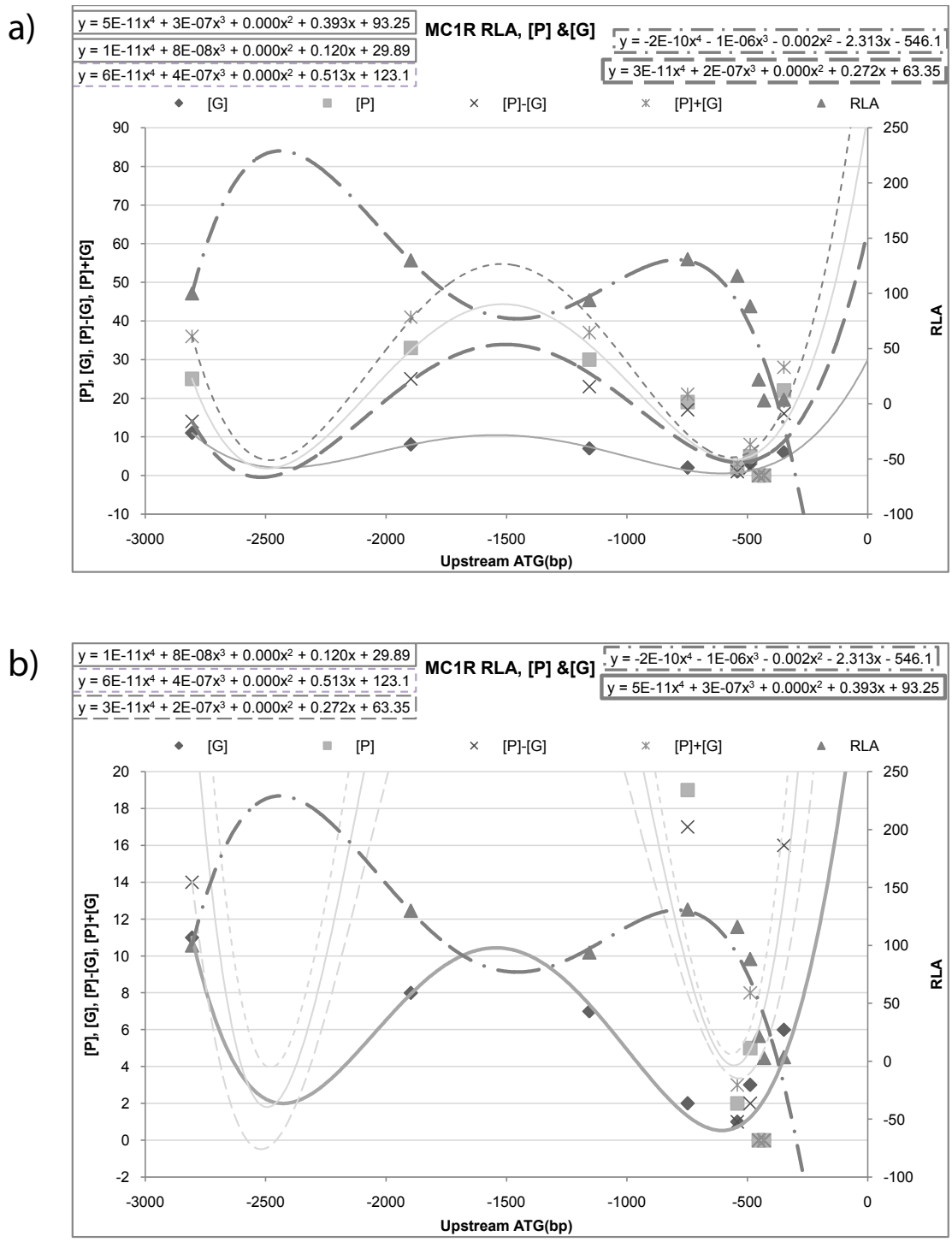

Figure 3: RLA data of MC1R and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model.

For MC1R, plot the RLA data and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model on the same chart, the polynomial behavior of is: trend line of RLA is ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$); trend line of [G] is ($y=1E-11x^4+8E-08x^3+0.000x^2+0.120x+29.89$); trend line of [P]-[G] is ($y=3E-11x^4+2E-07x^3+0.000x^2+0.272x+63.35$); trend line of [P] is ($y=5E-11x^4+3E-07x^3+0.000x^2+0.393x+93.25$); trend line of [P]+[G] is ($y=6E-11x^4+4E-07x^3+0.000x^2+0.513x+123.1$). Trend line RLA and trend line of ([G], [P], [P] ± [G]) give an inverted image to each other. In detail, the sign of leading coefficient is opposite to each other, and sign of coefficient of corresponding degree are also opposite to each other. Furthermore, trend line of RLA ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$) and trend line of [P]-[G] ($y=3E-11x^4+2E-07x^3+0.000x^2+0.272x+63.35$) tend to have a relation of negative reciprocal (a). Concurrently, trend line of RLA ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$) and trend line of [G] ($y=1E-11x^4+8E-08x^3+0.000x^2+0.120x+29.89$) tend to have a relation of negative reciprocal as well (b).

