Effects of background mutations and single nucleotide polymorphisms (SNPs) on the Disc1 L100P behavioral phenotype associated with schizophrenia in mice

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Abstract

Background: Disrupted-in-schizophrenia 1 (DISC1) is a promising candidate susceptibility gene for psychiatric disorders, including schizophrenia, bipolar disorder and major depression. Several previous studies reported that mice with N-ethyl-N-nitrosourea (ENU)-induced L100P mutation in Disc1 showed some schizophrenia-related behavioral phenotypes. This line originally carried several thousands of ENU-induced point mutations in the C57BL/6 J strain and single nucleotide polymorphisms (SNPs) from the DBA/2 J inbred strain.

Methods: To investigate the effect of Disc1 L100P, background mutations and SNPs on phenotypic characterization, we performed behavioral analyses to better understand phenotypes of Disc1 L100P mice and comprehensive genetic analyses using whole-exome resequencing and SNP panels to map ENU-induced mutations and strain-specific SNPs, respectively.

Results: We found no differences in spontaneous or methamphetamine-induced locomotor activity, sociability or social novelty preference among Disc1 L100P/L100P, L100P/+ mutants and wild-type littermates. Whole-exome resequencing of the original G1 mouse identified 117 ENU-induced variants, including Disc1 L100P per se. Two females and three males from the congenic L100P strain after backcrossing to C57BL/6 J were deposited to RIKEN BioResource Center in 2008. We genotyped them with DBA/2 J × C57BL/6 J SNPs and found a number of the checked SNPs still remained.

Conclusion: These results suggest that causal attribution of the discrepancy in behavioral phenotypes to the Disc1 L100P mutant mouse line existing among different research groups needs to be cautiously investigated in further study by taking into account the effect(s) of other ENU-induced mutations and/or SNPs from DBA/2 J.

Keywords: Disrupted-in-schizophrenia 1 (DISC1), ENU mutagenesis, Locomotor activity, Methamphetamine, Social interaction, Whole-exome resequencing, Strain-specific SNPs

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Background

Schizophrenia is a devastating mental disorder with a significant genetic component that affects approximately 0.5–1.0% of the general population. Based on genetic epidemiological studies, it was estimated that schizophrenia has a heritability of 60–80% [1,2]. Disrupted-in-schizophrenia 1 (DISC1) is one of the candidate susceptibility genes for a spectrum of major psychiatric disorders. DISC1 was originally identified on chromosome 1 by analyzing a large Scottish pedigree showing a heavy burden of major psychiatric disorders associated with DISC1. DISC1 is implicated in the genetic risk for many aspects of quantitative cognitive traits of psychotic patients [3-4]. DISC1 is related to schizophrenia 1 (DISC1) in the human genome, ENU mutant mouse lines may harbor unknown functional mutations other than those are assumed to contribute to the phenovariance of interest [22,23,28,29]. Additionally, ENU-mutagenized C57BL/6 J male mice at RIKEN were out-crossed to untreated DBA/2 J female mice to produce the original progeny (G1). Although heterozygous mice resulting from repeated backcrossing to the C57BL/6 J background were finally intercrossed to generate L100P/L100P homozygous mice, remaining ENU-induced mutations outside Disc1 and/or single nucleotide polymorphisms (SNPs) from DBA/2 J background may confound the causal relationship between L100P mutation and the behavioral phenotype. Disclosure of this information would provide a justification for the usage of ENU-mutagenesis to fully explain the phenotypic manifestation of the main causative mutation. Herein, we performed three experiments to better understand behavioral phenotypes of Disc1 L100P mice with reference to our new genetic data. First, we investigated the genetic background of the L100P strain that was reversely-deposited back to the Experimental Animal Division (EAD), RIKEN BioResource Center (BRC) by Roder’s group [24]. The genetic background of the deposited L100P/L100P homozygous pairs and their offspring born via intercrossing between the homozygotes were investigated. Second, L100P/L100P, L100P/+ and wild-type littermates (+/+), which were derived from the deposited L100P/L100P homozygous mice mated with the C57BL/6 J mice (details in Materials and Methods), were examined for schizophrenia-related behavioral phenotypes using open field and social interaction tests. Third, we conducted whole-exome resequencing analysis of the original G1 genome to screen for ENU-induced mutations in addition to the Disc1 L100P mutation. The results obtained were discussed in light of the validity and issue of Disc1 L100P mutant model.

