Brief Data Reports

Mammalian Genome

Mammalian Genome 7, 864–869 (1996).

The methylenetetrahydrofolate reductase (*Mthfr*) gene maps to distal mouse Chromosome 4

P. Frosst, Z.-X. Zhang, A. Pai, R. Rozen

Departments of Human Genetics, Pediatrics and Biology, McGill University—Montreal Children's Hospital Research Institute, 4060 Ste. Catherine Street West, Room 242, Montreal, Canada H3Z 2Z3

Received: 15 May 1996 / Accepted: 15 July 1996

Species: Mouse
Locus name: 5,10-Methylenetetrahydrofolate reductase
Locus symbol: *Mthfr*
Map position: Distal Chromosome (Chr) 4, Centromere–*D4Mit13*–1.06 cM ± 0.75–*Mthfr–1.06* ± 1.06–*D4Mit42*
Method of mapping: *Mthfr* was localized by RFLP analysis of 94 animals from an interspecific backcross panel ((C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei) provided by The Jackson Laboratory, Bar Harbor, Me. (BSS panel) [1]. To verify the localization, RFLP analysis was performed on 94 animals from the reciprocal cross ((C57BL/6J × *Mus spretus*)F_1 × C57BL/6J) (BSB panel) (Fig. 1) [1].
Database deposit information: The data are available from the Mouse Genome Database, accession number MGD-CREX-672.
Molecular reagents: A 727-bp mouse cDNA was obtained by reverse transcription/PCR of mouse brain RNA, with primer sequences based on sequence data of a mouse genomic clone (Pai et al. unpublished). The 5′ primer (5′-ATGGTGAACGAGGC-CAGAGGAA-3′) began at the ATG translation initiation start site, and the 3′ primer (5′-GCAGGCCTTCACAAAGCTGAAGA-3′) ended at bp 727 of the mouse cDNA. Sequence analysis verified the identity of the PCR product (Frosst, results not shown). The mouse cDNA was labeled by random priming and hybridized to Southern blots of *Taq*I-digested mouse genomic DNA.
Allele detection: Allele detection was performed by RFLP analysis of a *Taq*I polymorphism. The C57BL/6J strain has an allele of approximately 6.5 kb, while the *Mus spretus* strain has an allele of approximately 4.2 kb. A constant band of approximately 3.3 kb was seen in both strains.
Previously identified homologs: Human MTHFR has been mapped to the p36.3–p36.2 region of Chr 1 by *in situ* hybridization [2].
Discussion: MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate. 5-Methyltetrahydrofolate serves as the carbon donor for homocysteine remethylation to methionine. Recently, a common mutation in MTHFR (a C → T substitution at bp 677 resulting in an alanine to valine missense mutation) was proposed as a genetic risk factor for neural tube defects in man [3,4,5]. This mutation requires low folate status in plasma for the development of mild hyperhomocysteinemia [6]. This genetic-nutrient interactive effect is consistent with the multifactorial model for neural tube defects and with the established role of folic acid in reducing the recurrence and occurrence of this developmental anomaly.

The curly-tail (*ct*) mouse is a mouse model for neural tube defects; it is thought to closely mimic the human disorder with respect to the location of defects, pathology, and the effects of various teratogens [7]. A recent study of *ct* mice has shown that a diet low in folate or methionine can affect the incidence of tail defects [8]. A major gene that predisposes to neural tube defects in the *ct* strain has been localized to distal Chr 4, to a region approximately 3 cM proximal to the *D4Mit13* marker [7,9]. In this study, *Mthfr* was mapped to a region approximately 1 cM distal to the *D4Mit13* marker with one backcross panel; no crossovers were observed between *Mthfr* and *D4Mit13* with the second panel. The metabolic relevance of *Mthfr* to folate-dependent methionine synthesis and the localization of *Mthfr* to distal Chr 4 suggest that *Mthfr*, or a closely linked gene in this pathway, should be evaluated as a candidate for the major gene that predisposes to neural tube defects in the *ct* strain.

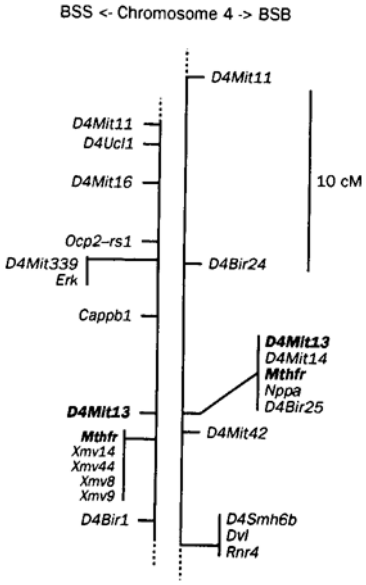

Fig. 1. The localization of *Mthfr* to distal Chr 4. The markers on the left are from the BSS backcross panel, while the markers on the right are from the BSB panel. These panel data and references for mapping the other loci are publicly available from The Jackson Laboratory Mapping Resource at the World Wide Web address: http://www.jax.org/resources/documents/cmdata.

