

Note

Deletion Polymorphism of *Disc1* Is Common to All 129 Mouse Substrains: Implications for Gene-Targeting Studies of Brain Function

Steven J. Clapcote^{*,1} and John C. Roder^{*,†}

^{*}Mount Sinai Hospital Research Institute, Toronto, Ontario M5G 1X5, Canada and [†]Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Manuscript received May 17, 2006
Accepted for publication May 29, 2006

ABSTRACT

We report that the *Disc1* gene in all extant 129 mouse inbred substrains has a deletion, previously considered specific to the 129S6/SvEv substrain, which is predicted to abolish production of the full-length protein. This finding has implications for the study of knockout mice generated from 129-derived embryonic stem cells.

DISCI (disrupted in schizophrenia 1) was directly implicated as a candidate gene for psychiatric disease by the identification of truncation mutations cosegregating with schizophrenia and affective disorder in two distinct Scottish (MILLAR *et al.* 2000) and American (SACHS *et al.* 2005) families. Other polymorphisms of DISCI have shown association with these diseases in broader populations in the United States (HODGKINSON *et al.* 2004; CALLICOTT *et al.* 2005) and northern Europe (CANNON *et al.* 2005; ZHANG *et al.* 2006). DISCI protein is present in multiple brain regions and in several other tissues, including heart and kidney (MILLAR *et al.* 2004). Although the functions of DISCI are not understood in detail, it has been shown to interact with multiple proteins required for neuronal functions such as neurite extension, neurotransmission, signal transduction, and the cytoskeleton (PORTEOUS and MILLAR 2006).

In the original Scottish DISCI truncation family, MILLAR *et al.* (2005) found a 50% reduction of the normal level of DISCI in cell lines derived from mutation hemizygotes, but could find no evidence for a stable, truncated DISCI protein product. In an analogous finding in the mouse, KOIKE *et al.* (2006) reported that the 129S6/SvEv (129S6) inbred strain bred by Taconic Farms is homozygous for a 25-bp deletion in exon 6 of *Disc1* that induces a frame shift in the reading frame of *Disc1*, resulting in 13 novel

amino acids, followed by a premature stop codon. Neither full-length *Disc1* nor the predicted C-terminally truncated protein was detectable in 129S6 brain tissue (KOIKE *et al.* 2006), suggesting that the deletion is a null allele. After backcrossing the deletion onto the C57BL/6J inbred strain to obtain a uniform genetic background, KOIKE *et al.* (2006) found that both heterozygous and homozygous mutants showed a consistent impairment in a working memory test, suggesting that *Disc1* is haplo-insufficient in relation to this trait. Consistent with this haplo-insufficiency model, KAMIYA *et al.* (2005) have shown that reduction of endogenous *Disc1* expression in mice disturbs proper neuronal migration and arborization of dendritic neuronal processes in the developing cerebral cortex.

Fixation of spontaneous null mutations within mouse production colonies has been reported previously. C57BL/6J^{OlaHsd} mice supplied by Harlan UK have a deletion of the α -synuclein locus (SPECHT and SCHOEPFER 2001), while C57BL/6J mice supplied by JAX Research Systems have a deletion in the nicotinamide nucleotide transhydrogenase gene (HUANG *et al.* 2006). Neither deletion has been detected in other C57BL/6 substrains. As the *Disc1* deletion was found to be absent in the AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, CBA/J, and DBA/2J strains, KOIKE *et al.* (2006) concluded that it represents a 129S6-specific polymorphism. However, the 129S6 is just one of several inbred substrains within the complex genealogy of the 129 strain family, which includes the parental, steel, *Ter* (teratoma susceptible), and X (genetically contaminated) lineages (FESTING *et al.* 1999; BECK *et al.* 2000). As KOIKE *et al.* (2006) did not examine any other 129 substrains for

¹Corresponding author: Room 860, Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, ON M5G 1X5, Canada.
E-mail: clapcote@mshri.on.ca