Methods

Animals

Male and female homozygous Disc1 L100P/L100P mutant mice backcrossed to C57BL/6 J were deposited at the EAD at RIKEN BRC (Tsukuba, Japan, http://www.brc.riken.jp/lab/animal/en/) by another research group [24], and their offspring was bred to maintain the homozygosity (L100P/L100P). These L100P/L100P mutant male mice (Disc1 < Rgsc1390>) were obtained from RIKEN and then backcrossed to an inbred C57BL/6 J female (Japan Clea Co., Tokyo Japan) for one generation. The resultant L100P/+ progeny were intercrossed to generate L100P/L100P, L100P/+ and +/- littermates. Mice were weaned at postnatal day 25–28 and segregated by sex; were housed 2–4 per cage in a temperature-controlled (25 ± 1°C) and light-controlled room (light on 0600–1800 h) in plastic cages with ad libitum access to food and water.
PCR-based genotyping was conducted with a primer pair (common forward primer: 5'-CCTGTCCCCAGGACTGGCATC-3'; reverse primer for L100L wild-type: 5'-CAGGGGAGGGAGCTCTTGCA-3'; reverse primer for L100P mutants: 5'-CAGGGGACAGGGAGCTCT TC-3') and genomic DNA extracted from tail biopsies using Hot Sodium Hydroxide and Tris (HotSHOT) method [30]. Age-matched male L100P/L100P, L100P/+ and +/+ mice (12–16 weeks old) were compared in behavioral analyses. To compare wild-type littermates with inbred C57BL/6 J mice, 12–16-week-old male C57BL/6 J mice were purchased from Japan Clea Co. (Tokyo, Japan). All experiments were performed in accordance with the Guidelines for Care and Use of Laboratory Animals, Dokkyo University School of Medicine and conformed to all Japanese federal rules and guidelines.

Drug treatment
Methamphetamine hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan) was dissolved in saline and administered subcutaneously (s.c.) in a volume of 10 ml/kg.

Behavioral analysis
All behavioral analyses were recorded with a CCD camera and analyzed using video tracking software (ANY-maze ver. 4.82; Stoelting Co., USA). The apparatus was illuminated at approximately 300 lux. Mice were transferred to the experimental site at least 30 min before testing.

Spontaneous locomotor activity
Mice were placed individually in grey plastic cages (42 × 42 × 30 cm). Spontaneous locomotor activity was recorded in 5-min sessions during a 90-min test period, with distance traveled as the primary outcome measure.

Methamphetamine-induced locomotor activity
Mice were placed individually in grey plastic cages (42 × 42 × 30 cm) for a 30-min habituation session and then injected subcutaneously with methamphetamine (0.2, 0.5 or 1.0 mg/kg) or saline. Locomotor activity was recorded continuously during the 30-min habituation period and for 60 min after injection of saline or methamphetamine. To verify effects of genetic characteristics on behavioral phenotypes, methamphetamine-induced locomotor activity was compared among mice in the following experiments. In experiment 1, effects of L100P on methamphetamine-induced locomotor activity were compared in age-matched male littermates (L100P/L100P, L100P/+ and +/+) in mice with the results of wild-type littermates born to Disc1 L100P mutant line obtained from the experiment 1.

Sociability and social novelty preference tests
Effects of the L100P point mutation on social behavior were assessed according to previously described methods [24,31] with minor modifications. A three-chamber apparatus was constructed of clear acrylic sheets; each chamber had a size of 19 × 40 × 25 cm (width × depth × height). Each side chamber contained a wire-bar cup (Galaxy Cup, Spectrum Diversified Designs, Inc., OH, USA) placed on either side of the arena. Two dividing walls containing doors allowed access to each of the side chambers from the center chamber. The behavioral test consisted of three sessions: (1) habituation, (2) sociability and (3) social novelty preference. In the habituation session (1), the subject mouse was placed in the center chamber and allowed to freely explore all three chambers for 10 min. In the sociability session (2), following the habituation session, the subject mouse was introduced in the center chamber for 1 min with access to the side chambers blocked by white partitions. An unfamiliar male C57BL/6 J mouse (Stranger 1) was enclosed in the wire-bar cup in one of the side chambers. The location of Stranger 1 alternated among subject mice. On removal of partitions, the subject mouse was allowed to freely explore the entire apparatus for 10 min. Time spent within the interaction zone (an oblong area of 19 × 15 cm containing the wire-bar cup) and the number of entries into each chamber was measured. In the social novelty preference test (3) conducted after the sociability test, the subject mouse was placed in the center chamber for 1 min. An unfamiliar intruder male C57BL/6 J mouse (Stranger 2) was enclosed in the wire-bar cup in the other side chamber. Time spent in the interaction zones and the number of entries into each chamber was measured.