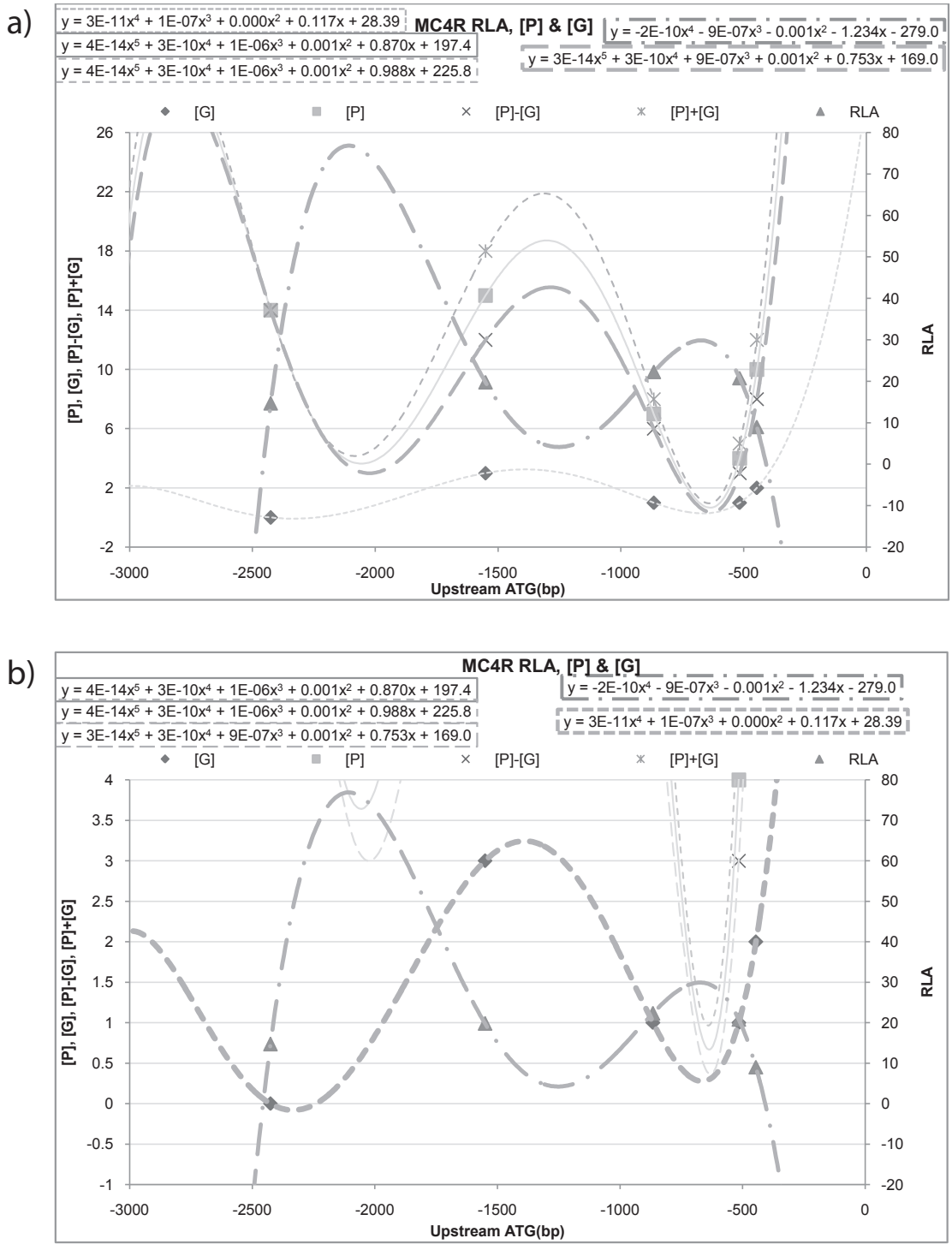

Figure 4: RLA data of MC4R and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model.

For MC4R, plot the RLA data and {the glycosylation site [G], phosphorylation site [P], sum of [P]+[G], difference of [P]-[G] site} of TF for promoter model on the same chart, the polynomial behavior of MC4R is: trend line of RLA is ($y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$); trend line of [G] is ($y=3E-11x^4+1E-07x^3+0.000x^2+0.117x+28.39$); trend line of [P]-[G] is ($y=3E-14x^5+3E-10x^4+9E-07x^3+0.001x^2+0.753x+169.0$); trend line of [P] is ($y=4E-14x^5+3E-10x^4+1E-06x^3+0.001x^2+0.870x+197.4$); trend line of [P]+[G] is ($y=4E-14x^5+3E-10x^4+1E-06x^3+0.001x^2+0.988x+225.8$). Trend line RLA and trend line of ([G], [P], [P] ± [G]) give an inverted image to each other. In detail, the sign of leading coefficient is opposite to each other, and sign of coefficient of corresponding degree are also opposite to each other. Furthermore, trend line of RLA and trend line of [P]-[G] tend to have a relation of negative reciprocal (a). Concurrently, trend line of RLA and trend line of [G] tend to have a relation of negative reciprocal as well (b).

