

種の遺伝子の発現変動が認められたが、授乳後 1 年目の仔マウス脳中では変動する遺伝子が認められなかった。従って、胎生期のメチル水銀曝露による遺伝子発現の変動は新生仔時期に起こり、1 年後には正常値に回復することが示唆された。さらに、メチル水銀と水銀蒸気の複合曝露によって新生仔脳中で発現が変動した遺伝子は、野生型マウスおよびメタロチオネイン-I/II 欠損マウスともにそれぞれ異なった遺伝子が 1 種ずつであった。しかも、これらの遺伝子は、メチル水銀単独曝露によって発現が変動した遺伝子とは一致しなかった。従って、胎生期のメチル水銀曝露による新生仔脳での遺伝子発現の変動は、水銀蒸気の複合曝露によって修飾される可能性が示された。

【結論】

DNA マイクロアレイ法およびリアルタイム RT-PCR 法の解析により、カドミウムあるいはメチル水銀の胎生期曝露によって新生仔脳で Trfr (カドミウム曝露で変動)あるいは TIMP4 (メチル水銀曝露で変動)の発現がそれぞれ促進されることが明らかとなった。

【研究発表】

- 1) 本田晶子, 長谷川達也, 瀬子義幸, 鈴木純子, 遠山千春, 佐藤雅彦, 永瀬久光. カドミウム曝露による母体および胎仔へのカドミウム蓄積に及ぼすメタロチオネインの影響. フォーラム 2004: 衛生薬学・環境トキシコロジー (2004 年 10 月 25-26 日, 千葉市).
- 2) 佐藤雅彦, 本田晶子, 吉田 稔, 渡辺知保. 周生期メチル水銀およびカドミウム投与マウス脳における遺伝子発現: マイクロアレイおよび real time RT-PCR による検討. 第 75 回日本衛生学会総会 (2005 年 3 月 27-30 日, 新潟市).
- 3) 本田晶子, 大西沙樹, 長谷川達也, 瀬子義幸, 鈴木純子, 遠山千春, 佐藤雅彦, 永瀬久光. 胎盤中カドミウムの存在状態におよぼすメタロチオネインの関与. 日本薬学会第 125 年会 (2005 年 3 月 29-31 日, 東京).

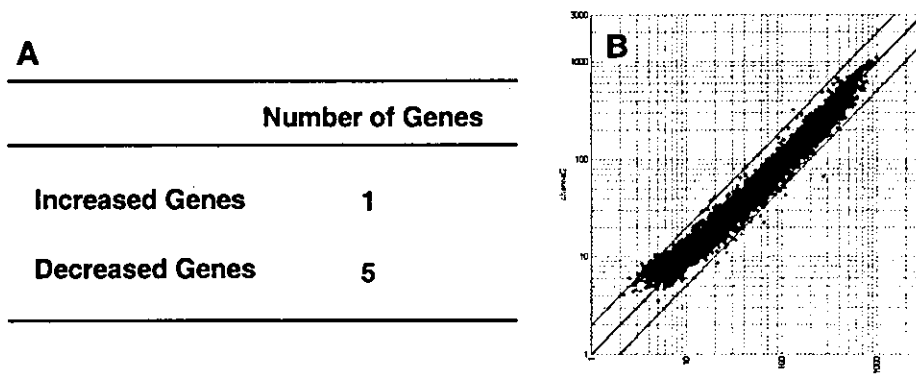


Fig.1 Gene expression altered by MT-1/1 null mutation in neonatal brain of untreated mice from microarray data
A : Number of genes altered by MT-1/1 null mutation in untreated mice.
B : Scatter plot of control group (Cy3) of wild-type mice vs control group (Cy5) of MT-1/1 null mice fluorescence intensity signal for each gene.
 X-axis : Control group (Cy3) of wild-type mice, Y-axis : Control group (Cy5) MT-1/1 null mice.

Table 1 Altered gene expression from microarray data of control group (Cy3) of wild-type mice vs control group (Cy5) of MT-1/1 null mice hybridization

Gene Description	Ratio
<u>MT-1/1 null (Control) / Wild-type (Control) > 2</u>	
Def8 [expressed sequence ai449518]	2.4
<u>MT-1/1 null (Control) / Wild-type (Control) < 0.5</u>	
unknown (cDNA sequence AF233884)	0.24
hypothetical protein (RIKEN cDNA 1700019B01 gene)	0.39
hypothetical protein (RIKEN cDNA 9130008F23 gene)	0.47
Bax [bcl2-associated x protein]	0.48
Plcb3 [phospholipase c, beta 3]	0.49

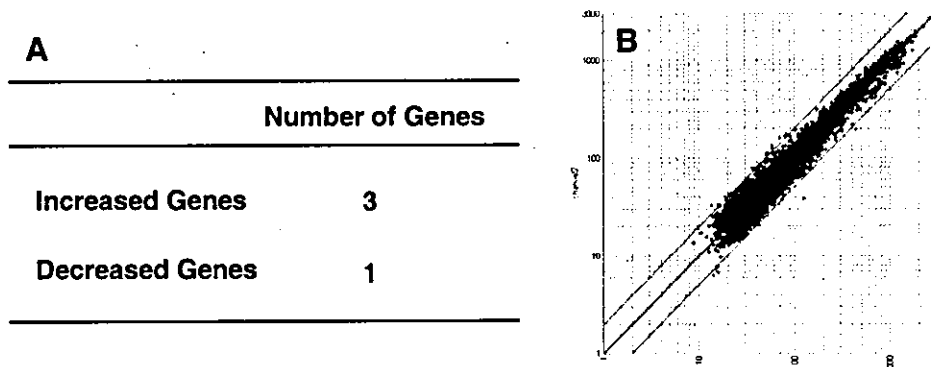


Fig. 2 Gene expression altered by cadmium exposure in neonatal brain of wild-type mice from microarray data
A : Number of genes altered by cadmium exposure in wild-type mice.
B : Scatter plot of control group (Cy3) of wild-type mice vs Cd-exposure group (Cy5) of wild-type mice fluorescence intensity signal for each gene.
 X-axis : Control group (Cy3) of wild-type mice, Y-axis : Cd-exposure group (Cy5) of wild-type mice.

