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Legends for Table and Figures

Table 1 Sequences of primers and fluorescent hybridization probes used for quantitative polymerase chain reaction.

Figure 1 TetHeLaG2m cell line.

(A) Schematic figure of the GluR2 minigene with pTRE-Tight Vector.

(B) Editing efficiencies at the Q/R site of GluR2 minigene pre-mRNA in conventional HeLa and TetHeLaG2m cell lines cultured in a dish for 48 h. Each symbol represents the extent of Q/R site-editing of GluR2 minigene pre-mRNA isolated from a single culture dish. Each large symbol represents the results of five culture dishes. For each cell line, the mean ±SEM (n=15-30) is also indicated.

(C) Culture time-dependent changes of editing efficiency at the Q/R site of GluR2 minigene pre-mRNA and expression levels of ADAR2 mRNA and GluR2 pre-mRNA, and relative abundance of ADAR2 mRNA to GluR2 pre-mRNA in the TetHeLaG2m cell line. TetHeLaG2m cells were plated at a low concentration (5×10^5 cells/well in 6-well plate). The relative abundance of ADAR2 mRNA to GluR2 pre-mRNA increases in parallel with the editing efficiency at the GluR2 Q/R site in a time-dependent manner. For each culture time, the mean ± SEM (n=6) is also indicated.

Figure 2 Method of detecting editing efficiency at the Q/R site of GluR2.

(A) Scheme for detecting editing efficiency at the Q/R site of GluR2. Open bars represent nested PCR products. Intrinsic *BbvI* recognition sites are indicated by vertical solid arrowheads. The sizes of the DNA fragments generated by restriction digestion are indicated.

(B) Example of the quantification of editing efficiency using a 2100 Bioanalyzer. The molarity of the 129-bp (derived from edited GluR2 pre-mRNA) and 71-bp (derived from both edited and unedited GluR2 pre-mRNA) bands was quantified after restriction digestion with *BbvI* for 12 h, and the editing efficiency was calculated as the ratio of the former to the latter. The lower figure indicates gel-like image produced by the 2100 Bioanalyzer.

GluR, glutamate receptor; Q, glutamine; R, arginine; PCR, polymerase chain reaction

Figure 3 Editing extent of GluR2 Q/R site

Editing efficiency in TetHeLaG2m cells after treatment with antidepressants (0-10 μM) for 24 h is expressed as mean ± SEM (n = 5 to 8). Statistical analysis was performed by the one-way ANOVA test followed by Dunnett's multiple comparison test (*P<0.05, **P<0.01 and ***P<0.001).

GluR, glutamate receptor; Q, glutamine; R, arginine; ANOVA, analysis of variance

Figure 4 Expression levels of ADAR2 mRNA, GluR2 mRNA and GluR2 pre-mRNA

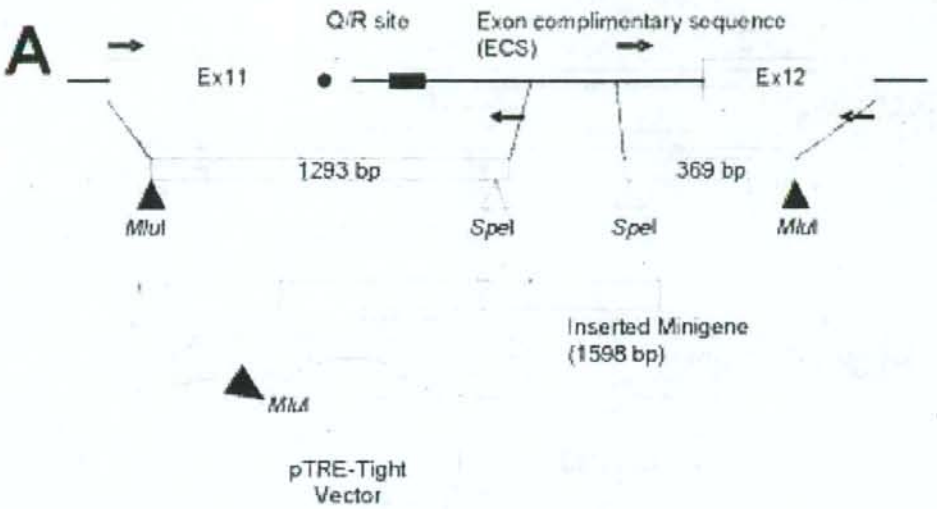
Amounts of RNA that were upregulated by seven out of eight antidepressants that upregulated by GluR2 Q/R site-editing (A, B, C, D, E, F, and G) were quantitatively analyzed. The amounts of ADAR2 mRNA, GluR2 mRNA, and GluR2 pre-mRNA are expressed as values relative to that of β-actin mRNA. The amount of ADAR2 mRNA is also expressed as a value relative to that of GluR2 mRNA. Mean ± SEM of at least 5-8 wells are displayed. Statistical analysis was performed with Steel's multiple comparison test (*P<0.05, **P<0.01, ***P<0.001).

