Single Nucleotide Polymorphism (−468 Gly to Ala) at the Promoter Region of Sterol Regulatory Element-binding Protein-1c Associates with Genetic Defect of Fructose-induced Hepatic Lipogenesis* 

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To evaluate the genetic susceptibility to metabolic disorders induced by high fructose diet, we investigated the metabolic characteristics in 10 strains of inbred mice and found that they were separated into CBA and DBA groups according to the response to high fructose diet. The hepatic mRNA expression of the sterol regulatory element-binding protein-1 (SREBP-1) in CBA/JN was remarkably enhanced by high fructose diet but not in DBA/2N. Similar results were observed in primary hepatocytes after exposure to fructose. The nucleotide sequence at −468 bp from the putative starting point of the SREBP-1c gene was adenine in the DBA group while it was guanine in the CBA group. In hepatocytes from CBA/JN, the activity of CBA-SREBP-1c promoter was significantly increased by 2.4- and 2.2-fold, in response to 30 mM fructose or 10 nM insulin, respectively, whereas the activity of DBA-SREBP-1c promoter responded to insulin but not to fructose. In hepatocytes from DBA/2N, both types of SREBP-1c promoter activities in response to insulin were attenuated. Furthermore, electrophoretic mobility shift assay revealed an unidentified nuclear protein bound to the oligonucleotides made from the region between −453 to −480 bp of the SREBP-1c promoter of CBA/JN but not to the probe from DBA/2N. Thus, in DBA/2N, the reduced mRNA expression of SREBP-1 after fructose refeeding appeared to associate with two independent mechanisms, 1) loss of binding of unidentified proteins to the region between −453 to −480 bp of the SREBP-1c promoter and 2) impaired insulin stimulation of SREBP-1c promoter activity.

The prevalence of type 2 diabetes has been rising over the last few decades (1). An important correlate of this alarming phenomenon is the recent increase in prevalence among the population of metabolic syndrome, which consists of several metabolic disorders including hyperlipidemia, visceral obesity, impaired glucose tolerance, and hyperinsulinemia (2, 3). Metabolic derangements in rats fed a high fructose diet. The hepatic mRNA expression of the sterol regulatory element-binding protein-1 (SREBP-1c) is influenced by high fructose diet but not in DBA/2N. Similar results were observed in primary hepatocytes after exposure to fructose. The nucleotide sequence at −468 bp from the putative starting point of the SREBP-1c gene was adenine in the DBA group while it was guanine in the CBA group. In hepatocytes from CBA/JN, the activity of CBA-SREBP-1c promoter was significantly increased by 2.4- and 2.2-fold, in response to 30 mM fructose or 10 nM insulin, respectively, whereas the activity of DBA-SREBP-1c promoter responded to insulin but not to fructose. In hepatocytes from DBA/2N, both types of SREBP-1c promoter activities in response to insulin were attenuated. Furthermore, electrophoretic mobility shift assay revealed an unidentified nuclear protein bound to the oligonucleotides made from the region between −453 to −480 bp of the SREBP-1c promoter of CBA/JN but not to the probe from DBA/2N. Thus, in DBA/2N, the reduced mRNA expression of SREBP-1 after fructose refeeding appeared to associate with two independent mechanisms, 1) loss of binding of unidentified proteins to the region between −453 to −480 bp of the SREBP-1c promoter and 2) impaired insulin stimulation of SREBP-1c promoter activity.

To investigate the genetic heterogeneity in the regulation of hepatic SREBP-1 and PPARα gene expressions after consumption of high fructose diet, we selected two inbred mouse strains, one of which is highly responsive to a high fructose diet. We found that there were marked differences in CBA/JN mice and DBA/2N mice between the hepatic mRNA expressions of SREBP-1 in response to a high fructose diet. We also observed a single nucleotide mutation in DBA/2N mice from guanine to adenine at −468 bp from the putative starting point of the SREBP-1c gene, which caused impaired activation of SREBP-1c gene transcription in response to fructose. These results indicate that the loss of nuclear protein binding to the specific site at the promoter region of SREBP-1c may cause impaired hepatic lipogenesis in mice.

EXPERIMENTAL PROCEDURES

Materials—All materials were of reagent grade and were purchased from Nacalai Tesque (Kyoto, Japan) or Sigma unless otherwise indicated.