Table 2 Altered gene expression from microarray data of control group (Cy3) of wild-type mice vs Cd-exposure group (Cy5) of wild-type mice hybridization

Gene Description	Ratio
<u>Wild-type (Cd-exposure) / Wild-type (Control) > 2</u>	
Trfr [transferrin receptor]	2.3
Itgb4 [integrin beta 4 subunit]	2.1
Sema5a [sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (tm) and short cytoplasmic domain]	2.1
<u>Wild-type (Cd-exposure) / Wild-type (Control) < 0.5</u>	
Sep15 [15-kda selenoprotein]	0.49

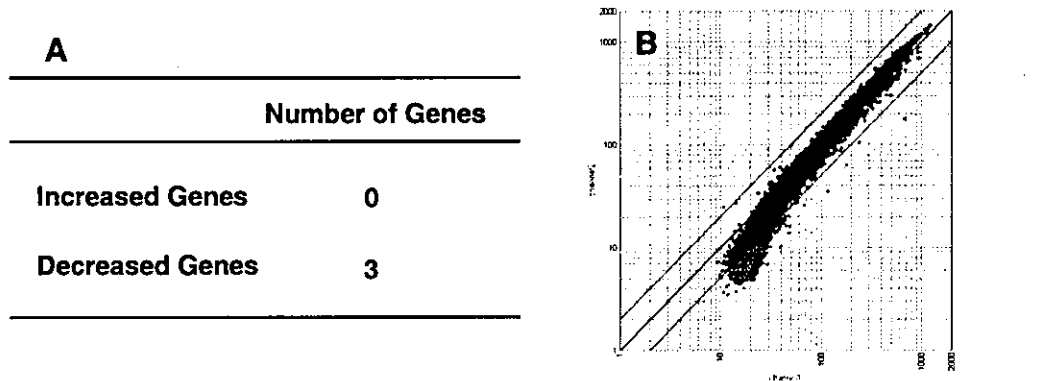


Fig. 3 Gene expression altered by cadmium exposure in neonatal brain of MT-1/1 null mice from microarray data
A : Number of genes altered by cadmium exposure in MT-1/1 null mice.
B : Scatter plot of control group (Cy3) of MT-1/1 null mice vs Cd-exposure group (Cy5) of MT-1/1 null mice fluorescence intensity signal for each gene.
 X-axis : Control group (Cy3) of MT-1/1 null mice, Y-axis : Cd-exposure group (Cy5) of MT-1/1 null mice.

Table 3 Altered gene expression from microarray data of control group (Cy3) of MT-1/1 null mice vs Cd-exposure group (Cy5) of MT-1/1 null mice hybridization

Gene Description	Ratio
<u>MT-1/1 null (Cd) / MT-1/1 null (Control) > 2</u>	
None	
<u>MT-1/1 null (Cd) / MT-1/1 null (Control) < 0.5</u>	
Mmp24 [matrix metalloproteinase 24]	0.43
Akt1s1 [matrix protein (ma), p15 containing protein data source:pfam, source key:pf01140, evidence:iss putative]	0.26
Def8 [expressed sequence ai449518]	0.34

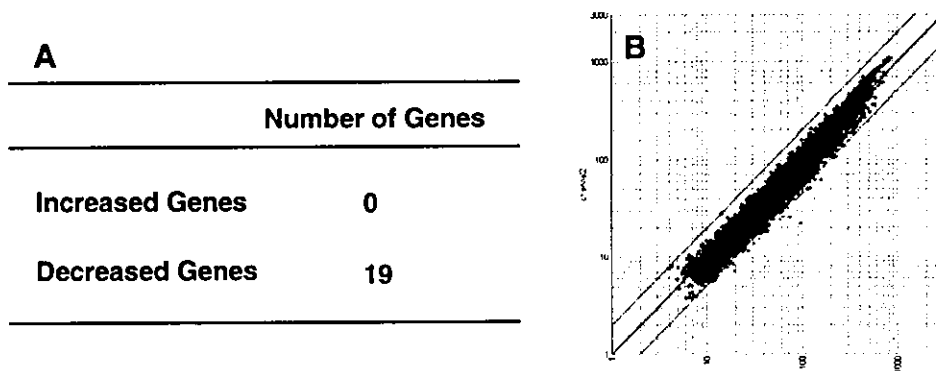


Fig. 4 Gene expression altered by MT-1/1 null mutation in neonatal brain of cadmium-exposed mice from microarray data
A : Number of genes altered by MT-1/1 null mutation in cadmium-exposed mice.
B : Scatter plot of control group (Cy3) of MT-1/1 null mice vs Cd-exposure group (Cy5) of MT-1/1 null mice fluorescence intensity signal for each gene.

X-axis : Cd-exposure group (Cy3) of wild-type mice, Y-axis : Cd-exposure group (Cy5) of MT-1/1 null mice.