SNP-typing for genetic background of L100P/L100P mutant mice
We genotyped 117 SNPs, which were openly available in the SNP database of RIKEN BRC (http://ja.brc.riken.jp/lab/jmc/mapping.html), distributed at approximately 15-cM intervals over the entire mouse genome in 8 mice; 3 males and 1 female of the L100P/L100P deposited mice to the RIKEN EAD and 1 male and 3 female progeny from the deposited pairs to distinguish between C57BL/6 J and DBA/2 J genetic backgrounds.

Exome re-sequencing of the original G1 carrying the L100P mutation
We used the SureSelect Mouse All Exon Kit (Agilent, Santa Clara, CA, USA) to enrich whole exons from genomic DNA of the G1 mouse. We performed resequencing using SOLiD4 (Life Technologies, Carlsbad, CA, USA) as reported previously [32].
Statistical analysis
Statistical analysis was conducted using SPSS software (ver. 19, IBM Japan). Data were analyzed using paired t-tests, one-way ANOVA, two-way ANOVA or two-way repeated measures ANOVA as appropriate. The Greenhouse–Geisser correction for repeated measures was applied as necessary.

Results

Spontaneous locomotor activity
Spontaneous locomotor activity in the open field declined to a similar extent in all three genotypes (L100P/L100P, L100P/+ and +/+). Two-way repeated measures ANOVA revealed no significant genotype × time interaction (F(20.169, 554.636) = 0.884, \( p = 0.609 \)). Moreover, total distance traveled was similar among genotypes (Figure 1B). ANOVA revealed no main effect of genotype on total distance traveled (F(2, 55) = 0.785, \( p = 0.981 \)).

Methamphetamine-induced locomotor activity
Methamphetamine (0.5 and 1.0 mg/kg) significantly increased total distance traveled in all genotypes. ANOVA revealed a significant effect of drug treatment (F(3, 117) = 19.844, \( p < 0.001 \)); Tukey post hoc analysis showed a significant effect of 0.5 mg/kg (\( p < 0.01 \)) and 1.0 mg/kg (\( p < 0.001 \)) methamphetamine versus saline.

To test for effects of putatively existing genetic elements inherited from the DBA/2 J background on spontaneous and methamphetamine-induced locomotion, we compared wild-type progeny born to Disc1 L100P/+ mutants and inbred C57BL/6 J mice. There was no difference in either spontaneous or methamphetamine-induced locomotor activity (Figure 2G–H). ANOVA revealed no significant drug × genetic background interaction in either the 30-min habituation period (F(3, 59) = 0.779, \( p = 0.511 \)) or the 60-min post-injection period (F(3, 59) = 0.195, \( p = 0.898 \)). There was no main effect of genetic background on locomotor activity in either the habitation period (F(1,59) = 0.806, \( p = 0.373 \)) or the post-injection period (F(1.59) = 0.195, \( p = 0.660 \)). There was no main effect of drug treatment on total distance traveled during the habituation period (F(3, 59) = 0.244, \( p = 0.866 \)) (Figure 2G). Methamphetamine (0.5 and 1.0 mg/kg) significantly increased locomotor activity in both wild-type progeny of Disc1 L100P/+ mice and inbred C57BL/6 J mice (Figure 2H). There was a significant main effect of drug treatment on total distance traveled after the drug administration (F(3, 59) = 12.114, \( p < 0.001 \)) and significant effects of both methamphetamine 0.5 mg/kg (\( p < 0.01 \)) and 1.0 mg/kg (\( p < 0.001 \)) versus saline as revealed by Dunnett’s T3 post hoc analysis.