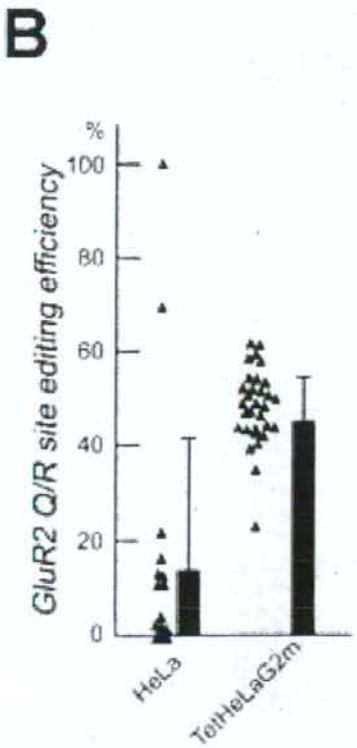
GluR, glutamate receptor; ADAR, adenosine deaminase acting on RNA; Q, glutamine; R, arginine

Table 1

	Oligonucleotide sequence
GluR2	
Forward primer	5'-ATGCGATATTTCGCCAAGA-3'
Reverse primer	5'-CAGTCAGGAAGGCAGCTAAGTT-3'
Universal Probe Library probe #63	5'-CTCCTCCT-3'
pre-GluR2	
Forward primer	5'-GATGGTGTCTCCCATCGAAA-3'
Reverse primer	5'-TCCATAAGCAATTTCTGTTTGCT-3'
Universal Probe Library probe #73	5'-GCTGAGGA-3'
ADAR2	
Forward primer	5'-GTGTAAGCACGCGTTGTACTG-3'
Reverse primer	5'-CGTAGTAAGTGGGAGGGAACC-3'
Universal Probe Library probe #42	5'-GCTGGATG-3'
β-actin	
Forward primer	5'-CCAACCGCGAGAAGATGA-3'
Reverse primer	5'-CCAGAGGCGTACAGGGATAG-3'
Universal Probe Library probe #64	5'-CCAGGCTG-3'

Figure 1


A
Q/R site
Exon complimentary sequence
(ECS)
Ex11
Ex12
1293 bp
369 bp
MluI
SpeI
SpeI
MluI
Inserted Minigene
(1598 bp)
MluI
pTRE-Tight
Vector