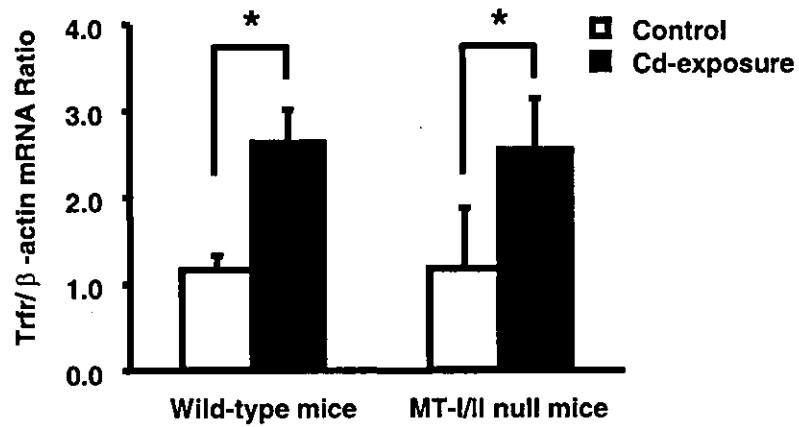
Table 4-1 Altered gene expression from microarray data of Cd-exposure group (Cy3) of wild-type mice vs Cd-exposure group (Cy5) of MT-1/1 null mice hybridization

Gene Description	Ratio
<u>MT-1/1 null (Cd) / Wild-type (Cd) > 2</u>	
None	
<u>MT-1/1 null (Cd) / Wild-type (Cd) < 0.5</u>	
Hmg20b [high mobility group protein 20 b]	0.23
Nfat5 [nuclear factor of activated t-cells 5]	0.39
Zfp346 [zinc finger protein 346]	0.42
hypothetical protein (RIKEN cDNA 1700019B01)	0.42
Ndr2 [n-myc downstream regulated 2]	0.44
related to o-linked glcnac transferase	0.45
hypothetical protein (RIKEN cDNA 4933437N03)	0.45

Table 4-2 Altered gene expression from microarray data of Cd-exposure group (Cy3) of wild-type mice vs Cd-exposure group (Cy5) of MT-1/1 null mice hybridization (Continued)

Gene Description	Ratio
MT-1/1 null (Cd) / Wild-type (Cd) < 0.5	
homolog to npd012	0.45
Skat2 [zinc finger protein skat2]	0.46
unclassifiable transcript	0.47
Arhgap6 [rho gtpase activating protein 6]	0.48
riken cdna 2310042n02	0.48
hypothetical protein (RIKEN cDNA C030011J08)	0.49
unclassifiable (RIKEN cDNA 2310007L24 gene)	0.49
Ptgfr [prostaglandin f receptor]	0.49
Htr5b [5-hydroxytryptamine (serotonin) receptor 5b]	0.49
hypothetical protein (RIKEN cDNA 2700046G09)	0.49
Dctn5 [dynactin 4]	0.50
hypothetical protein (RIKEN cDNA 2310079G19)	0.50
riken cdna 2310033p09	0.50

Trfr



Itgb4

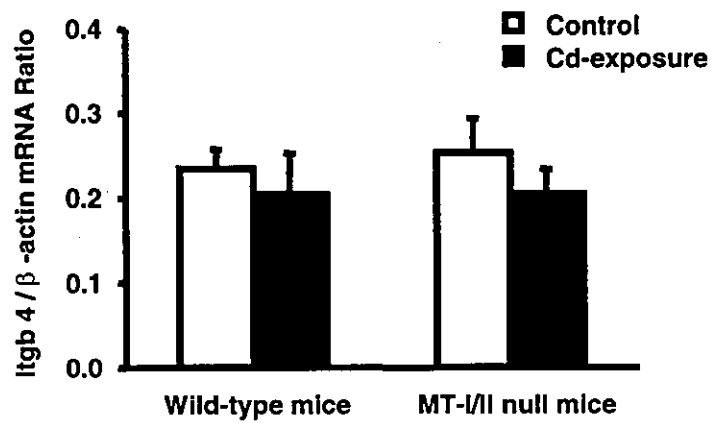
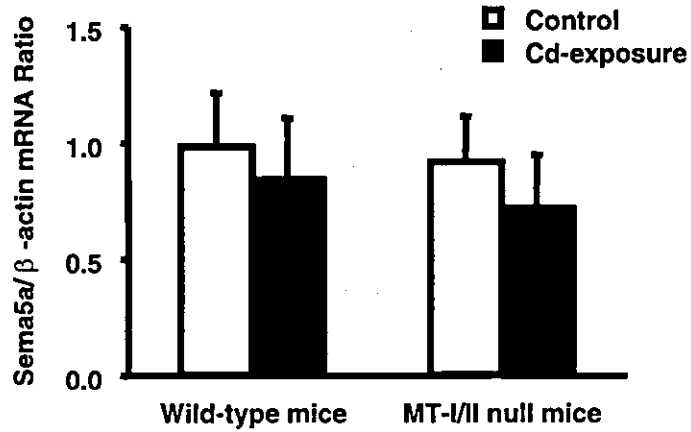


Fig. 5 Expression of Trfr [transferrin receptor] and Itgb4 [integrin beta 4 subunit] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to cadmium during gestation and lactation

Real-time RT-PCR analysis was performed on Trfr and Itgb4 genes. * P<0.05.

