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

Toshinobu Miyamoto¹, Kazuo Sengoku¹, Hiroaki Hayashi¹, Yoshihito Sasaki¹, Yoshihiro Jinno² and Mutsuo Ishikawa¹

¹Asahikawa Medical College, Department of Obstetrics and Gynecology, Asahikawa, Hokkaido, Japan. ²Ryukyu University School of Medicine, Department of Molecular Biology, Nishihara, Okinawa, Japan.

**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.

Received: January 23, 2004; Accepted: November 17, 2004.
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, 1×Taq polymerase buffer (1.5 mM MgCl2), and 0.25 units of AmpliTag 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/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; Guillelmo et al., 1995).

Acknowledgments

We thank Mr. S. Bayley for the reading of our manuscript. The present study was supported by a Grant-in-Aid for Scientific Research (n. 16390471 and n. 16790934) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References