Promoter Model	Sequence	bp	P	TF	Promoter Model	Sequence	bp	P	TF
					P3		-1178		
PBXC_MYOD_01	GCAGAGGCTGACAGCTT	-3177	P	V$PBXC	CAAT_AP1F_01	CCCTGACCAATCA	-1049	P	V$AP1F
PBXC_MYOD_01	GGCTGACAGCTTGTGGA	-3172	P	V$MYOD	CAAT_AP1F_01	CTGACCAATCAAGAG	-1046	P	V$CAAT
P1		-3114			CAAT_CAAT_01	CTGACCAATCAAGAG	-1046	P	V$CAAT
E2FF_SP1F_01	GCTCAGGGTGGGTTTGG	-3054	P	V$SP1F	CAAT_CAAT_01	GGAGCCAAACACTCT	-1020	P	V$CAAT
E2FF_SP1F_01	GGGAGGCGGGGATCTGC	-3036	P	V$E2FF	P4		-978		
ETSF_ETSF_02	CTGTGAGGGGAAGTTGAGATC	-2897	P	V$ETSF	NEUR_LHXF_02	ATGTCAACTGCTC	-965	P	V$NEUR
IRFF_ETSF_02	CTGTGAGGGGAAGTTGAGATC	-2897	P	V$ETSF	NEUR_LHXF_02	TAAACGTGCAATTTAATAAGTCT	-899	P	V$LHXF
IRFF_ETSF_02	AGGGGAAGTTGAGATCCATCC	-2892	P	V$IRFF	STAT_IRFF_01	ACATAAAAATGAAACCTGCAC	-866	P	V$IRFF
ETSF_ETSF_02	ACAAGCCAGGATGGATCTCAA	-2884	P	V$ETSF	STAT_IRFF_01	TTATTTCCAAGAACACATA	-851	P	V$STAT
EGRF_NFAT_01	GCTGAGGAAATTATTTTAA	-2815	P	V$NFAT	STAT_FKHD_01	TGTGTTCTTGGAAATAAAC	-849	P	V$STAT
CEBP_CREB_01	CCCAGCTGAGGAAATTATTTT	-2812	P	V$CREB	STAT_FKHD_01	TGGAAATAAACAAAAAT	-842	P	V$FKHD
CEBP_CREB_01	CAGCTGAGGAAATTA	-2811	P	V$CEBP	SORY_SORY_02	AATAAACAAAAATTGGTGTAATTCT	-834	P	V$SORY
HAND_GATA_02	ATTTCCTCAGCTGGGAGATTT	-2806	P	V$HAND	SORY_SORY_02	CACGAGAATTACACCAATTTTTGTT	-830	P	V$SORY
HAND_GATA_02	TATGGATATCCTA	-2789	P	V$GATA	PBXC_MYOD_01	TTCCTGGGTGACAGCTG	-783	P	V$PBXC
GATA_MYOD_01	GGGCCACAGTTGGCTGT	-2741	P	V$MYOD	PBXC_MYOD_01	GGGTGACAGCTGCAATA	-778	P	V$MYOD
GATA_MYOD_01	GATCGATACCCGT	-2683	P	V$GATA	SORY_ETSF_01	CATTGACAATATTGCAGCTGTCACC	-773	P	V$SORY
SMAD_AP1F_03	CTGTGAGTCCAGA	-2651	P	V$AP1F	P5		-765		
OCT1_CEBP_01	ACTATAGTAATTTGA	-2593	P	V$OCT1	ETSF_IRFF_01	TGGAGGAAGAGAAATCAGAGA	-738	P	V$IRFF
OCT1_CEBP_01	ATAGTTTTGAAAATT	-2583	P	V$CEBP	IRFF_ETSF_01	TGGAGGAAGAGAAATCAGAGA	-738	P	V$IRFF
NFKB_SP1F_04	GATGTTTATTCCCTC	-2472	P	V$NFKB	ETSF_IRFF_01	AGGATGGAGGAAGAGAAATCA	-734	P	V$ETSF
SMAD_AP1F_03	GAAGTCTGGAG	-2462	P	V$SMAD	IRFF_ETSF_01	AGGATGGAGGAAGAGAAATCA	-734	P	V$ETSF
SMAD_EBOX_02	GAAGTCTGGAG	-2462	P	V$SMAD	SORY_ETSF_01	AGGATGGAGGAAGAGAAATCA	-734	P	V$ETSF
NFKB_SP1F_04	GCCCTGGGCAGAGGCCC	-2438	P	V$SP1F	SP1F_YY1F_01	TTCCTCCATCCTAGACGCTGG	-725	P	V$YY1F
EGRF_SP1F_01	GATGTGGGCGGGACCAA	-2383	P	V$SP1F	ETSF_SP1F_05	GGCGGTGGCGGGGGTCG	-704	P	V$SP1F
KLFS_SP1F_01	GATGTGGGCGGGACCAA	-2383	P	V$SP1F	SP1F_YY1F_01	GGCGGTGGCGGGGGTCG	-704	P	V$SP1F
KLFS_SP1F_01	AAGGTTGGGGCTGAAGG	-2367	P	V$KLFS	E2FF_SP1F_01	CGGTGGCGGGGGTCGGG	-702	P	V$E2FF
NR2F_SF1F_01	TTGCCAAGGACCCGT	-2278	P	V$SP1F	ETSF_SP1F_05	GGGTCGGGGGAAGGGCTGGGC	-691	P	V$ETSF
NR2F_SF1F_01	GGGTCCTTGGCAAAGAACACCTCCC	-2271	P	V$NR2F	SP1F_ETSF_01	GGGTCGGGGGAAGGGCTGGGC	-691	P	V$ETSF
NF1F_NR2F_01	TCCTTGGCAAAGAACACCTCC	-2270	P	V$NF1F	E2FF_SP1F_01	GGGAAGGGCTGGGCCAG	-686	P	V$SP1F
NF1F_NR2F_01	ATCATGGGGGCAAAGGGGAGGTGTT	-2256	P	V$NR2F	SP1F_ETSF_01	GGGAAGGGCTGGGCCAG	-686	P	V$SP1F
EBOX_SREB_01	AGATCATGGGGGCAA	-2249	P	V$SREB	P6		-604		
EBOX_SREB_01	AAATCAGGAGATCAT	-2241	P	V$SREB	CAAT_SREB_02	TGCTCACCTGCGCTC	-542	P	V$SREB
P2		-2170			SP1F_MYOD_01	CGAGCGCAGGTGAGCAG	-542	P	V$MYOD
CEBP_EBOX_01	TCTAATAAGTAAGGA	-1992	P	V$CEBP	SP1F_ETSF_04	TGCAGTCCGGACTGCTCACCT	-533	P	V$ETSF
CEBP_EBOX_01	TAGACACGTGCTC	-1982	P	V$EBOX	SP1F_ETSF_04	GCAGGGGGCGCCAATCT	-517	P	V$SP1F
CREB_EBOX_02	GGTAGAGCACGTGTCTAATAA	-1982	P	V$CREB	SP1F_MYOD_01	GCAGGGGGCGCCAATCT	-517	P	V$SP1F
CREB_EBOX_02	TAGACACGTGCTC	-1982	P	V$EBOX	CAAT_SREB_02	GGCGCCAATCTGCTT	-512	P	V$CAAT
EBOX_SREB_01	TAGACACGTGCTC	-1982	P	V$EBOX	PBXC_MYOD_01	TGACAGCAGGTCGGGGC	-471	P	V$MYOD
EBOX_SREB_01	TAGACACGTGCTC	-1982	P	V$EBOX	PBXC_MYOD_01	TGTCTGACTGACAGCAG	-463	P	V$PBXC
SMAD_EBOX_02	TAGACACGTGCTC	-1982	P	V$EBOX	P7		-373		
EBOX_SREB_01	AGAGCACGTGTCT	-1981	P	V$EBOX	NFAT_GATA_01	GCAAAGGAAAGTTCTTTCT	-202	P	V$NFAT
EBOX_SREB_01	AGAGCACGTGTCT	-1981	P	V$EBOX	IRFF_NFAT_01	GCAAAGGAAAGTTCTTTCT	-202	P	V$NFAT

EBOX_SREB_01	TCATCTCCAAATCAG	-1726	P	V$SREB	IRFF_NFAT_01	TGGAGACATAGAAAGAACTTT	-194	P	V$IRFF
EBOX_SREB_01	AAATCAGGTTGCAGT	-1718	P	V$SREB	NFAT_GATA_01	GATAGATAGAGAG	-166	P	V$GATA
SORY_SF1F_01	AACCCAAGGCTACAT	-1373	P	V$SF1F	GATA_GATA_GATA_01	GATAGATAGAGAG	-166	P	V$GATA
SORY_SF1F_01	TGCCTGCAATCCCTGCAGTCGGCAC	-1317	P	V$SORY	GATA_GATA_GATA_01	GATAGATAGATAG	-150	P	V$GATA
NF1F_NR2F_01	CATAGTGAGTTCAAAGCCAGCCTGG	-1250	P	V$NR2F	GATA_GATA_GATA_01	GATAGATAGATAG	-146	P	V$GATA
NF1F_NR2F_01	GGGTATCACCTATGCCAGGCT	-1234	P	V$NF1F	GATA_GATA_GATA_01	GAGAGATAGATAG	-138	P	V$GATA