Sema5a



Sep15

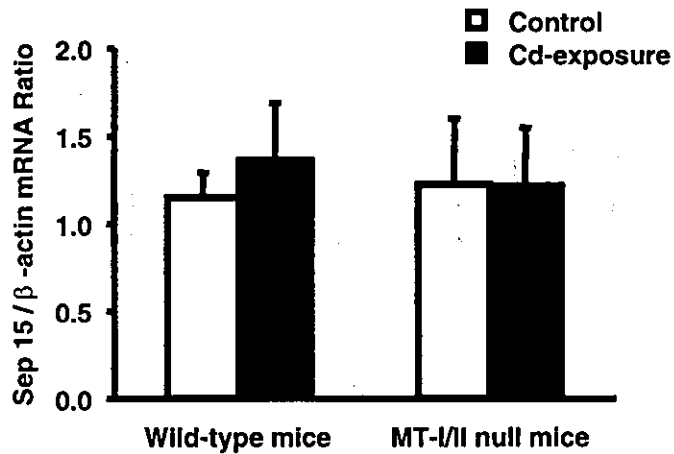
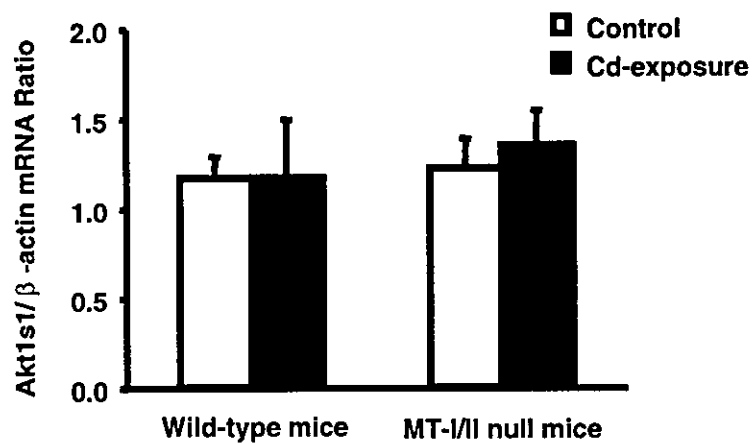


Fig. 6 Expression of Sema5a [sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (tm) and short cytoplasmic domain] and Sep15 [15-kda selenoprotein] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to cadmium during gestation and lactation

Real-time RT-PCR analysis was performed on Sema5a and Sep15 genes.

Akt1s1



Def8

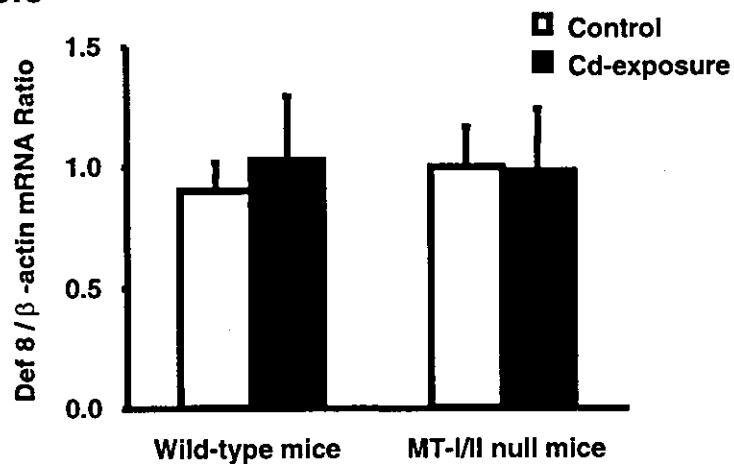
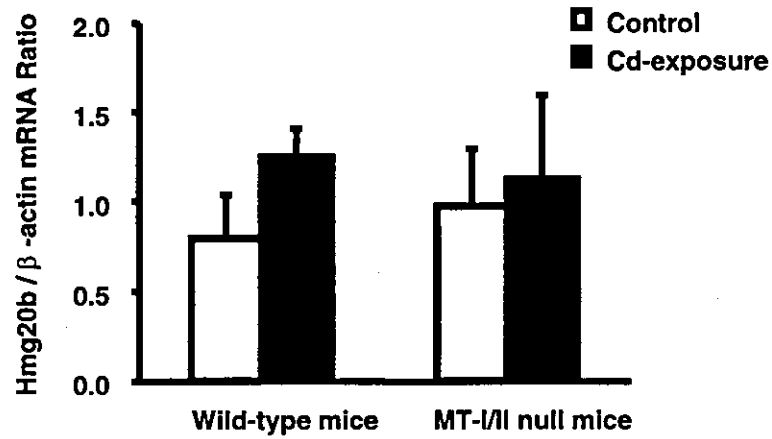


Fig. 7 Expression of Akt1s1 [matrix protein (ma), p15 containing protein data source:pfam, source key:pf01140, evidence:iss putative] and Def8 [expressed sequence al449518] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to cadmium during gestation and lactation

Real-time RT-PCR analysis was performed on Akt1s1 and Def8 genes.

Hmg20b



Nfat5

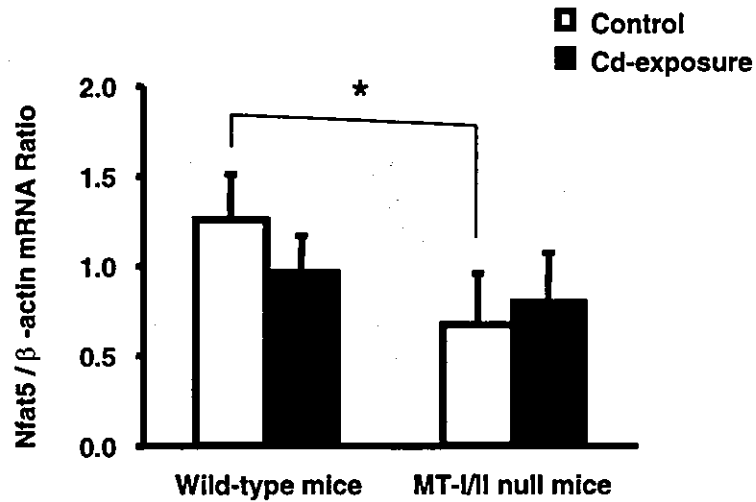


Fig. 8 Expression of Hmg20b [high mobility group protein 20 b] and Nfat5 [nuclear factor of activated t-cells 5] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to cadmium during gestation and lactation

Real-time RT-PCR analysis was performed on Hmg20b and Nfat5 genes. *P<0.05.