Sociability and social preference test
In the sociability session, two-way ANOVA revealed a significant interaction between genotype and the chambers in time spent in the interaction zones (F(2, 50 = 3.495, \( p < 0.05 \)). Post hoc analyses revealed no significant main effect of genotype for either side, but did show a significant main effect of preference for the stranger.
mouse (Stranger 1) for all genotypes (+/+: $p < 0.01$, L100P/+: $p < 0.001$, L100P/L100P: $p < 0.05$) on stay duration (Figure 3A). Two-way repeated measures ANOVA showed neither a significant genotype × chamber interaction for the number of entries nor a main effect of genotype, but did show a significant main effect of the chamber ($F(1.533, 76.638) = 403.012$, $p < 0.001$). Post hoc analyses revealed a significant main effect of preference for the center zone for the combined group (Empty: $p < 0.001$, Stranger 1: $p < 0.001$), but the number of transitions into the chamber containing Stranger 1 did not differ from the number of transitions into the empty chamber. In the social novelty preference session, two-way repeated measures ANOVA showed neither a significant genotype × side interaction in time spent in the interaction zones nor a significant main effect of genotype, but did reveal a
significant main effect of both sides ($F(1, 50) = 17.247, p < 0.001$). Post hoc analyses revealed a significant main effect of preference for the unfamiliar intruder (Stranger 2) compared with the by-then familiar mouse (Stranger 1) for the combined group ($p < 0.001$) (Figure 3B). Two-way repeated measures ANOVA showed neither a significant genotype × chamber interaction for the number of entries nor a significant main effect of genotype, but did show a significant main effect of the chamber ($F(1.669, 83.456) = 346.789, p < 0.001$). Post hoc analyses revealed a significant main effect of preference for the center zone for the combined group (Stranger 1: $p < 0.001$, Stranger 2: $p < 0.001$), but the number of transitions into the chamber containing Stranger 1 did not differ from the number of transitions into the chamber containing Stranger 2.

SNP typing for genetic background of L100P/L100P mutant mouse line (Disc1 < Rgsc1390>)

Despite backcrosses, all the genotyped eight L100P/L100P homozygotes harbored DBA/2 J-derived SNP alleles, which were either homozygous or heterozygous variant (Additional file 1: Figure S1). Genome-wide SNP panel analysis revealed that the numbers of SNPs indicative of homozygosity or heterozygosity for DBA/2 J ancestry varied among individual mice. Each mouse harbored 1–5 homozygous and 8–12 heterozygous loci for DBA/2 J-derived SNP alleles (Additional file 2: Table S1).