Animals—Five-week-old male mice of CBA/JN, DBA/2N, C57BL/6N, C57BL/6J, C3H/HeJ, and DBA/2N strains were purchased fromCLEA Japan, Inc. (Tokyo, Japan). Mice of C57BL/6 strain were purchased from Charles River Japan (Kanagawa, Japan). Mice of C3H/He, C3H/HeJ, and BALB/c strains were purchased from SLC Japan, Inc. (Shi-...
Mouse Primary Hepatocytes—Mouse primary hepatocytes were isolated from the livers of male mice by perfusion in situ via the portal vein with 150 ml of Krebs-Ringer buffer followed by 100 ml of Krebs-Ringer buffer containing collagenase (Sigma–Aldrich). The cells were dispersed in an equal volume of ice-cold William’s E medium supplemented with 10% fetal calf serum, 1 nM insulin, 1 nM dexamethasone, and 100 μg/ml streptomycin. Two million liver cells were plated onto 6-well rat collagen I-coated dishes (Asahi Techno Glass, Chiba, Japan). After incubation for 2 h at 37 °C in 9% CO2, the cells were cultured with William’s E medium supplemented with 10% fetal calf serum, 1 μM insulin, 1 μM dexamethasone, 100 μg/ml streptomycin, and 100 μg/ml penicillin.

Northern Blot Analysis—Total hepatic RNA was isolated from the livers with TRIzol reagent (Invitrogen) after perfusion of ice-cold phosphate-buffered saline (−15) in situ via the portal vein. In cases of primary hepatocytes they were starved in William’s E medium supplemented with 10% fetal calf serum, 1 μM insulin, 1 μM dexamethasone, and 100 μg/ml streptomycin for 24 h before isolation.

Sequencing of SREBP-1c Promoter Region—DNA sequences were analyzed by the dideoxynucleotide chain termination method.

Characteristics of Experimental Animals—In 10 inbred mouse strains (BALB/c, C3H/He, C3H/HeJ, C3H/HeN, C57BL/6, C57BL/6N, C57BL/6J, DBA/2N) we investigated the responses of metabolic characteristics to the high fructose diet. The metabolic characteristics of the six rat representatives are shown in Table I. Compared with the control mice fed a normal laboratory chow, C3H/He showed significantly increased body weights after feeding of the high fructose diet for 8 weeks. The epididymal fat weights for all strains except the DBA/2N and DBA/2JN strains showed significant increase after the high fructose diet for 8 weeks compared with those of the control animals. The levels of blood glucose did not significantly differ in the control and the high fructose diet groups for any strains.

The mRNA Expression of SREBP-1 in the Liver—To explore the molecular mechanisms of the differences in response to the high fructose diet, we compared the hepatic mRNA expressions...
of SREBP-1, a key transcription factor regulating fatty acid synthesis in various strains of mice. As shown in Fig. 1, A and B, the hepatic mRNA expression of SREBP-1 in the CBA/JN mice was increased after feeding. In the control-postprandial group, it was stimulated by 3.9-fold (p < 0.001) as compared with that of the control-fasting group. The fructose-fasting group showed stimulation by 1.9-fold (p < 0.05) as compared with the control-fasting group. Furthermore, the fructose-postprandial group showed enhancement by 23-fold (p < 0.001) compared with the control-fasting group. Thus, the fructose diet stimulated the level of SREBP-1 mRNA expression by 11-fold (p < 0.001) compared with fructose-fasting levels and by 6-fold (p < 0.001) compared with the control-postprandial group.

Similar changes in the hepatic mRNA expression of SREBP-1 were observed in the C3H/He strain (Fig. 1, C and D). The hepatic mRNA expression of SREBP-1 in the C3H/He control-postprandial group was stimulated by 3.8-fold (p < 0.05) compared with that in the control-fasting group. The level in the fructose postprandial group was enhanced by 5.8-fold (p < 0.001) compared with the control-fasting group. As a result, a fructose diet stimulated the level of SREBP-1 mRNA expression by 3.5-fold (p < 0.001) compared with the fructose-fasting level and by 1.5-fold (p < 0.001) compared with the control-postprandial group. In addition, compared with that in the control-fasting group, the hepatic mRNA expression of SREBP-1 in the BALB/c strain was also significantly increased by 4.2-fold (p < 0.05) or by 6.1-fold (p < 0.001), respectively, after feeding the control or the high fructose diet.