Htr5b

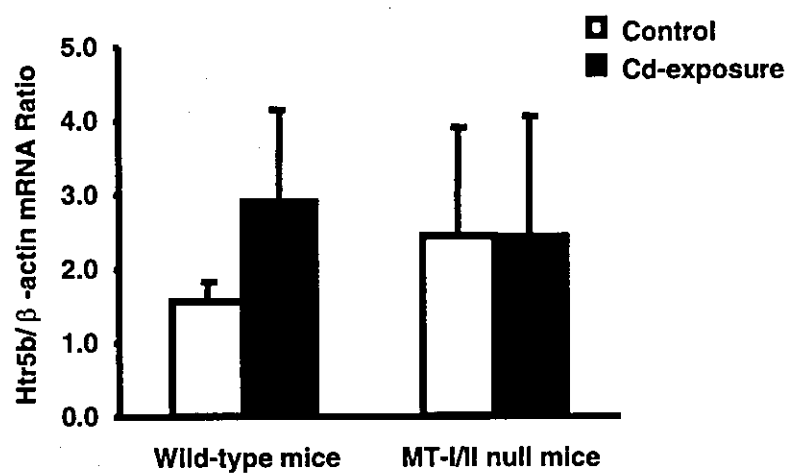


Fig. 9 Expression of Htr5b [5-hydroxytryptamine (serotonin) receptor 5b] gene in neonatal brain of wild-type mice and MT-1/1 null mice exposed to cadmium during gestation and lactation

Real-time RT-PCR analysis was performed on Htr5b gene.

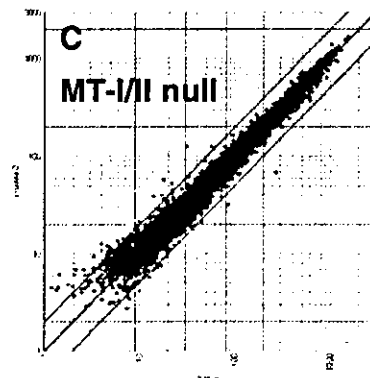
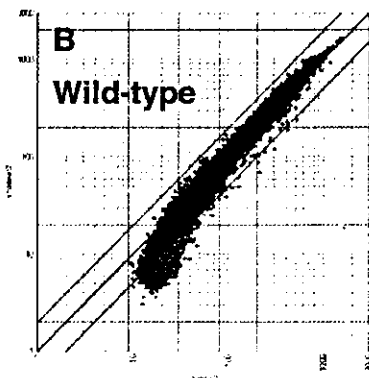
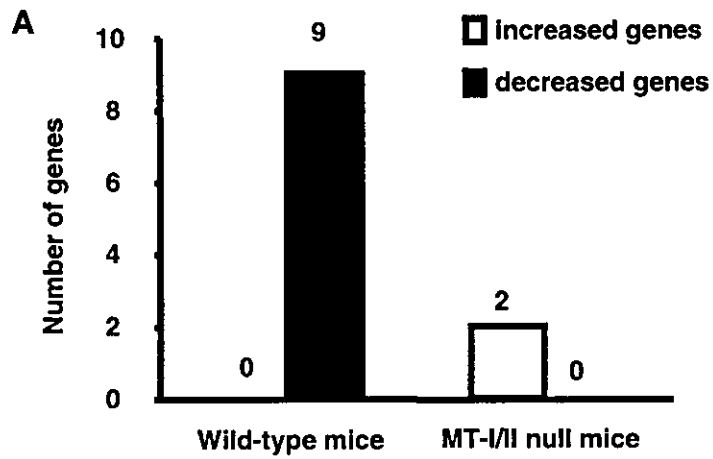


Fig. 10 Gene expression altered by methyl mercury exposure in neonatal brain of wild-type mice and MT-1/1 null mice from microarray data
A : Number of genes altered by methyl mercury exposure in wild-type mice and MT-1/1 null mice.

B : Scatter plot of control group (Cy3) of wild-type mice vs MeHg-exposure group (Cy5) of wild-type mice fluorescence intensity signal for each gene.
C : Scatter plot of control group (Cy3) of MT-1/1 null mice vs MeHg-exposure group (Cy5) of MT-1/1 null mice fluorescence intensity signal for each gene.

X-axis : Control group (Cy3), Y-axis : MeHg-exposure group (Cy5).

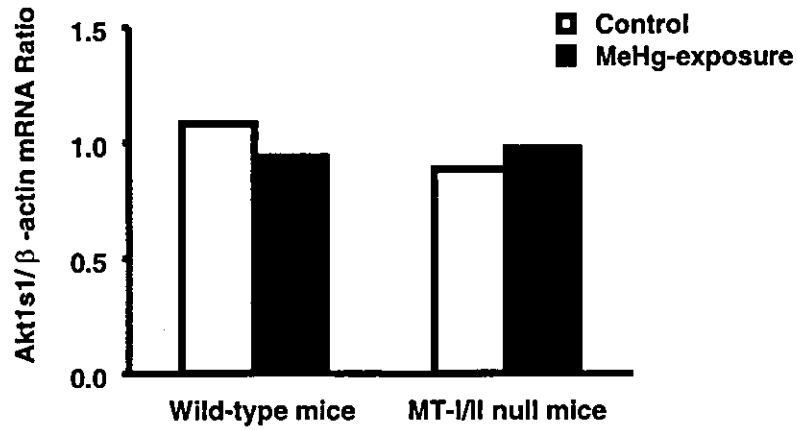
Table 5 Altered gene expression from microarray data of control group (Cy3) of wild-type mice vs MeHg-exposure group (Cy5) of wild-type mice hybridization