Acknowledgments. The technical assistance to Renate Milos and the secretarial assistance of Carolyn Mandel are gratefully acknowledged. We also appreciate the expert advice and assistance of Lucy Rowe and Mary Barter from The Jackson Laboratory Backcross DNA Panel Map Resource. This work was supported by the Medical Research Council of Canada.

References

1. Rowe, L.B., Nadeau J.H., Turner, R., Frankel, W.N., Letts, V.A., Eppig, J.T., Ko, M.S.H., Thurston, S.J., Birkenmeier, E.H. (1994). Mamm. Genome 5, 253–274.
2. Goyette, P., Sumner, J.S., Milos, R., Duncan, A.M.V., Rosenblatt, D.S., Matthews, R.G., Rozen, R. (1994). Nature Genet. 7, 195–200.
3. Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J.H., den Heijer, M., Kluijtmans, L.A.J., van den Heuvel, L.P., Rozen, R. (1995). Nature Genet. 10, 111–113.
4. van der Put, N.M.J., Steegers-Theunissen, R.P.M., Frosst, P., Trijbels, F.J.M., Eskes, T.K.A.B., ven den Heuvel, L.P., Mariman, E.C.M., den Heyer, M., Rozen, R., Blom, H.J. (1995). Lancet 346, 1070–1071.

Correspondence to: R. Rozen

5. Whitehead, A.S., Gallagher, P., Mills, J.L., Kirke, P.N., Burke, H., Molloy, A.M., Weir, D.G., Shields, D.C., Scott, J.M. (1995). Q. J. Med. 88, 763–766.
6. Jacques, P.F., Bostom, A.G., Williams, R.R., Ellison, R.C., Eckfeldt, J.H., Rosenberg, I.H., Selhub, J., Rozen, R. (1996). Circulation 93, 7–9.
7. Neumann, P.E., Frankel, W.N., Letts, V.A., Coffin, J.M., Copp, A.J., Bernfield, M. (1994). Nature Genet. 6, 357–362.
8. Lanoue, L., Sulik, K.K. (1995). Teratology 51, 162.
9. Beier, D.R., Dushkin, H., Telle, T. (1995). Mamm. Genome 6, 269–272.

A major polymorphism in the rat S_A gene caused by the insertion of a LINE element

S.A. Frantz, A.S. Thiara, D. Lodwick, N.J. Samani

Departments of Medicine and Therapeutics, and Cardiology, University of Leicester, Leicester, LE2 7LX, UK

Received: 3 June 1996 / Accepted: 19 July 1996

Species: *Rattus norvegicus*
Locus name: S_A gene
Locus symbol: S_A
Chromosomal location: Chromosome (Chr) 1 [1]
Molecular reagents: Primers (forward: 5′ TGGCTTTCTCTC-CCATTAA 3′ position 7–26 in the cDNA sequence [2]; reverse: 5′ TCCTGGTGTCCACCTCCTTGTGTGAGAAG 3′ position 157–129), located in the first second exons of the S_A gene respectively, were designed with the help of OLIGO [3] and used to amplify the first intron of the rat S_A gene by Long and Accurate Polymerase Chain Reaction (LA-PCR) [4]. The PCR products were cloned directly into pGEM-T (Promega, Southampton, UK), and the region of the DNA containing the polymorphism was subcloned into pBluescript (Stratagene, Cambridge, UK). These subclones were sequenced by the dideoxy chain termination method with the T7 Sequencing kit (Pharmacia, Milton Keynes, UK), with primers (designed as before) "walking" along the DNA. A 695-bp *XhoI/Bam*HI fragment located within the observed insertion was used as a probe for Southern blots.
Previously identified sequence: S_A cDNA sequence [2] and *Rattus norvegicus* (S_A) gene microsatellite SA3CA sequence [5].
Discussion: The S_A gene, of unknown function, was identified owing to its higher expression in the Spontaneously Hypertensive Rat (SHR) compared with the normotensive Wistar Kyoto (WKY) rat strain [2], and this gene locus was shown to cosegregate with blood pressure in F_2 populations of various crosses of hypertensive and normotensive inbred rat strains [1,6–8]. Restriction Fragment Length Polymorphism analysis of SHR and WKY genomic DNA showed that there was a significant degree of polymorphism between these two S_A alleles [2]. To identify any structural differences in the genomic DNA that could explain this, PCR primers flanking the first intron were used to amplify this area of the gene from SHR and WKY rats; this resulted in products of approximately 6.5 kb and 5.1 kb from SHR and WKY rat DNA respectively.

