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Short Communication

GATM, the human ortholog of the mouse imprinted *Gatm* gene, escapes genomic imprinting in placenta

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Abstract

The *GATM* gene encodes L-arginine:glycine amidinotransferase, which catalyzes the conversion of L-arginine into guanidinoacetate, the rate-limiting step in the synthesis of creatine. Since, deficiencies in creatine synthesis and transport lead to certain forms of mental retardation in human, the human *GATM* gene appears to be involved in brain development. Recently it has been demonstrated that the mouse *Gatm* is expressed during development and is imprinted with maternal expression in the placenta and yolk sac, but not in embryonic tissues. We investigated the imprinting status of the human *GATM* by analyzing its expression in four human placentas. *GATM* was biallelically expressed, thus suggesting that this gene escapes genomic imprinting in placentas, differently from what has been reported in mouse extra-embryonic tissues.

Key words: *GATM* gene, genomic imprinting, DNA polymorphism.

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The *GATM* gene encodes L-arginine: glycine amidinotransferase, which catalyzes the rate-limiting step in the synthesis of creatine, *i.e.*, the conversion of L-arginine into guanidinoacetate (Wyss and Kaddurah-Daouk, 2000). Phosphocreatine, the phosphorylated form of creatine, serves as a reservoir for high-energy phosphate in ATP synthesis and is, therefore, critically involved in maintaining the energy balance (Ellington, 2001). In the mouse, the *Gatm* gene is expressed during development and is imprinted, so the only maternal allele is expressed exclusively in the placenta and yolk sac, but not in embryonic tissues (Sandell *et al*., 2003). It maps to mouse chromosome 2 in a region not previously shown to contain imprinted genes. In humans, the *GATM* gene, located on chromosome 15, appears to be important for brain development, since deficiencies in creatine synthesis and transport have been implicated in certain forms of mental retardation (Bianchi *et al*., 2000; van der Knaap *et al*., 2000). Mutations in the *GATM* gene itself (Item *et al*., 2001), as well as in other genes required for the synthesis or transport of creatine (Carducci *et al*., 2000; Leuzzi *et al*., 2000; Salomons *et al*., 2001; Stockler *et al*., 1996), have been described in such cases. However, the imprinting status of *GATM* is unknown. Human *GATM* cDNA was isolated in 1994 by Humm, and the nucleotide sequence is available from GenBank under accession number BC004141. This 2342bp cDNA spans an open reading frame from nucleotide 70 to 1341, encoding a putative 423-amino acid protein.

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Herein we report an investigation on the imprinting status of the *GATM* gene in human placentas. To determine the allelic expression status of the human *GATM* gene, nucleotide polymorphisms in the transcribed region were used. Four single-nucleotide polymorphisms (SNPs) at position 1904 (T/G), 1941 (G/A), 2056 (C/T) and 2067 (C/A) have been previously identified in the 3'UTR region (NM-001482).

Twenty placentas from healthy Japanese volunteers were studied. Written informed consent was obtained in each case. The placentas, with 7 to 40 weeks of gestation, were washed with saline solution, rapidly frozen in liquid nitrogen, and ground to a powder. Part of the powdered tissue was used for obtaining DNA, using standard proteinase K and SDS treatment, phenol/chloroform extraction and ethanol precipitation. Three primers were constructed encompassing the target region, and PCR was performed with the primers GATMFFF1(5'-GGGCAAGGTTCATTCTC CTG-3') and GATMRR2 (5'-AAGGATGGGAACTGA TAGCG-3'). Then, semi-nested PCR was done with the primers GATMFFF2 (5'-AACAGGGGTCGTAAGCCTG

G-3') and GATMRR2. PCR was carried out in a total volume of 25 μL, containing 50 ng genomic DNA, 5 pmol of each primer, 1XTaq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of Amplitaq DNA polymerase (Perkin Elmer, Foster City, U.S.A.), under the following conditions: initial denaturation at 95 °C for 150 s, followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 90 s, and extension at 72 °C for 90 s. Semi-nested PCR was carried out under the same conditions, but for only 20 cycles. DNA was also purified and amplified from maternal peripheral blood.

RNA was extracted with guanidinium thiocyanate followed by centrifugation in a caesium chloride gradient. Total RNA was treated with *DNase* I (Roche, Tokyo, Japan) at 37 °C for 30 min to prevent contamination. The cDNA was synthesized from total RNA, using a cDNA synthesis kit (Roche). RT-PCR was carried out with total RNA, under the same conditions as PCR with the genomic DNA. Negative controls without oligo (dT) primer or reverse transcriptase were used.

Sequencing was performed with the primer GATMFF2 (5'-GCAGCTTGAAATGTTGGTCC-3'), using an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA).

Sequence analyses of the genomic DNA from the 20 placentas did not reveal either T/G heterozygotes at position 1904 (1904 T/G) or at 2067 C/A. Four samples were heterozygous for both 1941 G/A and 2056 C/T polymorphisms. Gestation ages were seven weeks (one placenta), eight weeks (two), and 13 weeks (one). The alleles carried by the mothers were determined.

To examine the allelic expression of the human *GATM* gene in these four informative cases, RT-PCR products were sequenced. Both alleles were detected, demonstrating that the genes of both parental alleles were expressed equally in these placentas. These data suggest that there is a difference in the imprinting status between the human *GATM* and the mouse *Gatm* genes in extra-embryonic tissues. A similar discrepancy has been reported in the human and mouse *ASCL2* (*HASH2*)/*Ascl2* (*Mash2*) gene (Miyamoto *et al*., 2002; Guillemot *et al*., 1995).

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