TABLE 1
129 mouse substrains assayed for the *Disc1* deletion polymorphism

Strain name	Abbreviation	Lineage	ES cell lines
129P1/ReJ	129P1	Parental (P)	
129P2/OlaHsd	129P2	Parental (P)	E14TG2a, HM-1
129P3/J	129P3	Parental (P)	mEMS32
129P4/RrRkJ	129P4	Parental (P)	
129S1/SvImJ	129S1	Steel (S)	CJ7, W9.5
129S2/SvPas	129S2	Steel (S)	D3
129S4/SvJae	129S4	Steel (S)	AK7, J1
129S5/SvEvBrd	129S5	Steel (S)	Lex-1
129S7/SvEvBrd	129S7	Steel (S)	AB1, AB2.1
129S8/SvEv	129S8	Steel (S)	
129T1/Sv	129T1	<i>Ter</i> (T)	
129T2/SvEmsJ	129T2	<i>Ter</i> (T)	
129X1/SvJ	129X1	Contaminated (X)	Pat5, PJ1-5, RW-4

We assayed all extant inbred substrains of 129 listed by FESTING *et al.* (1999) and BECK *et al.* (2000). DNA samples from 129P1, 129P3, 129P4, 129S1, 129S4, 129S8, 129T1, 129T2, and C57BL/6J mice were obtained from the Mouse DNA Resource of The Jackson Laboratory (Bar Harbor, ME). DNA samples from 129S5 and 129S7 mice were obtained from Roswell Park Cancer Institute (Buffalo) and B&K Universal (Hull, Yorkshire, UK), respectively. DNA was isolated from tail biopsies from 129S6 and B6129F1 mice supplied by Taconic Farms (Germantown, NY), from 129P2 mice supplied by Harlan (Indianapolis), and from 129S2 mice supplied by Charles River (Saint-Constant, Quebec, Canada). ES cell line progenitor strains were identified using the MOUSE GENOME DATABASE (2006). 129S1/SvImJ was previously named 129S3/SvImJ (JACKSON LABORATORY 2001). 129/Sv (JAX 000094), a cryopreserved strain that is heterozygous at many loci (SIMPSON *et al.* 1997), was excluded as it is clearly not inbred.

the *Disc1* deletion, they could not determine whether it arose *de novo* within the 129S6 colony at Taconic Farms.

As virtually all embryonic stem (ES) cell lines for gene targeting are derived from substrains of the 129 mouse (Table 1), the potential presence of a genetic ablation known to confer a cognitive impairment in the heterozygous state (KOIKE *et al.* 2006) deserves the attention of scientists using knockout mice to analyze brain function. *Disc1* deficiency in these mice may have previously unforeseen consequent effects on the >50 putative *Disc1* interacting proteins that have been reported (PORTEOUS and MILLAR 2006). The objective of this study was thus to establish the prevalence of the *Disc1*

deletion among the other inbred substrains of 129, including the progenitors of 14 ES cell lines (Table 1).

To simultaneously detect the 25-bp deletion of 129S6 and the wild-type allele of C57BL/6 mice, we devised a PCR-based genotyping assay (Figure 1). A trial PCR with control DNA of known genotype revealed that C57BL/6J had a single band of 196 bp, 129S6 had a single band of 171 bp, and the B6129F1 (C57BL/6N × 129S6) hybrid had two bands of 196 and 171 bp (Figure 2), which was consistent with the predicted action of the primers.

Application of the genotyping assay to 13 other substrains of 129 (Table 1) revealed that each had a single band of the same size as that of the 129S6

F primer 5'-GCTGTGACCTGATGGCACT>>>
C57BL/6 5'-GCTGTGACCTGATGGCACTGGTGGCCAGATGTCCCCAGCCAGCTGCAGGAGGTCAGCAAGGCC
129S6 5'-GCTGTGACCTGATGGCACTGGTGGCCAGATGTCCCCAGCCAGCTGCAGGAGGTCAGCAAGGCC

C57BL/6 TTGGGAGAGACCCCTGACCTCTGCCAACCCAGGCTCCCTTCCAGGTTGGAGCCACCTGAGACCCCTCAG
129S6 TTGGGAGAGACCCCTGACCTCTGCCAACCC.....TGAGACCCCTCAG

C57BL/6 GAGGTACTGGTGACTTTCTGAGTTTCCAACCTTTACCTCTTTCTCCTGGTTATTGAGGTGACTTTGC-3'
129S6 GAGGTACTGGTGACTTTCTGAGTTTCCAACCTTTACCTCTTTCTCCTGGTTATTGAGGTGACTTTGC-3'
R primer <<<ACCAATAACTCCACTGAAACG-5'