Gene Description	Ratio
<u>Wild-type (MeHg-exposure) / Wild-type (Control) > 2</u>	
None	
<u>Wild-type (MeHg-exposure) / Wild-type (Control) < 0.5</u>	
RPL32 [ribosomal protein l32]	0.38
Akt1s1 [matrix protein (ma), p15 containing protein data source:pfam, source key:pf01140, evidence:iss putative]	0.39
RPS12 [ribosomal protein s12]	0.43
RPS20 [ribosomal protein s20]	0.44
PLP [6.8 kda mitochondrial proteolipid]	0.45
RPL38 [ribosomal protein L38]	0.46
Nme2 [expressed in non-metastatic cells 2, protein (nm23b)]	0.47
RPS29 [ribosomal protein s29]	0.48
Cox7b [cytochrome c oxidase subunit viib]	0.50

Table 6 Altered gene expression from microarray data of control group (Cy3) of MT-1/1 null mice vs MeHg-exposure group (Cy5) of MT-1/1 null mice hybridization

Gene Description	Ratio
<u>MT-1/1 null (MeHg-exposure) / MT-1/1 null (Control) > 2</u>	
Xist [nuclear-localized inactive x-specific transcript]	4.0
TIMP4 [tissue inhibitor of metalloproteinase 4]	2.2
<u>MT-1/1 null (MeHg-exposure) / MT-1/1 null (Control) < 0.5</u>	
None	

Akt1s1



RPS12

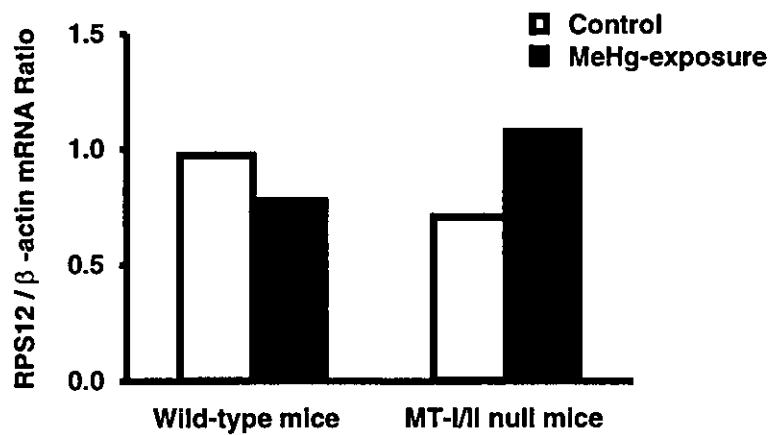
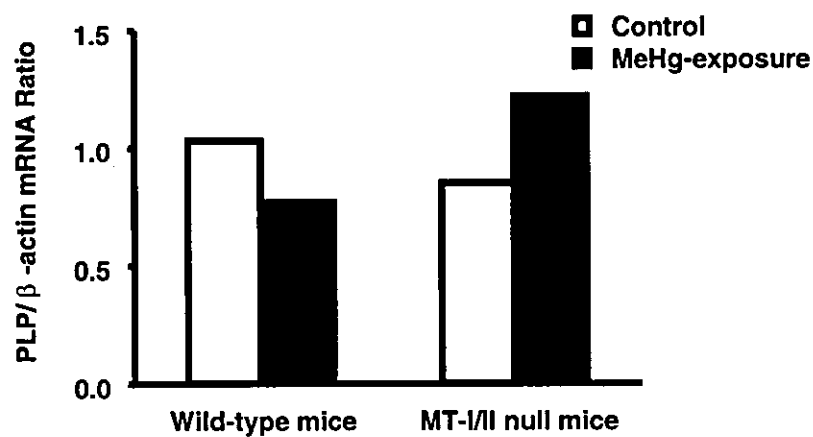


Fig. 11 Expression of Akt1s1 [matrix protein (ma), p15 containing protein data source:pfam, source key:pf01140, evidence:iss putative] and RPS12 [ribosomal protein s12] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to methyl mercury during gestation and lactation

Real-time RT-PCR analysis was performed on Akt1s1 and RPS12 genes.

PLP



RPS20

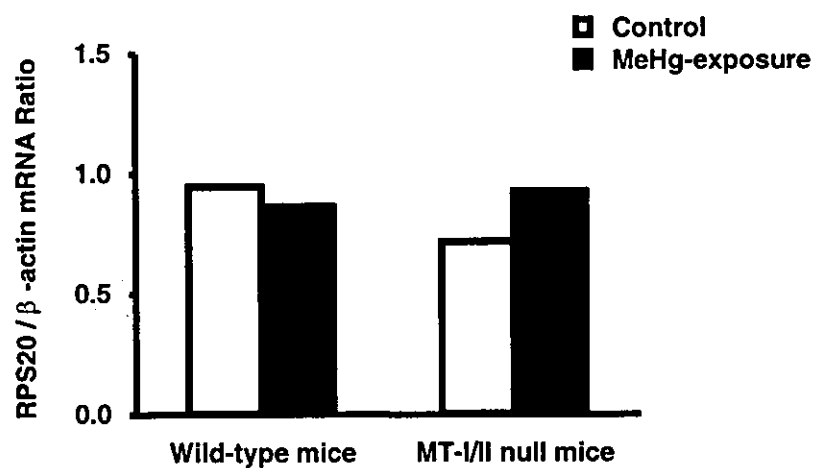
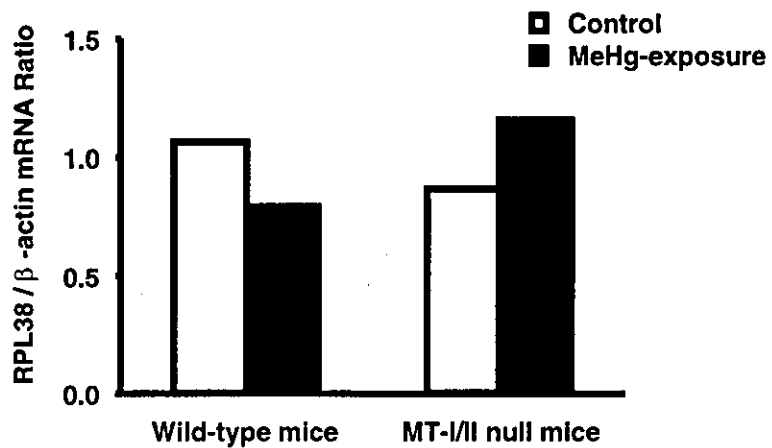