Table 2: Phosphorylation site on promoter model of mMC3r

Promoter Model	Sequence	bp	G	TF
P1		-3200		
SP1F_SP1F_04	CGTCTGGGTGGCTTCAG	-3183	G	V$SP1F
CREB_EBOX_02	GGCCCATCACGTGAATGGTGT	-3074	G	V$CREB
CREB_EBOX_02	CATTCACGTGATG	-3074	G	V$EBOX
CREB_EBOX_02	CACCATTCACGTGATGGGCCA	-3073	G	V$CREB
CREB_EBOX_02	CCATCACGTGAAT	-3073	G	V$EBOX
SP1F_YY1F_01	CAGATGGGCTGGCAGCG	-3056	G	V$SP1F
GREF_OCT1_01	CACGCGCCTGTAGTTCCAG	-2742	G	V$GREF
NEUR_SP1F_01	AGATGGGGTGTGGGGGC	-2587	G	V$SP1F
SP1F_KLFS_01	AGATGGGGTGTGGGGGC	-2587	G	V$SP1F
SP1F_SP1F_04	AGATGGGGTGTGGGGGC	-2587	G	V$SP1F
EREF_GATA_01	ACCTGAGGTCAGGAGTTCAAGAC	-2521	G	V$EREF
P2		-2413		
NF1F_EBOX_01	CCTCCACCTTCTG	-2383	G	V$EBOX
CREB_NFKB_05	TTATCCTGACTCAGCTGGGAT	-1861	G	V$CREB
EREF_GATA_01	ACCTGAGGTCAGGAGTTCGAGAC	-1762	G	V$EREF
CREB_EBOX_01	AACTCCTGACCTCAGGTGATC	-1757	G	V$CREB
SF1F_CREB_01	AACTCCTGACCTCAGGTGATC	-1757	G	V$CREB
CREB_EBOX_01	GATCCACCCGCCT	-1744	G	V$EBOX
SORY_PAX3_01	CACTCACAAGCCACCTCCATGTGTG	-1572	G	V$SORY
SP1F_ETSF_01	GAAGCGGGAGGGGGATG	-1402	G	V$SP1F
P3		-1382		
SP1F_NFKB_02	CTCCAGGGCGTTCCCAG	-1347	G	V$SP1F
EBOX_SREB_01	GGGCCACGCCCGA	-1268	G	V$EBOX
SP1F_ETSF_02	CAGTCGGGCGTGGCCCC	-1267	G	V$SP1F
EBOX_AP2F_01	GTCGCACGTGGTG	-1233	G	V$EBOX
RXRF_EBOX_01	GTCGCACGTGGTG	-1233	G	V$EBOX
E2FF_SP1F_01	GGGGAGGGCGGGAGCAG	-1134	G	V$SP1F
ETSF_SP1F_03	GGGGAGGGCGGGAGCAG	-1134	G	V$SP1F
P4		-930		
ETSF_SP1F_05	GGAGAGGGCAGGTCCCG	-634	G	V$SP1F
ETSF_SP1F_03	TAGAGGGGCGGCCAGGT	-600	G	V$SP1F
P5		-566		
CREB_NFKB_05	TCTCCTTTACGTTAAAAAACA	-551	G	V$CREB
P6		-517		
AP2F_P53F_01	GGTGGCAGGCCGGGCCATGGTGG	-489	G	V$P53F

EGRF_SP1F_01	GCCGGGGGCGTGAGCAC	-469	G	V$SP1F
SP1F_AP2F_01	GCCGGGGGCGTGAGCAC	-469	G	V$SP1F
P7		-459		
P8		-447		
P9		-414		
P53F_SP1F_02	CTGGACTGGCTGGGCCATGCCTG	-199	G	V$P53F
P53F_SP1F_02	TGCCTGGGCTGACCTGT	-185	G	V$SP1F
CREB_NFKB_05	CTGGGCTGACCTGTCCAGCCA	-180	G	V$CREB
AP2F_P53F_01	GCTGACCTGTCCAGCCAGGGAGA	-175	G	V$P53F
RXRF_SP1F_RXRF_01	TGTGAGGGCAGATCTGG	-152	G	V$SP1F
GREF_STAT_01	CAGGTCCCCACAGTTCTTC	-50	G	V$GREF