6–7 is the reverse complement of nucleotides 128,021,642–128,021,662 of chromosome 8. Physical map distances between the primers were obtained using the UCSC Genome Browser (<http://genome.ucsc.edu/>; mouse assembly February 2006). The primers are designed to amplify a fragment of 171 bp from 129S6 and a fragment of 196 bp from C57BL/6. Each 25- μ l PCR contains 17.7 μ l nuclease-free water, 2.5 μ l 10X PCR buffer [500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°), 1% Triton X-100], 1.6 mM magnesium chloride, 0.2 mM each of dATP, dTTP, dCTP, and dGTP, 0.5 μ M of each primer, 1.5 units *Taq* DNA polymerase, and 50 ng genomic DNA. Reactions are incubated at 94° for 5 min, followed by 35 cycles of 94° for 20 sec, 54° for 1 min, and 72° for 40 sec, followed by 72° for 10 min.

FIGURE 1.—PCR genotyping assay to detect the *Disc1* deletion. C57BL/6 mice have a wild-type *Disc1* allele, while 129S6 mice have a 25-bp deletion (dots) in exon 6 of *Disc1*. The forward primer (5'-GCT GTG ACC TGA TGG CAC T-3') within exon 6 corresponds to nucleotides 128,021,467–128,021,485, and the reverse primer (5'-CTT CTC ACC TGA GCA CAC CT-3') within intron

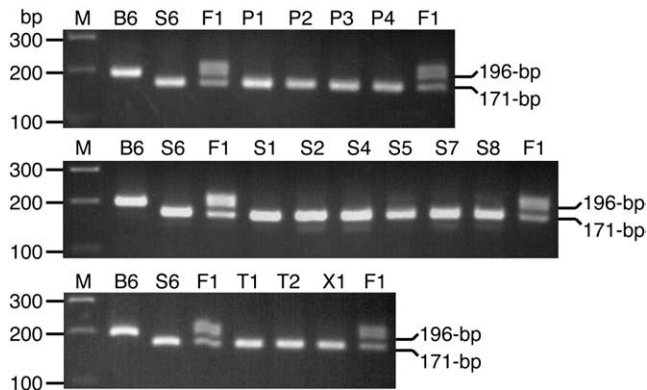


FIGURE 2.—*Disc1* deletion genotyping gel. M, 100-bp DNA ladder (1.0 μ g; 70 ng per band). B6, C57BL/6J. S6, 129S6. F1, B6129F1. P1, 129P1. P2, 129P2. P3, 129P3. P4, 129P4. S1, 129S1. S2, 129S2. S4, 129S4. S5, 129S5. S7, 129S7. S8, 129S8. T1, 129T1. T2, 129T2. X1, 129X1. Following thermocycling, 20 μ l of PCR product were electrophoresed at 8.5 V/cm through a 3% (w/v) agarose gel in 1X TAE buffer (40 mM tris-acetate, 2 mM EDTA) for 75 min. The sizes of the DNA ladder fragments and PCR products are indicated. The extra, upper band in the F1 (heterozygote) lanes is a heteroduplex of the wild-type and mutant strands, in which the 25-bp mismatch of the deletion forms a loop that slows migration through the gel.

control (Figure 2). Sequence analysis of the fragments from single representatives of the parental (129P2), steel (129S6), *Ter* (129T1), and X (129X1) lineages confirmed the presence of the deletion, which was absent in the C57BL/6J control (Figure 3). This result indicates uniform homozygosity for the *Disc1* deletion across all inbred lineages of 129.

As the *Disc1* deletion is common to all inbred substrains of 129, we conclude that it is not 129S6 specific. This is perhaps surprising given the substantial genetic heterogeneity between 129 substrains (SIMPSON *et al.* 1997; THREADGILL *et al.* 1997), even within the same lineage (SECHLER *et al.* 1997). The presence of the deletion in all lineages suggests that it was already fixed in the population of mice used to reestablish the Jackson Laboratory's 129 breeding colony in 1948, from which all the current substrains are descended (SIMPSON *et al.* 1997). Given the ancestral fixation of the *Disc1* deletion in the 129 strain, the numerous 129 substrain-derived ES cell lines established within the past 20 years are also likely to harbor the deletion (Table 1). In light of our findings, studies of working memory in knockout mice with isogenic or hybrid 129 genetic backgrounds should perhaps be reevaluated (*e.g.*, BJORKLUND *et al.* 2001; DELLER *et al.* 2003). To avoid potential confounds resulting from *Disc1* deficiency, scientists using gene targeting to analyze brain function would be advised to genotype their knockout lines for the *Disc1* deletion. Backcrossing to a wild-type *Disc1* strain, such as C57BL/6, for several generations should remove the deletion if it is unlinked to the targeted gene. On a more positive