B
GluR2 Q/R site editing efficiency
%
100
80
60
40
20
0
HeLa
TetHeLaG2m

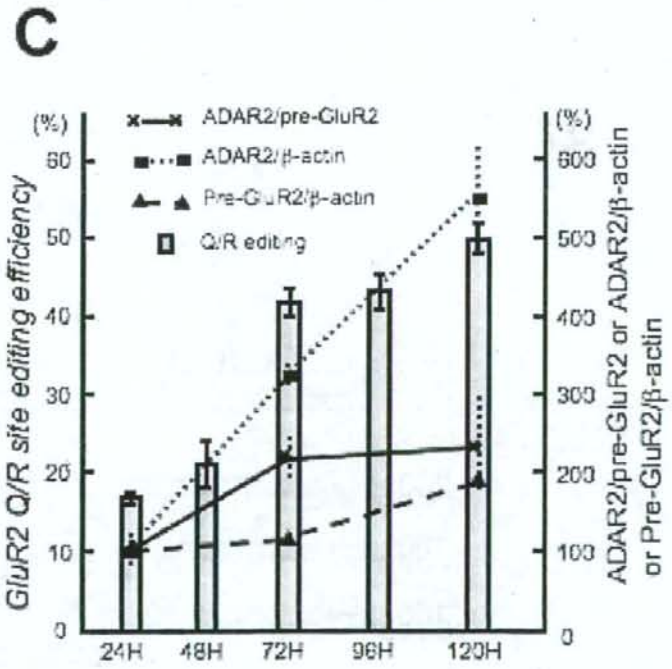
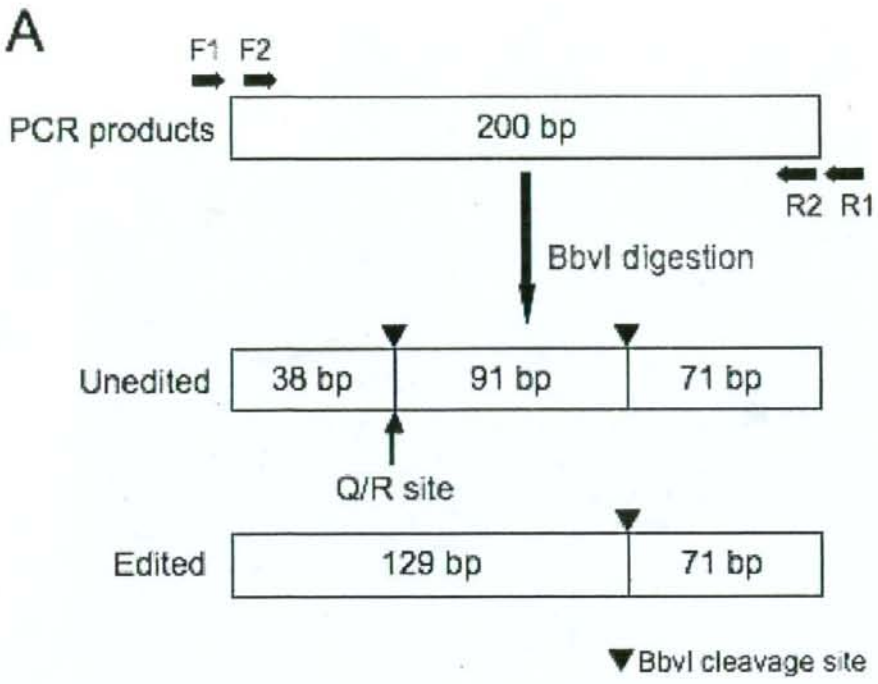

C
GluR2 Q/R site editing efficiency
(%)
ADAR2/pre-GluR2
ADAR2/β-actin
Pre-GluR2/β-actin
Q/R editing
(%)
ADAR2/pre-GluR2 or ADAR2/β-actin
or Pre-GluR2/β-actin
60
50
40
30
20
10
0
600
500
400
300
200
100
0
24H
48H
72H
96H
120H

Figure 2


A
F1 F2
PCR products
200 bp
R2 R1
BbvI digestion
Unedited
38 bp
91 bp
71 bp
Q/R site
Edited
129 bp
71 bp
▼BbvI cleavage site

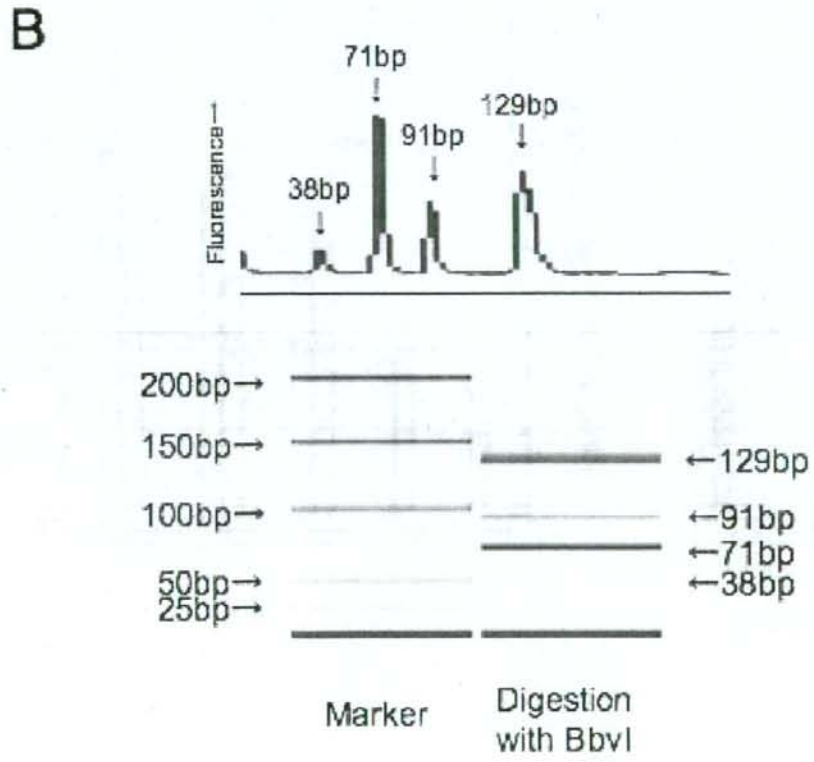

B
71bp
129bp
91bp
38bp
Fluorescence
200bp→
150bp→
100bp→
50bp→
25bp→
←129bp
←91bp
←71bp
←38bp
Marker
Digestion with BbvI