Table 3: Glycosylation site on promoter model of hMC1R

Promoter Model	Sequence	bp	P	TF	Promoter Model	Sequence	bp	P	TF
P1		-3200			SMAD_LEFF_01	GAGGTCTGCCT	-1209	P	V$SMAD
SP1F_SP1F_04	CGTCTGGGTGGCTTCAG	-3183	P	V$SP1F	KLFS_KLFS_01	CCACATCAAAGGCAGAC	-1203	P	V$KLFS
HIFF_HASF_01	CACCCAGACGTGGTCTG	-3176	P	V$HIFF	SMAD_LEFF_01	GCCACATCAAAGGCAGA	-1202	P	V$LEFF
NF1F_MYOD_01	TAGGCGAATCTCAGCCAGAGA	-3107	P	V$NF1F	ETSF_ETSF_01	TCCGCTGCGGAAGGCACCACA	-1168	P	V$ETSF
SP1F_YY1F_01	GGACACCATTCACGTGATGGG	-3076	P	V$YY1F	ETSF_ETSF_01	TCCGCAGCGGAAATGGCGCGC	-1158	P	V$ETSF
CREB_EBOX_02	GGCCCATCACGTGAATGGTGT	-3074	P	V$CREB	ETSF_SP1F_03	TCCGCAGCGGAAATGGCGCGC	-1158	P	V$ETSF
CREB_EBOX_02	CATTCACGTGATG	-3074	P	V$EBOX	E2FF_SP1F_01	AAATGGCGCGCCGCCCG	-1150	P	V$E2FF
CREB_EBOX_02	CACCATTCACGTGATGGGCCA	-3073	P	V$CREB	E2FF_SP1F_01	GGGGAGGGCGGGAGCAG	-1134	P	V$SP1F
CREB_EBOX_02	CCATCACGTGAAT	-3073	P	V$EBOX	ETSF_SP1F_03	GGGGAGGGCGGGAGCAG	-1134	P	V$SP1F
NF1F_MYOD_01	ATGGGCCAGATGGGCTG	-3062	P	V$MYOD	ETSF_HAML_03	CGCTCCCAGGAAGCGACACCC	-1033	P	V$ETSF
SP1F_YY1F_01	CAGATGGGCTGGCAGCG	-3056	P	V$SP1F	ETSF_KLFS_01	CGCTCCCAGGAAGCGACACCC	-1033	P	V$ETSF
TEAF_TEAF_01	CAACATACATGAA	-2931	P	V$TEAF	IRFF_ETSF_02	CGCTCCCAGGAAGCGACACCC	-1033	P	V$ETSF
TEAF_TEAF_01	AAACATTTTAGGG	-2907	P	V$TEAF	IRFF_ETSF_02	CCCAGGAAGCGACACCCCCAC	-1029	P	V$IRFF
E2FF_E2FF_03	GGGAGGCGGAGGCGGGA	-2854	P	V$E2FF	ETSF_KLFS_01	CTGTGGGGGTGTCGCTT	-1025	P	V$KLFS
E2FF_E2FF_03	CGGAGGCGGGAGGATCA	-2848	P	V$E2FF	ETSF_HAML_03	GGCTGTGGGGGTGTC	-1021	P	V$HAML
GREF_OCT1_01	CACGCGCCTGTAGTTCCAG	-2742	P	V$GREF	ETSF_IRFF_02	CGCTCCCAGGAAGCGACACCC	-983	P	V$ETSF
GREF_OCT1_01	GGTATGAGAATTACT	-2709	P	V$OCT1	NKXH_NKXH_CEBP_01	GCCCCCAAGGGCCCAGCCT	-965	P	V$NKXH
NEUR_SP1F_01	ACCCCATCTGCTT	-2593	P	V$NEUR	AP2F_YBXF_01	CAGAGTGGCCTCA	-943	P	$YBXF
SP1F_KLFS_01	CAGATGGGGTGTGGGGG	-2588	P	V$KLFS	AP2F_YBXF_01	AGAGCCTGAGGCCAC	-938	P	V$AP2F
NEUR_SP1F_01	AGATGGGGTGTGGGGGC	-2587	P	V$SP1F	P4		-930		
SP1F_KLFS_01	AGATGGGGTGTGGGGGC	-2587	P	V$SP1F	ETSF_IRFF_02	CAAGATGCCTGAAAACACCAA	-919	P	V$IRFF
SP1F_SP1F_04	AGATGGGGTGTGGGGGC	-2587	P	V$SP1F	ETSF_SP1F_03	GGTCCCGGGGAAGCTCCGGAC	-872	P	V$ETSF
EREF_GATA_01	CTGAGATTACAGG	-2567	P	V$GATA	ETSF_KLFS_01	CCCTGGCCGGAAGCCCCCTCA	-857	P	V$ETSF
RXRF_RXRF_01	TCACCTGAGGTCAGGAGTTCAAGAC	-2522	P	V$RXRF	KLFS_KLFS_NFKB_01	GAGGGGGCTTCCGGC	-855	P	V$NFKB
EREF_GATA_01	ACCTGAGGTCAGGAGTTCAAGAC	-2521	P	V$EREF	STAT_NFKB_06	GAGGGGGCTTCCGGC	-855	P	V$NFKB
RXRF_RXRF_01	CTAGTTGGGAGGCTGAGGCATAAGT	-2414	P	V$RXRF	KLFS_KLFS_NFKB_01	GGTGAGGGGGCTTCCGG	-853	P	V$KLFS
P2		-2413			ETSF_KLFS_01	CGCGCGGGGTGAGGGGG	-846	P	V$KLFS
NF1F_EBOX_01	CCTCCACCTTCTG	-2383	P	V$EBOX	STAT_NFKB_06	AAAGTTCTGGAAACTGAGT	-805	P	V$STAT
NF1F_EBOX_01	GAGGTTGCAGTGAGCCAAGAA	-2370	P	V$NF1F	OCT1_CEBP_01	GAACTTTAATTATTG	-795	P	V$OCT1
SREB_SREB_SREB_01	ACTGCACTCCAGCCT	-2225	P	V$SREB	PBXC_MYOD_01	GTGAACGTTGACAGCTG	-745	P	V$PBXC