Fig. 12 Expression of PLP [6.8 kda mitochondrial proteolipid] and RPS20 [ribosomal protein s20] genes In neonatal brain of wild-type mice and MT-1/1 null mice exposed to methyl mercury during gestation and lactation

Real-time RT-PCR analysis was performed on PLP and RPS20 genes.

RPL38



Nme2

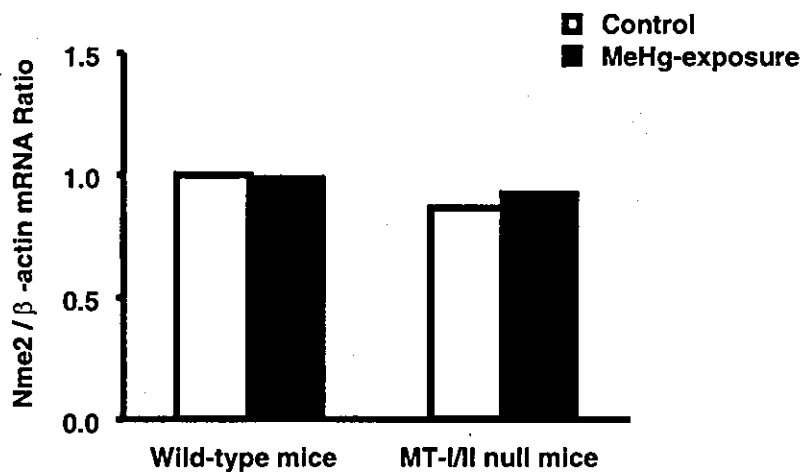


Fig. 13 Expression of RPL38 [ribosomal protein L38] and Nme2 [expressed in non-metastatic cells 2, protein (nm23b)] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to methyl mercury during gestation and lactation

Real-time RT-PCR analysis was performed on RPL38 and Nme2 genes.

Cox7b

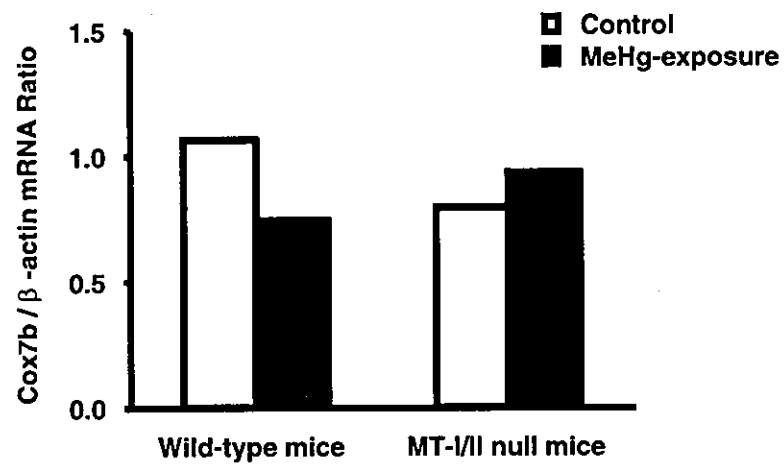
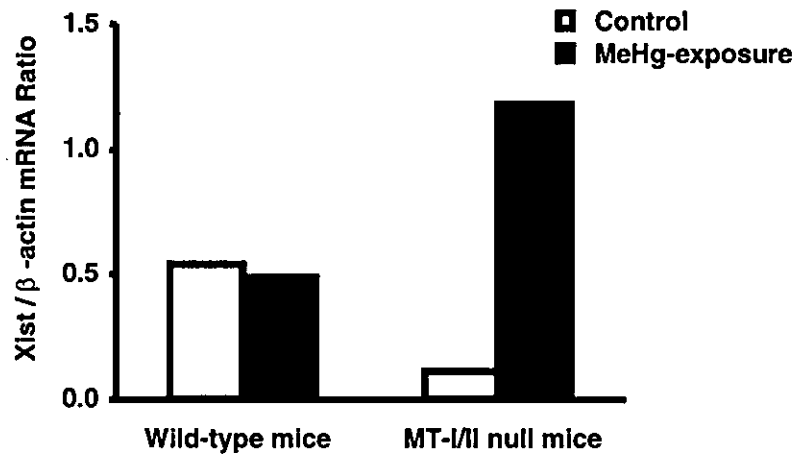


Fig. 14 Expression of Cox7b [cytochrome c oxidase subunit viib] gene in neonatal brain of wild-type mice and MT-1/1 null mice exposed to methyl mercury during gestation and lactation

Real-time RT-PCR analysis was performed on Cox7b gene.

