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P-15 The Gene Encoding Guanidinoacetate Methyltransferase (GAMT) Maps to Mouse Chromosome 10 near the locus of hesitant mutation affecting male fertility

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INTRODUCTION

Guanidinoacetate methyltransferase (GAMT) catalyzes the last step of creatine biosynthesis in mammals. Creatine is an important metabolite for cellular energy metabolism in a variety of tissues like skeletal/cardiac/smooth muscles and brain. High levels of creatine (Cr) and/or phosphocreatine (PCr) are also found in the extracellular spaces of the male reproductive tract, suggesting that Cr may be involved in reproductive functions. A neurologic disorder caused by the congenital deficiency of the enzyme was recently described in two patients (3). One 22-month-old male patient had muscular hypotonia and progressive extrapyramidal movement disorder, and another 4-year-old patient showed severe developmental delay with muscular hypotonia, ataxia, and intractable seizures. Both patients manifested low levels of creatine and simultaneous accumulation of guanidinoacetate in brain. Oral administration of creatine greatly alleviated the symptoms. However, the mechanism underlying the neurologic symptom is not clear at present.

MATERIALS & METHODS

We used the BSS backcross [(C57BL/6JEi x SPRET/Ei)F1 x SPRET/Ei) panel DNA (Jackson Laboratory, Bar Harbor, Maine) for mapping. The panel consists of DNA from 94 backcross animals and two parental controls. A GAMT gene-specific primer pair (forward primer, 5-CCTACACTGCCTGATGGTC-3; reverse primer, 5-AGTGTGCCAGGTCTCTTCA-3) was designed and used for the PCR-RFLP analysis of the panel DNA.

A human/rodent hybrid cell panel DNA (NIGMS, Camden, NJ) and a human radiation hybrid mapping panel DNA (GeneBridge 4, Whitehead Genome Center) ware analyzed for the mapping of human GAMT gene. A human specific primer set was designed and used for the analysis.

RESULTS & DISCUSSION

The resulting typing of the 94 BSS backcross panel DNAs for the *Sty* I RFLP indicated that the locus maps to the central part of mouse Chr 10 in a large cluster of cosegregating loci including many other genes and expressed sequences (raw data may be accessed at: http://www.jax.org/resources/documents/cmdata/).

The low resolution mapping of *GAMT* by typing the NIGMS somatic cell hybrid mapping panel 2 localized *GAMT* to human Chr 19. A human-specific band of approximately 420bp was detected in control DNA from human cells but not in DNAs from mouse or hamster cells. The same size of band was detected in a lane of hybrid cell DNA retaining only human Chr 19 but not in any other lanes of hybrid cell DNA.

The result of the analysis of GeneBridge 4 panel DNA indicates that GAMT is tightly linked to the marker WI-6480 ($0.1cR_{3000}$ from the marker with a lod>3.0) and probably in the same RH bin as WI-6480. Since the WI-6480 is in Chr 19p13.3 (GDB, http://gdbwww.gdb.org), *GAMT* also should be located in the same region.

Cayman type cerebellar ataxia (ATCAY), a human genetic disorder exhibiting some overlapping symptoms with that of GAMT deficiency, has been mapped to human Chr 19p13.3 (4). Mouse mutations jittery (*ji*) and hesitant (ji^{hes})are allelic mutations sharing some common neurologic symptoms with that of human GAMT deficiency (1). Both *Gamt* and *ji* are closely linked to markers at the central Chr 10 region where syntenic homology to human Chr 19p13.3 has been demonstrated (MGD). Both the human mutation (ATCAY) and mouse mutation (jittery) manifest some similar neurologic symptoms to that of the GAMT deficiency beginning at a young

age: retarded growth, progressive ataxia, and seizures. Another interesting symptom of the *ji*^{hes} mutant mouse is the reduced fertility in males(7). If the jittery mutation is indeed caused by the mutation in GAMT gene, this implies that GAMT or Cr play an important role in male reproduction. Recently, Jenne *et al.* reported the localization of human GAMT gene to chromosome 19p13.3 by physical mapping, conforming present mapping data. They also sepuenced the coding region of the mouse GAMT gene and found no difference between the sequences of wild type mice and those of *ji* mouse. Thus, jittery mutation seems not to be caused by *Gamt* structural mutation, but it is still possible that mutation in the regulatory region of *Gamt* might prevent the expression of GAMT in *ji* mice.

SUMMARY

Guanidinoacetate methyltransferase (GAMT) catalyzes the last step of creatine biosynthesis in mammals. Creatine plays an important role in cellular energy metabolism in variety of tissues including brain and male reproductive tract. Congenital deficiency of the enzyme leads to a neurologic disorder in humans.

We used an interspecific backcross DNA panel to map *Gamt* to the central region of mouse Chromosome (Chr) 10 near the locus of hesitant mutation affecting male fertility. We assigned the human GAMT gene to Chr 19 by PCR analysis of a human/rodent somatic hybrid cell line DNA panel, and further localized the human gene to Chr 19 at band p13.3 by PCR analysis of a human radiation hybrid DNA panel. Human Chr 19p13.3 is homologous to the central part of mouse Chr 10 where mouse *Gamt* is located. Furthermore, this part of mouse Chr 10 contains mutant loci the phenotype of which is similar to the GAMT deficiency in human.

REFERENCES

Kapfhamer D, Sweet HO, Sufalko D, Warren S, Johnson KR, and Burmeister M (1996) *Genomics* 35: 533-538.

Stockler S, Hanefeld F, and Frahm J (1996) *Lancet* 348: 789-790.

Wallimann T and Hemmer W (1994) *Molecular and Cellular Biochemistry* 133: 193-220.

Eppig JT et al (1991) *Mouse Genome* 89(4): 844.