SREB_SREB_ SREB_01	AGATCACACCACTGC	-2215	P	V$SREB	PBXC_MYOD_01	CGTTGACAGCTGAGTTG	-740	P	V$MYOD
SREB_SREB_ SREB_01	GGCTCACTGCAACCT	-2199	P	V$SREB	KLFS_KLFS_NFKB_01	CACGCATGGAGCAGCAA	-726	P	V$KLFS
RXRF_RXRF_01	CTACTTGGGAGGCTGAGGCAGAATT	-2160	P	V$RXRF	NF1F_NR2F_01	GCTTTGGCTGAGAGCAGAGGG	-708	P	V$NF1F
RXRF_RXRF_01	GATCATGAGGTCAGGAGTTCAAGAC	-2051	P	V$RXRF	NF1F_NR2F_01	TCAGGGAGGACAGGGGTCCCCTCTG	-692	P	V$NR2F
NR2F_GATA_01	CTCAGCCTCCCAAAGTGCTGAGATT	-2019	P	V$NR2F	ETSF_SP1F_05	GGAGAGGGCAGGTCCCG	-634	P	V$SP1F
SREB_SREB_01	TAATCTCAGCACTTT	-2013	P	V$SREB	NFKB_NFKB_04	CCGGGACCTGCCCTC	-632	P	V$NFKB
NR2F_GATA_01	CTGAGATTATAGG	-2008	P	V$GATA	ETSF_SP1F_05	GGTCCCGGGGAAGCTCCGGAC	-622	P	V$ETSF
CREB_NFKB_05	TTATCCTGACTCAGCTGGGAT	-1861	P	V$CREB	CREB_NFKB_05	TCCGGAGCTTCCCCG	-620	P	V$NFKB
CREB_NFKB_05	CGGGGTTTCTCCATG	-1793	P	V$NFKB	ETSF_SP1F_03	TAGAGGGGCGGCCAGGT	-600	P	V$SP1F
EREF_GATA_01	ACCTGAGGTCAGGAGTTCGAGAC	-1762	P	V$EREF	P5		-566		
CREB_EBOX_01	AACTCCTGACCTCAGGTGATC	-1757	P	V$CREB	HAML_CEBP_02	GACTGTGGTGTTTTT	-562	P	V$HAML
SF1F_CREB_01	AACTCCTGACCTCAGGTGATC	-1757	P	V$CREB	CREB_NFKB_05	TCTCCTTTACGTTAAAAAACA	-551	P	V$CREB
SREB_SREB_01	GGATCACCTGAGGTC	-1753	P	V$SREB	P6		-517		
CREB_EBOX_01	GATCCACCCGCCT	-1744	P	V$EBOX	AP2F_P53F_01	GCCGCCTGGTGGCAG	-500	P	V$AP2F
NR2F_SF1F_01	AGCCCAAGGCGGGTG	-1739	P	V$SF1F	SP1F_AP2F_01	CCGGCCTGCCACCAG	-495	P	V$AP2F
SF1F_CREB_01	AGCCCAAGGCGGGTG	-1739	P	V$SF1F	AP2F_P53F_01	GGTGGCAGGCCGGGCCATGGTGG	-489	P	V$P53F
IKRS_AP2F_01	CCCGCCTTGGGCTCC	-1737	P	V$AP2F	EGRF_SP1F_01	GCCGGGGGCGTGAGCAC	-469	P	V$SP1F
NR2F_GATA_01	CTTGGGCTCCCAAAGTGCTGGGATT	-1727	P	V$NR2F	SP1F_AP2F_01	GCCGGGGGCGTGAGCAC	-469	P	V$SP1F
NR2F_SF1F_01	CTTGGGCTCCCAAAGTGCTGGGATT	-1727	P	V$NR2F	P7		-459		
IKRS_AP2F_01	TGCTGGGATTACA	-1718	P	V$IKRS	P8		-447		
EREF_GATA_01	CTGGGATTACAGC	-1716	P	V$GATA	P9		-414		
NR2F_GATA_01	CTGGGATTACAGC	-1716	P	V$GATA	IRFF_ETSF_02	CTGGCCCAGGAAGGCAGGAGA	-361	P	V$ETSF
OCT1_PIT1_01	TGCTTTAAAATTTTT	-1675	P	V$OCT1	IRFF_ETSF_02	AGGAAGGCAGGAGACAGAGGC	-354	P	V$IRFF
OCT1_PIT1_01	AAGATTTATAAATTG	-1654	P	V$PIT1	NFKB_NFKB_04	CAGGGATTCTCACAA	-246.5	P	V$NFKB
SORY_PAX3_01	CACTCACAAGCCACCTCCATGTGTG	-1572	P	V$SORY	P53F_SP1F_02	CTGGACTGGCTGGGCCATGCCTG	-199	P	V$P53F
SORY_PAX3_01	CATTTCATGGTCAGAGTTT	-1543	P	V$PAX3	AP2F_P53F_01	CATGCCTGGGCTGAC	-188	P	V$AP2F
EBOX_SREB_01	CTCTCGCCCAACTGC	-1525	P	V$SREB	RXRF_SP1F_RXRF_01	TGGACAGGTCAGCCCAGGCATGGCC	-186	P	V$RXRF
SP1F_ETSF_02	CAGCTGCAGGAAAGGAGGCAA	-1440	P	V$ETSF	P53F_SP1F_02	TGCCTGGGCTGACCTGT	-185	P	V$SP1F
SP1F_ETSF_01	GAAGCGGGAGGGGGATG	-1402	P	V$SP1F	CREB_NFKB_05	CTGGGCTGACCTGTCCAGCCA	-180	P	V$CREB
SP1F_ETSF_01	TGAAGGGTGGAAGCGGGAGGG	-1395	P	V$ETSF	NF1F_MYOD_01	GCTGACCTGTCCAGCCAGGGA	-176	P	V$NF1F
P3		-1382			AP2F_P53F_01	GCTGACCTGTCCAGCCAGGGAGA	-175	P	V$P53F
SP1F_NFKB_02	CCAGGGCGTTCCCAG	-1348	P	V$NFKB	RXRF_SP1F_RXRF_01	TGTGAGGGCAGATCTGG	-152	P	V$SP1F
SP1F_NFKB_02	CTCCAGGGCGTTCCCAG	-1347	P	V$SP1F	RXRF_SP1F_RXRF_01	TCTGGGGGTGCCCAGATGGAAGGAG	-136	P	V$RXRF
EBOX_SREB_01	GGGCCACGCCCGA	-1268	P	V$EBOX	NF1F_MYOD_01	GGTGCCCAGATGGAAGG	-134	P	V$MYOD
SP1F_ETSF_02	CAGTCGGGCGTGGCCCC	-1267	P	V$SP1F	CREB_NFKB_05	TGGGGGACACCCAAG	-109	P	V$NFKB
KLFS_KLFS_01	GCAGTCGGGCGTGGCCC	-1266	P	V$KLFS	HAML_ETSF_01	CCTGGAGGGGAAGAACTGTGG	-58	P	V$ETSF
EBOX_AP2F_01	ACGGCCTGCGGAGCA	-1245	P	V$AP2F	GREF_STAT_01	CAGGTCCCCACAGTTCTTC	-50	P	V$GREF
HIFF_SMAD_01	GAGCACCACGTGCGACG	-1234	P	V$HIFF	HAML_ETSF_01	AACTGTGGGGACCTG	-48	P	V$HAML
EBOX_AP2F_01	GTCGCACGTGGTG	-1233	P	V$EBOX	ETSF_ETSF_05	AGGAAGCAGGAAGGAGTCGTT	-21	P	V$ETSF
RXRF_EBOX_01	GTCGCACGTGGTG	-1233	P	V$EBOX	IRFF_ETSF_02	CCAGGAAGCAGGAAGGAGTCG	-19	P	V$IRFF
RXRF_EBOX_01	ACGGCTGGAGGCGAGAGGTCTGCCT	-1216	P	V$RXRF	ETSF_ETSF_05	CCTGTCCAGGAAGCAGGAAGG	-14	P	V$ETSF
HIFF_SMAD_01	GAGGTCTGCCT	-1209	P	V$SMAD	IRFF_ETSF_02	CCTGTCCAGGAAGCAGGAAGG	-14	P	V$ETSF
					GREF_STAT_01	CTGCTTCCTGGACAGGACT	-10	P	V$STAT

Table 4: Phosphorylation site on promoter model of hMC1R

Promoter Model	Sequence	bp	G	TF
-3201				
NKXH_SRFF_01	GATATGAAGTGAACTCTCA	-3169	G	V$NKXH
NKXH_SRFF_01	TAGCCATCATAAGGATATG	-3156	G	V$SRFF
P1		-2926		
P2		-1926		
MYOD_SRFF_01	AGGACCATATCATGAAAGG	-1694	G	V$SRFF
SORY_SORY_02	CAGGAACAATACTTAGTATTGTCTC	-1506	G	V$SORY
SORY_SORY_02	CAGAGACAATACTAAGTATTGTTCC	-1504	G	V$SORY
P3		-1176		
ZFHX_ZFHX_NKXH_01	CTTCTTAATTTTTTGTTTT	-858	G	V$NKXH
P4		-556		
SP1F_ETSF_01	AGCAGAGGAGGAGCCAC	-498	G	V$SP1F
P5		-476		
GATA_NKXH_01	AAGCAGAAGTGAGAACAAG	-271	G	V$NKXH
CREB_HNF1_01	ACTCCCTGACCCAGGAGGTTA	-48	G	V$CREB