On the other hand, the hepatic mRNA expressions of SREBP-1 in the DBA/2N mice (Fig. 1, E and F) and the DBA/1JN mice (Fig. 1, G and H) were not affected by either the control or the high fructose diet. The hepatic mRNA expression of SREBP-1 in the C57BL/6N mice was increased significantly but to a lesser degree, i.e. the control postprandial group showed a 2.1-fold (p < 0.05) increase, and the fructose-postprandial group showed a 2.5-fold (p < 0.01) increase compared with the control fasting group, respectively. However, hepatic SREBP-1 mRNA expressions in either fasting or postprandial state were not different between the control and the high fructose diet groups.

The Hepatic mRNA Expression of Fatty Acid Synthase (FAS)—To investigate the effect of increased hepatic SREBP-1 expression on the downstream activation of the enzyme, we examined the mRNA expression of hepatic FAS, one of the target genes of SREBP-1, by Northern blot analysis. The hepatic mRNA expression of FAS from postprandial CBA/JN mice fed the high fructose diet was increased by 6-fold (p < 0.001) over that of the postprandial mice fed the control diet and by 4-fold (p < 0.001) when compared with that of the fructose-fasting mice (Fig. 2, A and B). In contrast, the hepatic mRNA expression of FAS in the DBA/2N mice was not significantly affected by either control diet or high fructose diet (Fig. 2, C and D).

The mRNA Expression of PPARα in the Livers from the CBA/JN and the DBA/2N Mice—As shown in Fig. 3, A and B, the mRNA expression of PPARα, a key transcription factor for fatty acid oxidation, in the livers from the CBA/JN mice was decreased after intake of either the control (p < 0.01) or the high fructose (p < 0.01) diet. Similar significant reductions of the hepatic mRNA expression of PPARα were also found in DBA/2N mice after intake of either diet (Fig. 3, C and D).

The mRNA Expression of SREBP-1 in Primary Cultured Hepatocytes—To study the direct effect of fructose on the expression of SREBP-1 in the liver, we examined the mRNA contents of SREBP-1 in primary cultured hepatocytes isolated from the CBA/JN (Fig. 4A) or the DBA/2N (Fig. 4B) mice in the presence or absence of 30 mM fructose. In this experiment we used cells cultured with 30 mM mannitol as a control for fructose-treated or insulin-treated cells, since 30 mM mannitol did not affect the mRNA expression of SREBP-1 in either CBA/JN or DBA/2N primary hepatocytes; the values for hepatocytes cultured with 5 mM glucose and with 30 mM mannitol were
As shown in Fig. 4A, compared with the controls, the primary cultured hepatocytes from the CBA/JN mice showed a 73% (\(p < 0.01\)) increase in the mRNA expression of SREBP-1 in the presence of 30 mM fructose in the media and 92% (\(p < 0.01\)) increase in the presence of 100 nM insulin. In contrast, as shown in Fig. 4B, the incubation with 30 mM fructose did not induce the mRNA expression of SREBP-1 in the primary hepatocytes isolated from the DBA/2N mice. However, 100 nM insulin significantly increased the expression of SREBP-1 mRNA by 24% (\(p < 0.01\)) in these hepatocytes. Thus, the effect of insulin on the SREBP-1 mRNA expression in hepatocytes from DBA/2N mice was significantly blunted compared with that on the hepatocytes from CBA/JN mice (\(p < 0.01\)).
The mRNA Expression of FAS in Primary Cultured Hepatocytes—The mRNA expressions of FAS in primary cultured hepatocytes from both DBA/2N and CBA/JN mice were studied in the presence or absence of 30 mM fructose. In this experiment we used cells cultured with 30 mM mannitol as the control for fructose-treated or insulin-treated cells, since the 30 mM mannitol did not affect the mRNA expression of FAS in either CBA/JN and DBA/2N primary hepatocytes; in the presence of 5...
mm glucose or 30 mm mannitol the expression was 1.0 ± 0.01 and 1.0 ± 0.1 for hepatocyte from CBA/JN (arbitrary units, n = 8, NS) and 1.0 ± 0.1 and 1.0 ± 0.2 for hepatocytes from DBA/2N (arbitrary units, n = 8, NS). Comparable with the results from the experiment on the mRNA expression of SREBP-1, both 30 mm fructose and 100 nM insulin significantly increased (p < 0.01) the mRNA expression of FAS in the hepatocytes from CBA/JN mice, by 100 and 96%, respectively (Fig. 5A). On the
other hand, the mRNA expression of FAS in primary hepatocytes from DBA/2N mice was not affected by 30 mM fructose (Fig. 5B). However, a 32% (p<0.01) increase in mRNA expression of FAS was induced by 100 nM insulin; the effect of insulin on the FAS mRNA expression was reduced significantly (p<0.01) compared with that on the hepatocytes from CBA/J mice.