Xist



TIMP4

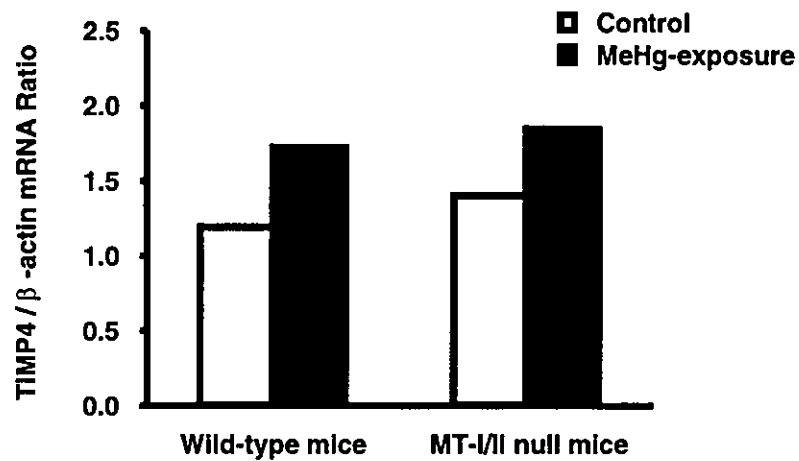


Fig. 15 Expression of Xist [nuclear-localized in active x-specific transcript] and TIMP4 [tissue inhibitor of metalloproteinase 4] genes in neonatal brain of wild-type mice and MT-1/1 null mice exposed to methyl mercury during gestation and lactation

Real-time RT-PCR analysis was performed on Xist and TIMP4 genes.

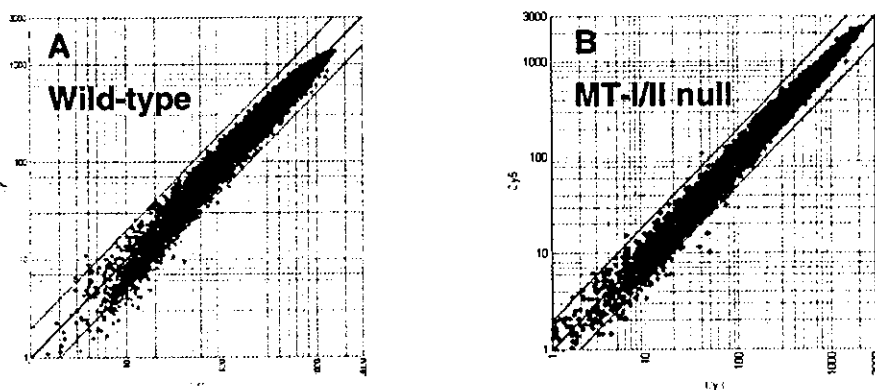


Fig. 16 Gene expression altered by methyl mercury exposure in brain of wild-type mice and MT-1/1 null mice from microarray data
A : Scatter plot of control group (Cy3) of wild-type mice vs MeHg-exposure group (Cy5) of wild-type mice fluorescence intensity signal for each gene.
B : Scatter plot of control group (Cy3) of MT-1/1 null mice vs MeHg-exposure group (Cy5) of MT-1/1 null mice fluorescence intensity signal for each gene.

X-axis : Control group (Cy3), Y-axis : MeHg-exposure group (Cy5).

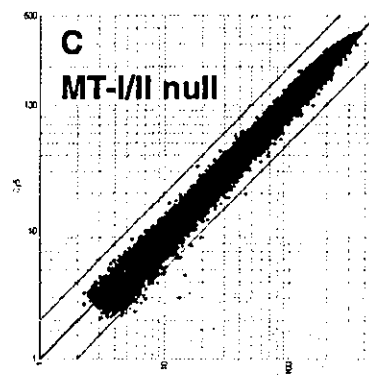
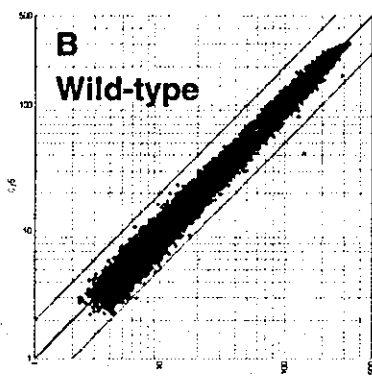
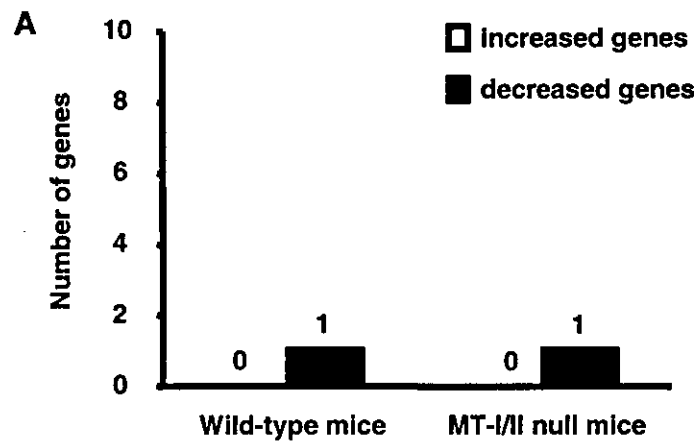


Fig. 17 Gene expression altered by combined exposure to methyl mercury and mercury vapor in neonatal brain of wild-type mice and MT-1/1 null mice from microarray data

A : Number of genes altered by combined exposure to methyl mercury and mercury vapor in wild-type mice and MT-1/1 null mice.

B : Scatter plot of control group (Cy3) of wild-type mice vs MeHg+Hg⁰-exposure group (Cy5) of wild-type mice fluorescence intensity signal for each gene.

C : Scatter plot of control group (Cy3) of MT-1/1 null mice vs MeHg+Hg⁰-exposure group (Cy5) of MT-1/1 null mice fluorescence intensity signal for each gene.

X-axis : Control group (Cy3), Y-axis : MeHg+Hg⁰-exposure group (Cy5).