In order to determine the nature of this size heterogeneity, these PCR products were cloned and characterized by restriction digest to localize the polymorphic region. A polymorphic *Sst*I fragment (3317 bp for SHR DNA and 1991 bp for WKY DNA) was subcloned into pBluescript SK and sequenced. The sequence data showed that the SHR sequence has greater than 95% homology with the *Rattus norvegicus* (S_A) gene microsatellite SA3CA sequence [5] over a 1669 bp overlap (at which point the published sequence ends) and contains an extra 1326 bp of DNA compared with the WKY PCR product. This additional 1326 bp shows over 91% homology to the 3′ end of a rat long interspersed repetitive element (LINE) [9] in an antisense orientation relative to the S_A gene; however, no homology to this LINE sequence was found in the corresponding WKY subclone. Moreover, the fact that the LINE element is flanked by a duplicated 16-bp target site for insertion, a property common with class II reterotransposons [10], implies that the nature of the size difference in the SHR S_A first intron is most likely to be the reterotranscriptional insertion of a LINE sequence.

To determine the distribution of the LINE insertion in the first intron of the S_A gene in other inbred rat strains, we prepared Southern blots from the PCR products of other strains and hybridized them to a ^{33}P-labeled LINE-specific DNA probe. This showed that several rat strains gave the larger PCR product (L in Table 1), all of which hybridized to the LINE-specific probe (+). However, those rat strains that gave the smaller PCR product (S) did not hybridize to the LINE-specific probe (−).

These results therefore demonstrate the presence of a novel insertional polymorphism in the S_A gene which is present in several inbred rat strains; the functional importance of this remains to be elucidated.

Acknowledgments: We thank Dr. John Armour for his advice regarding the LA-PCR protocol. This work was funded by the Wellcome Trust.

Correspondence to: N.J. Samani at Department of Cardiology, Clinical Sciences Wing, Glenfield Hospital, Groby Road, Leicester, LE3 9QP, UK.

References

1. Samani, N.J., Lodwick, D., Vincent, M., Dubay, C., Kaiser, M.A., Kelly, M.P., Lo, M., Harris, J., Sassard, J., Lathrop, M., Swales, J.D. (1993). J. Clin. Invest. 92, 1099–1103.
2. Iwai, N., Inagami, T. (1991). Hypertension 17, 161–169.
3. Rychlik, W., Rhoads, R.E. (1989). Nucleic Acids Res. 17, 8543–8551.
4. Cheng, S., Fockler, C., Barnes, W.M., Higuchi, R. (1994). Proc. Natl. Acad. Sci. USA 91, 5695–5699.
5. Gu, L., Dene, H., Rapp, J.P. (1995). Mamm. Genome 5, 833.
6. Iwai, N., Inagami, T. (1992). J. Hypertens. 10, 1155–1157.
7. Harris, E.L., Dene, H., Rapp, J.P. (1993). Am. J. Hypertens. 6, 330–334.
8. Lindpaintner, K., Hilbert, P., Ganten, D., Nadal-Ginard, B., Inagami, T., Iwai, N. (1993). J. Hypertens. 11, 19–23.
9. d'Ambrosio, E., Waitzkin, S.D., Witney, F.R., Salemme, A., Furano, A.V. (1986). Mol. Cell. Biol. 6, 411–424.
10. Fanning, T.G., Singer, M.F. (1987). Biochim. Biophys. Acta. 910, 203–212.

Table 1. Summary of occurrence of LINE insertion in different rat strains.

	Inbred Rat Strains										
	SHR	WKY	MHS	MNS	LH	LN	SS	SR	LEW	CDF	BN
PCR	L	S	S	L	L	L	L	L	S	L	L
Line	+	−	−	+	+	+	+	+	−	+	+

Abbreviations: SHR, Spontaneously hypertensive rat; WKY, Wistar Kyoto rat; MHS, Milan hypertensive strain; MNS, Milan normotensive strain; LH, Lyon hypertensive rat; LN, Lyon normotensive rat; SS, Dahl salt-sensitive rat; SR, Dahl salt-resistant rat; LEW, Lewis rat strain; CDF, CDF rat strain; BN, Brown Norway rat; PCR, PCR product size from amplification described in text; LINE, hybridization of LINE-specific sequence to the PCR products.