**Single Nucleotide Polymorphism in SREBP-1c Promoter Region**—We have cloned a 1.2-kilobase pair fragment of the 5′-upstream region of SREBP-1c gene from each inbred mouse strain and found a single nucleotide polymorphism at −468 bp from the putative starting point of the SREBP-1c gene. The nucleotide at −468 bp in the C3H/HeN, C3H/He, C3H/HeJ, BALB/c, and CBA/J mice is guanine, whereas it is adenine in the C57BL/6, C57BL/6J, C57BL/6N, DBA/1JN, and DBA/2N strains.

**Luciferase Activities of SREBP-1c Promoters**—To investigate the significance of the single nucleotide mutation from guanine to adenine at −468 bp of the SREBP-1c promoter, we analyzed the promoter activity using the luciferase reporter carrying the 1.2-kilobase pair SREBP-1c promoter region of the CBA/JN or DBA/2N mice with 5 individual experiments in a total of 12 determinations. The activity of SREBP-1c promoter in primary hepatocytes isolated from CBA/JN mice was significantly increased by 2.4-fold (p<0.01) by exposure of the cells to 30 mM fructose (Fig. 6A), but no increase was observed in primary hepatocytes isolated from the DBA/2N mice (Fig. 6B). The activity of the SREBP-1c promoter from DBA/2N mice was not
induced by 30 mM fructose in primary hepatocytes isolated from either the CBA/JN or the DBA/2N mice. We also investigated the effect of insulin with 5 individual experiments in a total of 12 determinations. As shown in Fig. 6C, the activity of SREBP-1c promoter from either the DBA/2N or CBA/JN strain in hepatocytes isolated from the CBA/JN mice was significantly increased by 2.4-fold (p < 0.01) or by 2.1-fold (p < 0.01), respectively, on the exposure of the cells to 10 nM insulin. These results indicate that a −468 Gly to Ala single nucleotide polymorphism did not affect the effect of insulin to SREBP-1c promoter. The activity of SREBP-1c promoter from either DBA/2N or CBA/JN strain in primary hepatocytes isolated from DBA/2N mice was increased only by 1.5-fold (p < 0.01) or by 1.4-fold (p < 0.01), respectively, with the exposure of the cells to 10 nM insulin. Thus, the effect of 10 nM insulin on the activity of SREBP-1c promoter from either DBA/2N or CBA/JN strain in hepatocytes isolated from DBA/2N mice compared with those in the cells from the CBA/JN mice.

The Effect of Insulin on Akt Phosphorylation and PPARα mRNA Expression in Primary Cultured Hepatocytes—Because the effect of insulin on the SREBP-1c promoter activities of both CBA/JN and DBA/2N in hepatocytes isolated from DBA/2N was reduced, we analyzed the level of phosphorylation of Akt to compare the differences of insulin signal transduction. As shown in Fig. 7A, 100 nM insulin stimulated the level of phosphorylation of Akt in both DBA/2N and CBA/JN primary hepatocytes to the same degree. We also examine the mRNA expression of PPARα, known as an insulin-regulated gene (12). Insulin decreased the level of mRNA expression on both DBA/2N and CBA/JN primary hepatocytes (Fig. 7B) equally. The values in medium without or with insulin were 1.0 ± 0.1 and 0.3 ± 0.2 (p < 0.001, n = 7) for hepatocytes from CBA/JN mice and 1.2 ± 0.2 and 0.3–0.1 (p < 0.001, n = 7) for hepatocytes from DBA/2N mice. There was no significant difference between the strains.