Table 5: Glycosylation site on promoter model of hMC4R

Promoter Model	Sequence	bp	P	TF	Promoter Model	Sequence	bp	P	TF
		-3201			MYBL_CEBP_01	GAACAACAGATTATA	-1351	P	V$MYBL
NKXH_SRFF_01	GATATGAAGTGAACTCTCA	-3169	P	V$NKXH	MYBL_CEBP_01	AATTAACTGAACAAC	-1343	P	V$MYBL
NKXH_SRFF_01	TAGCCATCATAAGGATATG	-3156	P	V$SRFF	MYBL_CEBP_01	TTAATTTGGAAAGTA	-1334	P	V$CEBP
TEAF_TEAF_01	TCACATTCTTGCT	-3110	P	V$TEAF	MYBL_CEBP_01	TTAATTTGGAAAGTA	-1334	P	V$CEBP
TEAF_TEAF_01	CCTCATACATTGC	-3085	P	V$TEAF	GATA_HNF1_01	AGTTAGTTATTGAAGTT	-1274	P	V$HNF1
STAT_MITF_01	TTGTTTTGAGGAACTGCCA	-3026	P	V$STAT	NF1F_NF1F_HNF1_01	TGATACTAGTTAGTTAT	-1267	P	V$NF1F
STAT_MITF_01	GTTATCATATGATTC	-3001	P	V$MITF	GATA_HNF1_01	TGGTGATACTAGT	-1262	P	V$GATA
STAT_MITF_01	TGAATCATATGATAA	-3000	P	V$MITF	NF1F_NF1F_HNF1_01	CTTTACTTATCAAGCCAAGAC	-1245	P	V$NF1F
STAT_MITF_01	TCCATTCCTGGTTGTGTAT	-2978	P	V$STAT	NF1F_NF1F_HNF1_01	CGTAAGTTGAACCGACAAATA	-1216	P	V$NF1F
MEF2_MYOD_02	ATGTGACAGATGATTTC	-2949	P	V$MYOD	P3		-1176		
MEF2_MYOD_02	AATTTGTCCATAAATATTCCTAG	-2926	P	V$MEF2	CEBP_MYBL_04	TTTTAACTGAACCAC	-895	P	V$MYBL
P1		-2926			ZFHX_ZFHX_NKXH_01	CTTCTTAATTTTTTGTTTT	-858	P	V$NKXH
ETSF_AP1F_05	CCAAAACTGGAAATAACCCAA	-2886	P	V$ETSF	ZFHX_ZFHX_NKXH_01	TATTTACCTTCTT	-848	P	V$ZFHX
ETSF_AP1F_05	AGTTGAGGAACAT	-2869	P	V$AP1F	ZFHX_ZFHX_NKXH_01	TATCTGTTTCAGG	-807	P	V$ZFHX
NR2F_GATA_01	CTGGGATTACAGG	-2805	P	V$GATA	CEBP_MYBL_04	ATTCTTATGTAAAAG	-744	P	V$CEBP
NR2F_GATA_01	CTCGGCCTCCCAAAGTGCTGGGATT	-2794	P	V$NR2F	STAT_IRFF_01	TTGTTTCCCTTGAAAACTT	-667	P	V$STAT
RXRF_RXRF_01	GGCGGGCGGATCATGAGGTCAGGAG	-2770	P	V$RXRF	STAT_IRFF_01	CTTAAAAAGGGAAATTCAGTC	-650	P	V$IRFF
NF1F_MYOD_01	GGACTACAGGTGCCCGC	-2688	P	V$MYOD	P4		-556		
RXRF_RXRF_01	TTGGGAGGCTGAGGCAGGAGAATGG	-2660	P	V$RXRF	CAAT_AP1F_01	CTGACCAATCCCAAT	-519	P	V$CAAT
NF1F_MYOD_01	CTCCTGGGTTCACGCCATTCT	-2644	P	V$NF1F	CAAT_AP1F_01	TTCTGACCAATCC	-516	P	V$AP1F
AP1F_CEBP_03	GGCTGAGTAATAT	-2504	P	V$AP1F	SP1F_ETSF_01	GGTCAGAAGGAAGCAGAGGAG	-507	P	V$ETSF
AP1F_CEBP_03	ATGGCTGAGTAATAT	-2503	P	V$CEBP	SP1F_ETSF_01	AGCAGAGGAGGAGCCAC	-498	P	V$SP1F
FKHD_NF1F_01	GGAATATTACTCAGCCATAAA	-2503	P	V$NF1F	P5		-476		
FKHD_NF1F_01	GGAATATTACTCAGCCATAAA	-2503	P	V$NF1F	NEUR_NEUR_02	GCGCCAGCTGCTG	-444	P	V$NEUR
FKHD_NF1F_01	GCCATAAAAACAAATCA	-2492	P	V$FKHD	SMAD_E2FF_01	TGTAGGCGCCAGCTGCT	-441	P	V$E2FF
FKHD_NF1F_01	GCCATAAAAACAAATCA	-2492	P	V$FKHD	SMAD_E2FF_01	AGGGGCTGTAG	-432	P	V$SMAD
P2		-1926			GATA_NKXH_01	ATAAGATTAAAGT	-289	P	V$GATA
MYOD_SRFF_01	AGGACCATATCATGAAAGG	-1694	P	V$SRFF	NEUR_NEUR_02	CTCACTTCTGCTT	-274	P	V$NEUR
MYOD_SRFF_01	ATGTTACAGGTGGCAGG	-1679	P	V$MYOD	GATA_NKXH_01	AAGCAGAAGTGAGAACAAG	-271	P	V$NKXH
MYOD_VTBP_01	ATGTTACAGGTGGCAGG	-1679	P	V$MYOD	HNF1_GATA_01	GACAGATAAAGAC	-86	P	V$GATA
MYOD_VTBP_01	TTGTGTAAAATTATGAA	-1636	P	O$VTBP	HNF1_GATA_01	TCGTCTCAGTTATTTCC	-66	P	V$HNF1
SORY_SORY_02	CAGGAACAATACTTAGTATTGTCTC	-1506	P	V$SORY	CREB_HNF1_01	ACTCCCTGACCCAGGAGGTTA	-48	P	V$CREB
SORY_SORY_02	CAGAGACAATACTAAGTATTGTTCC	-1504	P	V$SORY	CREB_HNF1_01	TGAATTGATTTAACCTC	-36	P	V$HNF1