Exome resequencing of the original G1 genome carrying L100P mutation

There was no single nucleotide mutation in the Disc1 exons other than L100P. In addition to the L100P mutation, exome resequencing analysis revealed 117 single nucleotide mutations in the exome and exome-flanking regions of the genomic DNA from the original G1 mouse, the F1 progenitor born to an ENU-treated G0 mouse (Table 1). Disc1 L100P and two other mutations (in Rab11fip1, known as Rab-coupling protein and Nr3c2) were found on chromosome 8. Other variants included synonymous coding variants (5), nonsynonymous coding (27 including ryr3, encoding the intracellular Ca2+ release channel ryanodine receptor 3 and Rab11fip1), knockout-equivalent nonsense (Aks2 and TiliI), essential splice site variants (3), 5′ UTR (3, including Dyx1c1, a susceptibility gene for dyslexia), 3′ UTR (9), and intronic mutations (49). Base substitution spectra of the 117 ENU-induced mutations were A/T to T/A transversions (26.5%), A/T to G/C transitions (39.3%), G/C to A/T transversions (18.8%), A/T to C/G transversions (6.8%), and G/C to T/A transversions (8.5%).
| Gene symbol | Entrez Gene ID | Chromosome | Position (MM9) | Base change | Spectrum | Classification | Amino acid substitution |
|-------------|---------------|------------|----------------|-------------|----------|----------------|------------------------|
| Pkhd1       | 241035        | 1          | 20,530,613     | T → A       | AT to TA transversions | Intronic               |                        |
| Trak2       | 70827         | 1          | 58,975,614     | A → G       | AT to GC transitions | Essential splice site  |                        |
| Als2        | 74018         | 1          | 59,235,933     | A → T       | AT to TA transversions | Stop gained           | Y- > X                 |
| Ngeff       | 53972         | 1          | 89,379,202     | A → T       | AT to TA transversions | Nonsynonymous          | I- > N                 |
| 2310035C23Rik| 227446       | 1          | 107,592,127    | A → G       | AT to GC transitions | Intronic               |                        |
| Ikbke       | 56489         | 1          | 133,172,385    | C → A       | GC to AT transitions | Nonsynonymous          | A- > S                 |
| Cdc73       | 226641        | 1          | 172,724,783    | G → A       | AT to TA transversions | Intronic               |                        |
| Nmnat2      | 12287         | 2          | 24,506,396     | A → G       | AT to GC transitions | Nonsynonymous          | G- > W                 |
| Atf6        | 2230035C23Rik | 2          | 30,140,520     | A → G       | AT to GC transitions | Nonsynonymous          | I- > N                 |
| Gm5706      | 435657        | 2          | 40,854,333     | A → C       | AT to GC transversions | Intronic               |                        |
| Iars2       | 381314        | 1          | 187,127,260    | A → T       | AT to TA transversions | Nonsynonymous          | I- > N                 |
| Dhtkd1      | 209692        | 2          | 5,831,429      | A → G       | AT to GC transitions | Synonymous             |                        |
| Cacna1b     | 12287         | 2          | 24,506,396     | A → G       | AT to GC transitions | Intronic               |                        |
| Dolk        | 227697        | 2          | 30,140,520     | A → G       | AT to GC transitions | Synonymous             |                        |
| Lrp1b       | 435657        | 2          | 40,854,333     | A → C       | AT to CG transversions | Intronic               |                        |
| Neb         | 17996         | 2          | 52,114,402     | C → A       | GC to TA transversions | Nonsynonymous          | R- > L                 |
| Gm13559     | 674940        | 2          | 58,593,845     | A → G       | AT to GC transitions | Genic                  |                        |
| Ttn         | 2230035C23Rik | 2          | 58,975,614     | A → G       | AT to GC transitions | Nonsynonymous          | M- > I                 |
| Olfr1270    | 258987        | 2          | 89,989,253     | T → C       | AT to GC transitions | Synonymous             |                        |
| Ext2        | 14043         | 2          | 93,558,064     | C → T       | GC to AT transitions | Intronic               |                        |
| Ryr3        | 20192         | 2          | 112,701,983    | C → A       | GC to TA transversions | Nonsynonymous          | G- > W                 |
| Fmn1        | 14260         | 2          | 113,528,228    | T → A       | AT to TA transversions | Intronic               |                        |
| Seil12      | 228684        | 2          | 140,071,126    | C → T       | GC to AT transitions | Nonsynonymous          | A- > T                 |
| Setd7       | 73251         | 2          | 51,334,212     | T → C       | AT to GC transitions | Intronic               |                        |
| Itf80       | 68259         | 3          | 68,794,587     | A → T       | AT to TA transversions | Intronic               |                        |
| Asb17       | 66772         | 3          | 153,516,522    | G → A       | GC to AT transversions | 3' UTR                 |                        |
| Esrp1       | 207920        | 4          | 11,277,315     | G → T       | GC to TA transversions | Intronic               |                        |
| Gm136       | 214568        | 4          | 34,699,557     | C → T       | GC to AT transitions | Nonsynonymous          | A- > T                 |
| Nfx1        | 74164         | 4          | 40,952,108     | T → A       | AT to TA transversions | Intronic               |                        |
| Gm12608     | 664785        | 4          | 89,109,036     | T → A       | AT to TA transversions | Genic                  |                        |
| Tek         | 21687         | 4          | 94,516,374     | T → A       | AT to TA transversions | Nonsynonymous          | S- > T                 |
| Dnaic6      | 72685         | 4          | 101,279,103    | C → T       | GC to AT transitions | Intronic               |                        |
| Texnc12     | 66073         | 4          | 108,533,802    | T → A       | AT to TA transversions | Intronic               |                        |
| Gm12901     | 194197        | 4          | 122,935,965    | T → C       | AT to GC transversions | Genic                  |                        |
| Tmprss11e   | 243084        | 5          | 87,156,236     | C → T       | GC to AT transversions | Intronic               |                        |
| Ccng2       | 12452         | 5          | 93,697,763     | T → C       | AT to GC transversions | Nonsynonymous          | I- > T                 |
| Calcr       | 12311         | 6          | 3,650,125      | A → G       | AT to GC transitions | Essential splice site  |                        |
| C130060K24Rik| 243407       | 6          | 65,406,380     | A → G       | AT to GC transversions | Nonsynonymous          | K- > E                 |
| Wnk1        | 232341        | 6          | 119,956,300    | A → T       | AT to TA transversions | Intronic               |                        |
| Vwf         | 22371         | 6          | 125,576,322    | G → A       | GC to AT transversions | Nonsynonymous          | M- > I                 |
| Klrk1       | 27007         | 6          | 129,566,794    | G → A       | GC to AT transversions | Intronic               |                        |
| Olfr1336    | 258917        | 7          | 6,413,462      | A → T       | AT to TA transversions | Nonsynonymous          | T- > S                 |
| Gene     | Chromosome | Position | Nature          | Type         | Category          | Description                        |
|----------|------------|----------|-----------------|--------------|------------------|------------------------------------|
| Nlrp9a   | 233001     | 7        | AT to CG        | Nonsynonymous| E- > A           | AT to CG transversions             |
| Arhgap33 | 233071     | 7        | GC to AT        | Synonymous   |                  | GC to AT transitions               |
| Gucy2d   | 14918      | 7        | AT to TA        | Exonic       |                  | AT to TA transversions             |
| Rab11fip1| 75767      | 7        | AT to TA        | Nonsynonymous| P- > L           | AT to TA transversions             |
| Nrlp9a   | 233001     | 7        | AT to CG        | Nonsynonymous| E- > A           | AT to CG transversions             |
| Arhgap33 | 233071     | 7        | GC to AT        | Synonymous   |                  | GC to AT transitions               |
| Gucy2d   | 14918      | 7        | AT to TA        | Exonic       |                  | AT to TA transversions             |
| Rab11fip1| 75767      | 7        | AT to TA        | Nonsynonymous| P- > L           | AT to TA transversions             |
| Disc1    | 110784     | 8        | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Prdm10   | 382066     | 9        | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Olfr909  | 100043200  | 9        | AT to GA        | Upstream     |                  | AT to GA transversions             |
| Olfr44   | 258716     | 9        | AT to CG        | Intronic     |                  | AT to CG transversions             |
| Pou2f3   | 18988      | 9        | GC to AT        | Essential splice site |              | GC to AT transitions               |
| Dyx1c1   | 67685      | 9        | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Unc13c   | 208898     | 9        | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Srebp1   | 215351     | 9        | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Heca     | 380629     | 10       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Olig3    | 94222      | 10       | AT to CG        | 3' UTR       |                  | AT to CG transversions             |
| Srebp1   | 109205     | 10       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Sf3a2    | 56442      | 10       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Cyp27b1  | 13115      | 10       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Tns3     | 319939     | 11       | AT to TA        | 5' UTR       |                  | AT to TA transversions             |
| Il5      | 16191      | 11       | AT to GA        | Intronic     |                  | AT to GA transversions             |
| 4930404A10Rik | 74847 | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Ulk2     | 29869      | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| S1c13e5  | 237831     | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Ap2b1    | 71770      | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Lrrc46   | 69297      | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Erbb2    | 13866      | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Krt40    | 40622      | 11       | AT to GA        | Intronic     |                  | AT to GA transversions             |
| Syngr2   | 20973      | 11       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Gm9229   | 665339     | 12       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Mark3    | 17169      | 12       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Igf      | 111507     | 12       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Tcrg-V4  | 21638      | 13       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| A0ah     | 27052      | 13       | AT to GA        | Intronic     |                  | AT to GA transversions             |
| Slc6a3   | 13162      | 13       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Gtzh2    | 23894      | 13       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Ccd1c25  | 76041      | 13       | AT to GA        | Intronic     |                  | AT to GA transversions             |
| 3425401B19Rik | 100504518 | 14      | AT to GA        | Intronic     |                  | AT to GA transversions             |
| Mcpt1    | 17224      | 14       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Atp8a2   | 50769      | 14       | AT to GA        | Intronic     |                  | AT to GA transversions             |
| Sorbs3   | 20410      | 14       | AT to TA        | Intronic     |                  | AT to TA transversions             |
| Epst1    | 108670     | 14       | AT to GA        | Intronic     |                  | AT to GA transversions             |
| Diap3    | 56419      | 14       | AT to GA        | Intronic     |                  | AT to GA transversions             |
Table 1 List of ENU-induced mutations in G1 mouse of Disc1<rgsc1390> (Continued)