EMSA of Nuclear Protein Binding to the Region at the Single Nucleotide Polymorphism—As shown in Fig. 8, A and B, EMSA of nuclear protein extracted from the livers of the CBA/JN mice revealed a specific band recognizing the oligonucleotides from −453 to −480 bp of the upstream region of SREBP-1c gene of the CBA/JN mice. In contrast, we could not detect this band clearly when we used the oligonucleotides from −453 to −480 bp of the upstream region of the SREBP-1c gene of DBA/2N mice as a probe (Fig. 8B). The band was hardly detectable with an EMSA of the nuclear protein extracted from the livers of DBA/2N mice using either CBA/JN or DBA/2N probe (Fig. 8, A and B). As shown in Fig. 8C, this band disappeared with the addition of an excess of unlabeled CBA/JN probe in dose-dependent manner. DBA/2N probe also competed with CBA/JN probe but less effectively. To assess the loading difference of the amount of the nuclear protein samples, we compared the content of OCT-1, a ubiquitously expressed transcriptional factor, between the samples from CBA/JN and DBA/2N. As shown in Fig. 8D, the binding activity of the hepatic nuclear protein isolated from DBA/2N mice to the oligonucleotides containing the consensus sequence of OCT-1 did not differ from that from CBA/JN mice.

Because the nucleotide sequences of the CBA/JN probe contain sequences similar to that of AP4, 5′-CAGCTG-3′, we examined whether or not the band detected with CBA/JN oligonucleotides was AP4. As shown in Fig. 8A, the band detected with CBA/JN probe was not competed by unlabeled AP4 consensus oligonucleotides or by antibody against AP4. Moreover, no band was detected when we used AP4 consensus oligonucleotides as the probe. In contrast, using a nuclear protein sample from HeLa cells we could detect the band with the AP4 consensus nucleotide as shown in Fig. 9B.
DISCUSSION

In the present study we found that the CBA/JN, C3H/HeN, C3H/He, C3H/HeJ, and BALB/c strains showed characteristics of metabolic syndrome, such as accumulation of visceral fat, postprandial hypertriglyceridemia, and hyperinsulinemia in response to the high fructose diet, whereas the DBA/2N, DBA/
1JN, C57BL/6, C57BL/6J, and C57BL/6N strains showed a lesser or no expression of such metabolic derangements. Both C3H and CBA strains are developed from a cross between a Bagg albino female and a DBA male, so they are rather closely related. BALB/c is established from a Bagg albino. The DBA/1 and DBA/2 strains are established from DBA, but they are regarded as different strains. The C57BL/6 strains have a less direct relationship compared with other strains (Mouse Genome Informatics, www.informatics.jax.org).

We previously reported that the high fructose diet induced metabolic disorders as well as overexpression of SREBP-1 mRNA and suppression of PPARα mRNA expression in the livers of Sprague-Dawley rats (7). We also found that the mRNA expression of SREBP-1 was greatly increased in the livers of the CBA/JN and C3H/He mice after feeding the high fructose diet but not in the livers of the DBA/2N and the DBA/1JN mice. The difference in SREBP-1 expressions induced by the high fructose diet in these mice was also suggested by the alteration in the mRNA expression of FAS, a target gene of transcriptional regulation by SREBP-1 (13), although the actual change in FAS mRNA seen at 4 h after feeding may be due to a separate mechanism because SREBP-1 mRNA but not protein may be elevated at this time point. Interestingly, inhibition of the hepatic mRNA expression of PPARα was observed in both the CBA/JN and DBA/2N mice fed the high fructose diet. These results indicate that the difference in postprandial serum triglyceride levels in the CBA/JN and DBA/2N mice is at least to some extent explained by the difference in the responses to fructose or insulin. Comparable results were also obtained with the primary cultured hepatocytes, isolated from the CBA/JN and DBA/2N strains, and exposed to the high fructose media or insulin. The mRNA expression of SREBP-1 in primary hepatocytes from DBA/2N mice showed the absence or reduced response to the high fructose medium or insulin, respectively. Those results indicate that the difference in the SREBP-1c mRNA expression between the CBA/JN and DBA/2N mice is at least to some extent explained by the difference in the responses to fructose or insulin.

It has been shown that the promoter activity of SREBP-1c is regulated by SREBP-1 itself (22) or the liver X receptor through a cluster of putative binding sites for several transcription factors (NF-Y, SRE, E-box, and Sp1 sites) and liver X receptor element binding sites (23). However, the mechanisms of the stimulation of SREBP-1c promoter activity by fructose or glucose are not fully under-
SNP in SREBP-1c Promoter in Mice

program (www.genomatix.de/cgi-bin/matinspector/matinspec-

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Temporal and spatial modulation of Rho GTPases during in vitro formation of capillary vascular network. Adherens junctions and myosin light chain as targets of Rac1 and RhoA.