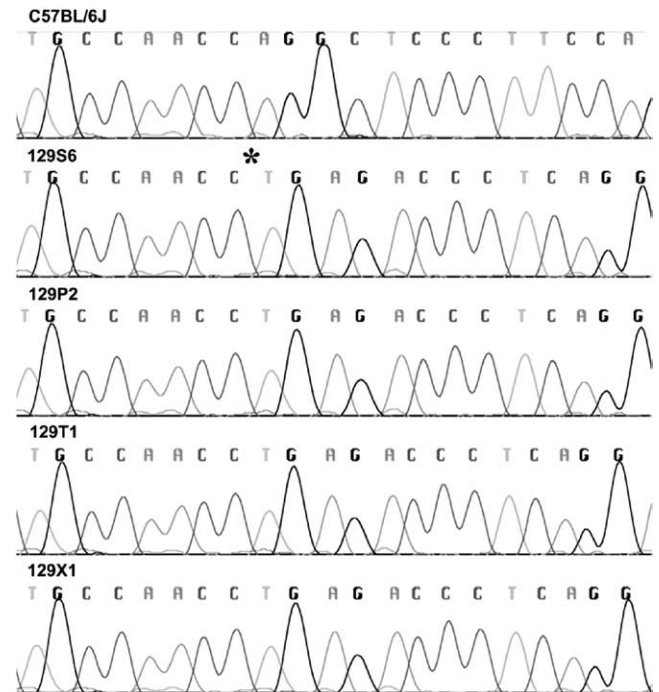


FIGURE 3.—Sequence chromatograms showing the 25-bp deletion of *Disc1* in representatives of the steel (129S6), parental (129P2), *Ter* (129T1), and X (129X1) lineages of the 129 inbred strain family. An asterisk indicates the location of the deletion in the 129S6 sequence. Alignment and comparisons with the wild-type C57BL/6J sequence were performed using Sequencher software (Gene Codes, Ann Arbor, MI).

note, investigators of psychiatric disease may welcome the news that *Disc1*-deficient mice can be obtained cheaply and easily by accessing any of the widely available 129 substrains.

We thank Y. Yu for 129S5/SvEvBrd DNA and D. Pomp for advice. This study was supported by grants-in-aid to J.C.R. from the Canadian Institutes of Health Research (GMH-79044) and the National Alliance for Research on Schizophrenia and Depression (NARSAD Distinguished Investigator Award). J.C.R. holds a Canada Research Chair. S.J.C. holds an Epilepsy Canada Research Fellowship.

LITERATURE CITED

- BECK, J. A., S. LLOYD, M. HAFEZPARAST, M. LENNON-PIERCE, J. T. EPPIG *et al.*, 2000 Genealogies of mouse inbred strains. *Nat. Genet.* **24**: 23–25.
- BJORKLUND, M., I. SIVERINA, T. HEIKKINEN, H. TANILA, J. SALLINEN *et al.*, 2001 Spatial working memory improvement by an alpha2-adrenoceptor agonist dexmedetomidine is not mediated through alpha2C-adrenoceptor. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **25**: 1539–1554.
- CALLICOTT, J. H., R. E. STRAUB, L. PEZAWAS, M. F. EGAN, V. S. MATTAY *et al.*, 2005 Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. *Proc. Natl. Acad. Sci. USA* **102**: 8627–8632.
- CANNON, T. D., W. HENNAH, T. G. M. VAN ERP, P. M. THOMPSON, J. LONNQVIST *et al.*, 2005 Association of DISC1/TRAX haplotypes with schizophrenia, reduced prefrontal gray matter, and impaired short- and long-term memory. *Arch. Gen. Psychiatry* **62**: 1205–1213.