| Gene     | Chromosome | Start (base) | End (base) | A → Base | Type | Region |
|----------|------------|--------------|------------|----------|------|--------|
| Vps13b   | 15         | 35,570,473   | 35,570,473 | A → G    | AT to GC transitions | Intronic |
| Ubr5     | 15         | 37,898,017   | 37,898,017 | A → G    | AT to GC transitions | 3' UTR |
| Micall1  | 15         | 78,960,399   | 78,960,399 | G → A    | GC to AT transitions | Intronic |
| Cyp2d12  | 15         | 82,387,610   | 82,387,610 | A → T    | AT to TA transitions | Intronic |
| Ttl1     | 15         | 83,320,020   | 83,320,020 | G → A    | GC to AT transitions | Stop gained |
| Prkag1   | 15         | 98,643,692   | 98,643,692 | A → G    | AT to GC transitions | 3' UTR |
| Gm7638   | 15         | 11,185,895   | 11,185,895 | C → T    | GC to AT transitions | Genic |
| Myh11    | 15         | 14,269,121   | 14,269,121 | A → G    | AT to GC transitions | Intronic |
| Gm6931   | 15         | 49,425,860   | 49,425,860 | T → A    | AT to TA transitions | Genic |
| Oifr125  | 17         | 37,972,895   | 37,972,895 | T → C    | AT to GC transitions | Nonsynonymous |
| Enpp5    | 17         | 44,222,094   | 44,222,094 | T → C    | AT to GC transitions | Intronic |
| Gm7334   | 17         | 50,838,499   | 50,838,499 | T → C    | AT to GC transitions | Genic |
| Sult1c1  | 17         | 54,101,361   | 54,101,361 | T → C    | AT to GC transitions | 3' UTR |
| 2700099C18Rik | 17 | 95,163,195 | 95,163,195 | C → T | GC to AT transitions | Genic |
| Fhod3    | 17         | 25,248,741   | 25,248,741 | A → G    | AT to GC transitions | Nonsynonymous |
| Kdm3b    | 17         | 34,982,966   | 34,982,966 | T → G    | AT to CG transitions | Intronic |
| Pcdhb6   | 18         | 37,493,679   | 37,493,679 | A → G    | AT to GC transitions | Upstream |
| Pcdha1   | 18         | 37,912,242   | 37,912,242 | C → T    | GC to AT transitions | Intronic |
| Spink10  | 18         | 62,820,759   | 62,820,759 | G → T    | GC to TA transitions | 3' UTR |
| Cst6     | 19         | 5,344,061    | 5,344,061  | A → T    | AT to TA transitions | Intronic |
| Ganab    | 19         | 8,987,570    | 8,987,570  | T → A    | AT to TA transitions | Intronic |
| Cpsf7    | 19         | 10,607,448   | 10,607,448 | G → T    | GC to TA transitions | Intronic |
| Ms4a5    | 19         | 11,358,379   | 11,358,379 | A → T    | AT to TA transitions | Upstream |
| Ms4a14   | 19         | 11,382,160   | 11,382,160 | A → T    | AT to TA transitions | Genic |
| Tjp2     | 19         | 24,194,331   | 24,194,331 | T → C    | AT to GC transitions | Intronic |
| Cyp2c29  | 19         | 39,405,010   | 39,405,010 | T → C    | AT to GC transitions | 3' UTR |
| Til2     | 19         | 41,257,593   | 41,257,593 | T → C    | AT to GC transitions | Intronic |
| Sorcs3   | 19         | 48,768,473   | 48,768,473 | A → C    | AT to CG transitions | Nonsynonymous |