Ilaria Cascone, Enrico Giraudo, Francesca Caccavari, Lucia Napione, Elisa Bertotti, John G. Collard, Guido Serini, and Federico Bussolino

Page 50711: Fig. 9D was inadvertently omitted. The figure and legend are shown on facing page.
FIG. 9. Effect of the expression of N17Rac1 on the dynamic of cell-to-cell adherens junctions. A, capillary ECs carrying vector alone or N17Rac1 were plated on Matrigel and dispersed with MatrIverse cell recovery solution at 30 min or at 2 h from the beginning of the morphogenetic process, lysed, and immunoprecipitated (IP) with mAb anti-β-catenin. Proteins were separated by SDS-PAGE (8%) and then immunoblotted with Abs anti-IQGAP-1 and anti-α-catenin and mAbs anti-Ve-cadherin and anti-β-catenin. The total amounts in vector-ECs of the indicated proteins are shown in the 1st lane. Equal results have been obtained in N17Rac1-ECs (not shown). B, ECs carrying vector alone or N17Rac1 were recovered as indicated above and lysed. After centrifugation, Triton X-100-insoluble pellets were solubilized in SDS buffer detailed under “Experimental Procedures” and boiled for 5 min. Solubilized proteins were separated on SDS-PAGE 8%) and immunoblotted with mAbs anti-Ve-cadherin or anti-β-catenin. Densitometric analysis of three independent experiments is shown as mean ± S.D. C, ECs carrying N17Rac1 were plated on Matrigel and after 2 h fixed and stained with TRICT-phalloidin (a). b shows the GFP expression in one of the two cells recorded in a. The negative GFP cell, which does not express N17Rac1, shows actin cable near and perpendicular to the cell protrusion forming the contact with a GFP-, N17Rac1-expressing cell. In this cell, F-actin is absent at the protrusion cone. Four experiments with similar results were performed. D, confluent cultured ECs carrying vector alone (a) or N17Rac1 (b) were treated with 0.01% trypsin in Hepes-buffered (TC treatment) or in Hepes-buffered saline without Ca²⁺ supplemented with 1 mM EDTA (TE treatment) for 15 min at 37 °C and dissociated through 10 times pipetting. The extent of dissociation cells was represented by the index $N_{TC}/N_{TE}$, where $N_{TC}$ and $N_{TE}$ are the number of clusters and single cells, respectively. This figure is representative of three experiments obtained with similar results.
Single nucleotide polymorphism (−468 Gly to Ala) at the promoter region of sterol regulatory element-binding protein-1c associates with genetic defect of fructose-induced hepatic lipogenesis.

Ryoko Nagata, Yoshihiko Nishio, Osamu Sekine, Yoshio Nagai, Yasuhiro Maeno, Satoshi Ugi, Hiroshi Maegawa, and Atsunori Kashiwagi

The title was incorrect. The correct title should be:

Single nucleotide polymorphism (−468 G to A) at the promoter region of SREBP-1c associates with genetic defect of fructose-induced hepatic lipogenesis

SREBP-1c mediates the insulin-dependent hepatic glucokinase expression.

So-Youn Kim, Ha-il Kim, Tae-Hyun Kim, Seung-Soon Im, Sang-Kyu Park, In-Kyu Lee, Kyung-Sup Kim, and Yong-Ho Ahn

Page 30827, Fig. 4B: In Fig. 4B, the sequence of oligonucleotide P-225/-150 is shown in the top line, and SREa and SREb are marked by underlines. The lower three lines are mutated oligonucleotides. There is a mistake in the second line, which indicates the oligonucleotide-containing mutation of SREa. The sequence of P-225/-150ma is equal to P-225/-150mb. “TT” of the SREb position should be changed by – –. The corrected figure is shown below.

```
|       | SREa | SREb   |
|-------|------|--------|
| P-225/-150-210 |      |        |
| P-225/-150ma-225 | GAAGGCTGGGGTGGGAGTGCCCAGGTCCAACCAT | --150 |
| P-225/-150mb-225 |           | TT--150 |
| P-225/-150mb-225 |           | TT--150 |
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