Figure 3

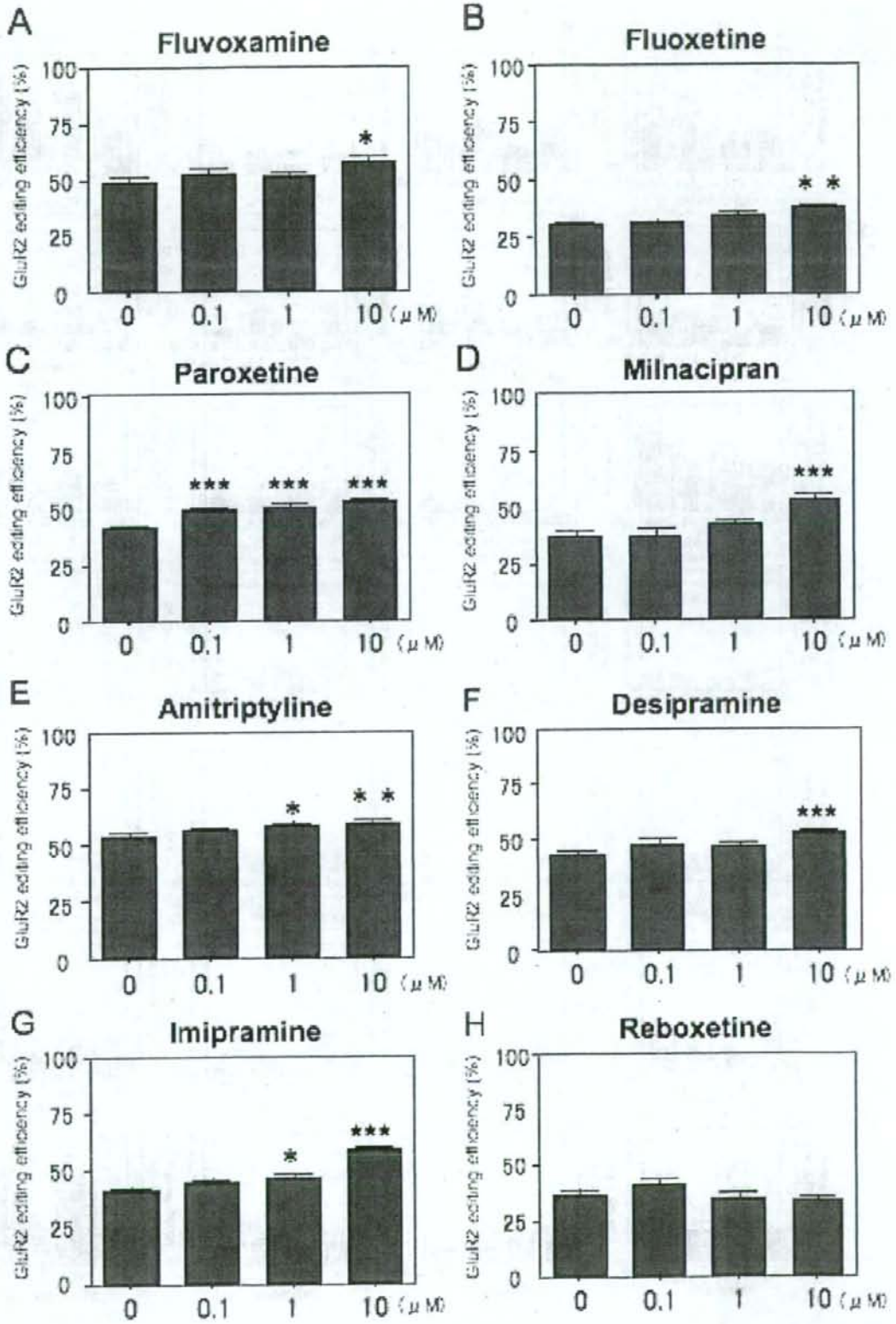
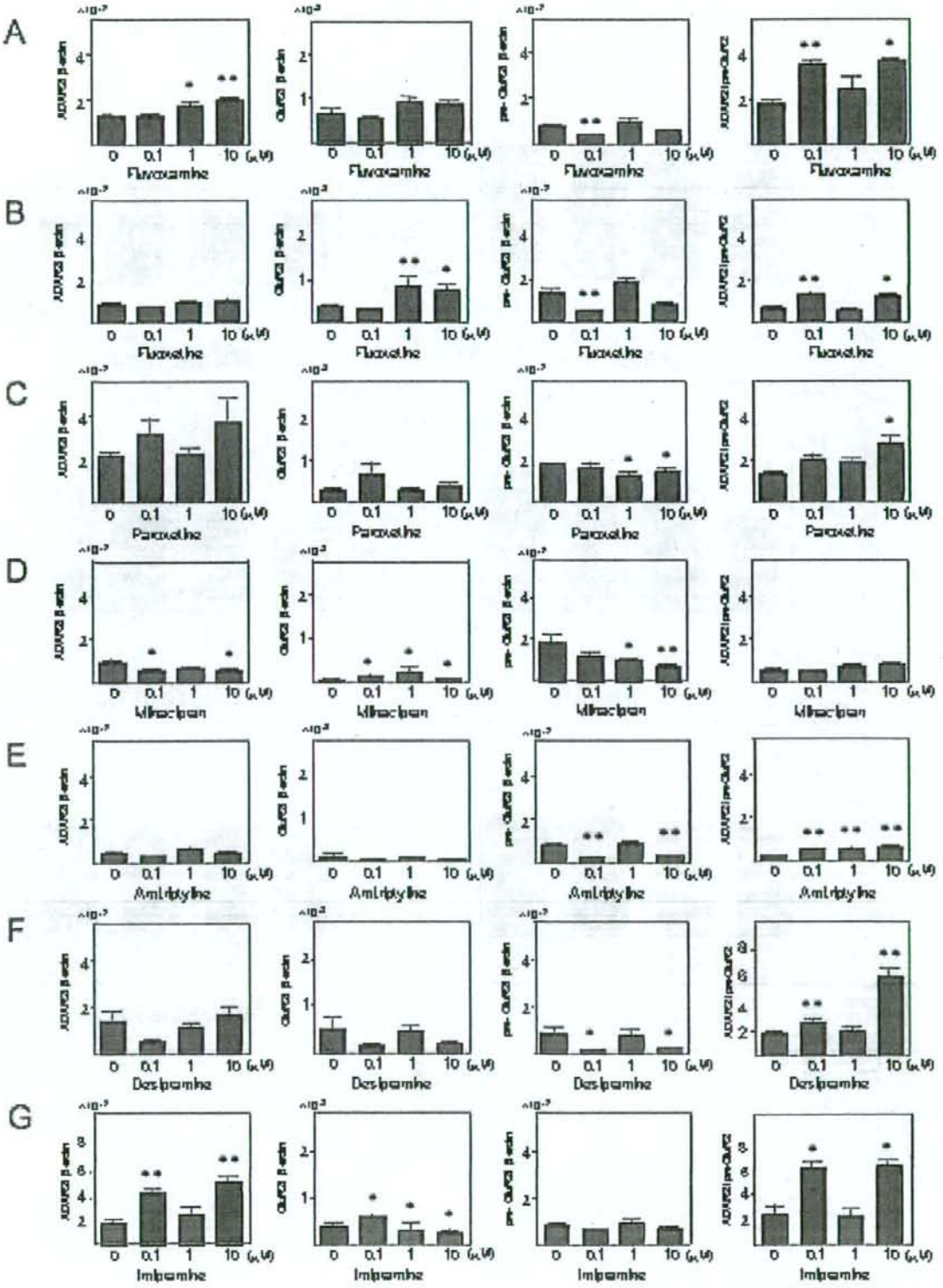

A
Fluvoxamine
B
Fluoxetine
C
Paroxetine
D
Milnacipran
E
Amitriptyline
F
Desipramine
G
Imipramine
H
Reboxetine
GluR2 editing efficiency (%)
100
75
50
25
0
0
0.1
1
10 (μM)
*
**
***

Figure 4


A
B
C
D
E
F
G
0 0.1 1 10 (μM)
Fluvoxamine
Fluoxetine
Paroxetine
Milnacipran
Amitriptyline
Desipramine
Imipramine