**Discussion**

The present study compared schizophrenia-related behavior among L100P mutant mice (L100P/L100P and L100P/+) and their wild-type littermates, and inbred C57BL/6J mice by testing spontaneous locomotor activity, methamphetamine-induced locomotor activity in the open field [33], and sociability/social novelty preference in the social interaction [34]. All behavior was comparable among L100P/L100P, L100P/+ and wild-type littermates; the results were partially inconsistent with previous studies using mice originated from the same G1 founder [24,25]. To assess effects of genetic background (C57BL/6J vs DBA/2J), we conducted comprehensive genotyping of the original G1 mouse and its homozygous offspring obtained by backcrossing to C57BL/6J; the same strain used for backcrossing in the previous report characterizing the L100P mutant [24]. Our genetic analyses demonstrated that both the L100P/L100P homozygous mice deposited by Roder's group [24] into the EAD at RIKEN and their homozygous progeny still harbored a small number of SNPs inherited from the DBA/2J strain (the mother of the G1 founder). Whole-exome resequencing of the original G1 genome also revealed an additional 116 single nucleotide variants induced by ENU.

Disc1 L100P was originally identified in one G1 progeny of several thousand screened for Disc1 mutants from a G1 male genomic DNA archive produced by breeding ENU-mutagenized C57BL/6J males and untreated DBA/2Jcrl females [24]. Live heterozygous mice carrying the L100P mutation were recovered by in vitro fertilization of C57BL/6J eggs with the cryopreserved sperm of the corresponding G1 progeny. In the study reported by Clapcote et al. [24], mutant mice were then backcrossed to the C57BL/6J background for at least six generations.
before intercrossing L100P/+ mice to generate homozygous mutants for behavioral tests. In theory, the backcrossed congenic mutant strain should have lost most of the other ENU-induced mutations and SNPs derived from DBA/2 J; however, the rate of SNP and mutant loss per generation has not been examined experimentally.