- DELLER, T., M. KORTE, S. CHABANIS, A. DRAKEW, H. SCHWEGLER *et al.*, 2003 Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity. *Proc. Natl. Acad. Sci. USA* **100**: 10494–10499.
- FESTING, M. F. W., E. M. SIMPSON, M. T. DAVISSON and L. E. MOBRAATEN, 1999 Revised nomenclature for strain 129 mice. *Mamm. Genome* **10**: 836.
- HODGKINSON, C. A., D. GOLDMAN, J. JAEGER, S. PERSAUD, J. M. KANE *et al.*, 2004 Disrupted in schizophrenia 1 (DISC1): association with schizophrenia, schizoaffective disorder, and bipolar disorder. *Am. J. Hum. Genet.* **75**: 862–872.
- HUANG, T. T., M. NAEEMUDDIN, S. ELCHURI, M. YAMAGUCHI, H. M. KOZY *et al.*, 2006 Genetic modifiers of the phenotype of mice deficient in mitochondrial superoxidase dismutase. *Hum. Mol. Genet.* **15**: 1187–1194.
- JACKSON LABORATORY, 2001 New 129 nomenclature—revised. JAX Bulletin, No. 1 (<http://jaxmice.jax.org/info/bulletin/bulletin01.html>). The Jackson Laboratory, Bar Harbor, ME.
- KAMIYA, A., K. KUBO, T. TOMODA, M. TAKAKI, R. YOUN *et al.*, 2005 A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat. Cell Biol.* **7**: 1067–1078.
- KOIKE, H., P. A. ARGUELLO, M. KVAJO, M. KARAYIORGOU and J. A. GOGOS, 2006 *Disc1* is mutated in the 129S6/SvEv strain and modulates working memory in mice. *Proc. Natl. Acad. Sci. USA* **103**: 3693–3697.
- MILLAR, J. K., J. C. WILSON-ANNAN, S. ANDERSON, S. CHRISTIE, M. S. TAYLOR *et al.*, 2000 Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* **9**: 1415–1423.
- MILLAR, J. K., R. JAMES, N. J. BRANDON and P. A. THOMSON, 2004 DISC1 and DISC2: discovering and dissecting molecular mechanisms underlying psychiatric disease. *Ann. Med.* **36**: 1–12.
- MILLAR, J. K., B. S. PICKARD, S. MACKIE, R. JAMES, S. CHRISTIE *et al.*, 2005 DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* **310**: 1187–1190.
- MOUSE GENOME DATABASE, 2006 ES cell line name and strain of origin. Mouse Genome Informatics (ftp://ftp.informatics.jax.org/pub/datasets/es_cell_lines.html). The Jackson Laboratory, Bar Harbor, ME.
- PORTEOUS, D. J., and J. K. MILLAR, 2006 Disrupted in schizophrenia 1: building brains and memories. *Trends Mol. Med.* **12**: 255–261.
- SACHS, N. A., A. SAWA, S. E. HOLMES, C. A. ROSS, L. E. DELISI *et al.*, 2005 A frameshift mutation in Disrupted in Schizophrenia 1 in an American family with schizophrenia and schizoaffective disorder. *Mol. Psychiatry* **10**: 758–764.
- SECHLER, J. M. G., J. C. YIP and A. S. ROSENBERG, 1997 Genetic variation among 129 substrains: practical consequences. *J. Immunol.* **159**: 5766–5768.
- SIMPSON, E. M., C. C. LINDER, E. E. SARGENT, M. T. DAVISSON, L. E. MOBRAATEN *et al.*, 1997 Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. *Nat. Genet.* **16**: 19–27.
- SPECHT, C. G., and R. SCHOEPFER, 2001 Deletion of the alpha-synuclein locus in a sub-population of C57BL/6J inbred mice. *BMC Neurosci.* **2**: 11.
- THREAGILL, D. W., D. YEE, A. MATIN, J. H. NADEAU and T. MAGNUSON, 1997 Genealogy of the 129 inbred strains: 129/SvJ is a contaminated inbred strain. *Mamm. Genome* **8**: 390–393.
- ZHANG, F., J. SARGINSON, C. CROMBIE, N. WALKER, D. ST. CLAIR *et al.*, 2006 Genetic association between schizophrenia and the DISC1 gene in the Scottish population. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **141B**: 155–159.

Communicating editor: T. R. MAGNUSON