Table 6: Phorsphorylation site on promoter model of hMC4R

	hMC1R	mMC3r	hMC4R
RLA	$y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$	$y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$	$y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$
[G]	$y=1E-11x^4+8E-08x^3+0.000x^2+0.120x+29.89$	$y=2E-14x^5+1E-10x^4+2E-07x^3+0.000x^2+0.033x+1.873$	$y=3E-11x^4+1E-07x^3+0.000x^2+0.117x+28.39$
[P]-[G]	$y=3E-11x^4+2E-07x^3+0.000x^2+0.272x+63.35$	$y=7E-14x^5+4E-10x^4+9E-07x^3+0.000x^2+0.337x+51.63$	$y=3E-14x^5+3E-10x^4+9E-07x^3+0.001x^2+0.753x+169.0$
[P]	$y=5E-11x^4+3E-07x^3+0.000x^2+0.393x+93.25$	$y=9E-14x^5+5E-10x^4+1E-06x^3+0.001x^2+0.371x+53.50$	$y=4E-14x^5+3E-10x^4+1E-06x^3+0.001x^2+0.870x+197.4$
[P]+[G]	$y=6E-11x^4+4E-07x^3+0.000x^2+0.513x+123.1$	$y=1E-13x^5+7E-10x^4+1E-06x^3+0.001x^2+0.404x+55.37$	$y=4E-14x^5+3E-10x^4+1E-06x^3+ 0.001x^2+0.988x+225.8$

Table 7: Polynormial schemes of trend line RLA, [P], [P] ± [G], [G] for three melanocortin receptors

Discussion

As a result, for MC1R, trend line of RLA ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$) and trend line of [G] ($y=1E-11x^4+8E-08x^3+0.000x^2+0.120x+29.89$) tend to have a relation of negative reciprocal (Figure 3b). At the same time, trend line of RLA ($y=-2E-10x^4-1E-06x^3-0.002x^2-2.313x-546.1$) and trend line of [P]-[G] ($y=3E-11x^4+2E-07x^3+0.000x^2+0.272x+63.35$) tend to have a relation of reciprocal as well (Figure 3a). For Mc3r, trend line of RLA ($y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$) and trend line of [G] ($y=2E-14x^5+1E-10x^4+2E-07x^3+0.000x^2+0.033x+1.873$) tend to have a relation of negative reciprocal (Figure 1b). At the same time, trend line of RLA ($y=-2E-11x^4-6E-08x^3-6E-05x^2-0.033x-3.146$) and trend line of [P]-[G] ($y=7E-14x^5+4E-10x^4+9E-07x^3+0.000x^2+0.337x+51.63$) tend to have a relation of negative reciprocal as well (Figure 1a). For MC4R, trend line of RLA ($y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$) and trend line of [G] ($y=3E-11x^4+1E-07x^3+0.000x^2+0.117x+28.39$) tend to have a relation of negative reciprocal (Figure 4b). At the same time, trend line of RLA ($y=-2E-10x^4-9E-07x^3-0.001x^2-1.234x-279.0$) and trend line of [P]-[G] ($y=3E-14x^5+3E-10x^4+9E-07x^3+0.001x^2+0.753x+169.0$) tend to have a relation of negative reciprocal as well (Figure 4a).

For all three receptors (MC1R, Mc3r and MC4R), not only the trend lines of RLA with [G] but also those of RLA with ([P]-[G]), tend to have a negative reciprocal relationship. Further investigations are being made to verify the reciprocal relationship.

As indicated from the figures that the trend line of RLA of all three receptors tend to have a relation of negative reciprocal to that the trend line of their [G] respectively (Figures 1b,3b and 4b), and for all of the three receptors, trend line of [G] is always the closest one (among the negative reciprocal relations) to its negative reciprocal RLA (Table 7). If it is true, Change of signal sensing phosphorylation [P] won't influence the negative reciprocal regulation of transcription activity by [G], therefore, [G] is independent from [P]. It helps us recall the theory: *O*-GlcNAc and *O*-phosphate exhibit a complex interplay on signaling, transcriptional, and cytoskeletal regulatory proteins within the cell, sometimes, *O*-GlcNAcylation and *O*-phosphorylation appear to be independently regulated [12]. Does this figure give specific digit evidence to "independently regulated" in the above theory?

In contrast, From the fact shown by the figures that trend line of RLA of all three receptors tend to have a relation of negative reciprocal to that the trend line of the corresponding ([P]-[G]) (Figures 1a,3a and 4a), and for all the three receptors, trend line of ([P]-[G]) is the second closest one (among the negative reciprocal relations) which approaching to its corresponding negative reciprocal RLA (Table 7). If it is true, the structure and nutritional element [G] will reduce the regulation of signal sensing phosphorylation [P] to the transcription activity; [P] and [G] coordinately play a negative reciprocal regulation to the transcription activity? Since *O*-GlcNAc and *O*-phosphate exhibit a complex interplay on signaling, transcriptional, and cytoskeletal regulatory proteins within the cell, one of the major functions of *O*-GlcNAc is to prevent *O*-phosphorylation and, by doing so, to modulate signaling and transcription in response to cellular nutrients or stress [14], does this negative reciprocal between trend line of ([P]-[G]) and that of RLA give a specific digit evidence to "*O*-GlcNAc prevent *O*-phosphorylation" in the above theory? Notice here is glycosylation, not *O*-GlcNAc.

Moreover, for all three receptors, as shown in Table 7, sequence of the trend line is: RLA~[G]<([P]-[G])<[P]<([P]+[G]), that the ([P]+[G]) is the farthest away from the RLA. From opposite aspect, which gives another specific digit proof to the above theory, major function of *O*-GlcNAc is to prevent *O*-phosphorylation. Since if [G] play a positive role in the [P], trend line of the ([P]+[G]) would be closer to that of RLA.

Now the question of investigation is does the nutritional and obesity related glycosylation and the signal sensing phosphorylation regulated the transcriptional activity in a negative reciprocal way for the MC1R, Mc3r and MC4R neural receptors? Besides, no matter such negative reciprocal exist or not in experimental world, simply from the negative reciprocal relationship indicated by the RLA ~([G] or [P]-[G]) figure, we can predict the transcriptional activity of the MC1R, Mc3r and MC4R in the same cell type as shown in their corresponding RLA, by counting the [P] and [G].

Declaration of Interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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