DBA/2 J and C57BL/6 J inbred strains were shown to exhibit differential sensitivity to psychostimulants [35,36] without significant differences in basal locomotor activity or sociability [31]. Thus, residual DBA/2 J SNPs, if any still exist in the analyzed mice, could conceivably influence the behavioral test results. Genomic analysis revealed a significant number of DBA/2 J SNPs remaining in the backcrossed Disc1 L100P mice. Among 117 loci tested, nineteen were still polymorphic in the eight L100P/L100P mice deposited at RIKEN and their progeny (Additional file 2: Table S1). The average frequency of DBA/2 J SNP alleles in the eight L100P/L100P mice was 6.63% (5.56%–8.12%, Additional file 2: Table S1); a rate of genetic vestige was 4.25-fold higher than the theoretical estimate of 1.56% [24]. The question arises as to whether any single DBA/2 J SNP or combination influences Disc1 L100P locomotor activity in the open field compared with the congenic background strain. An important feature of the present study was the inclusion of commercially available inbred C57BL/6 J mice in addition to wild-type littermates derived from Disc1 L100P mutants to assess effects of genetic background on behavioral phenotype. This experimental design could enhance the sensitivity for detecting effects of genetic background and ENU-induced mutations. However, our behavioral analyses showed that neither basal nor methamphetamine-induced locomotor activity differed between wild-type mice derived from Disc1 L100P mutants and inbred C57BL/6 J mice. This finding suggests that locomotor activity is not measurably affected by residual DBA/2 J SNPs. Subsequently, using the exome resequencing analysis, we identified 116 previously unreported mutations in the exome of the original G1 male, although L100P was the only single mutation found in the coding sequence of the Disc1 gene. It has been estimated that approximately 64%, 26% and 10% of ENU-induced single base pair mutations in coding regions are missense, synonymous, and nonsense mutations, respectively [28,37,38]. Intriguingly, the frequencies of base substitutions in the G1 exome decreased in the rank order of A/T to G/C transition > AT/TA transversion > G/C to A/T transition > G/C to T/A transversion > A/T to C/G transversion, consistent with estimates for the gene-driven approach [39]. Furthermore, the distribution of single base pair mutations in the G1 exome predicted to generate amino acid variants was approximately consistent with previous studies [37,38].

Locomotor activity in the open field did not differ between L100P mutant mice (homozygotes and heterozygotes) and wild-type littermates, consistent with the report by Shoji et al. [27]. In contrast, Clapcote et al. [24] and Lipina et al. [25] reported higher locomotor activity in L100P/L100P mice than in wild-type littermates during the first 30 min, after introduction into the open field (but not thereafter). The higher locomotor activity of L100P mice may represent slower habituation to a novel environment. In addition, although Lipina et al. [25] reported higher locomotor activity in L100P mice following injection of amphetamine compared with that of wild-type littermates, we did not observe enhanced locomotion after an acute challenge with methamphetamine. Although the reasons for this discrepancy with previous reports remains to be explored, one possibility is that critical mutation(s) other than L100P may have been missed during maintenance of the strain after submission to the RIKEN EAD and/or the single backcrossing of homozygous (L100P/L100P) mutant males obtained from RIKEN to female inbred C57BL/6 J mice. As shown by the distribution of SNPs, the genetic background in the deposited L100P homozygotes was heterozygous; therefore, such heterogeneity may be fixed to either allele in the offspring, even after many generations of maintenance.

The enhanced release of amphetamine-evoked dopamine consistently reported in neuroimaging studies of schizophrenia [40-42] may be of relevance to this model of schizophrenia. However, Lipina et al. [25] did not find greater striatal release of dopamine following amphetamine injection in L100P mice. In the adult brain, Disc1 is expressed in the hippocampus and not in the nucleus accumbens [43,44]. The hyperactivity of subcortical dopaminergic neurons is believed to be conveyed by glutamatergic afferents from the hippocampus to the nucleus accumbens, which in turn regulates release of dopamine and the activity of dopaminergic neurons in the ventral tegmental area [45,46]. Another possible mechanism relates to disruption of the interaction between the hippocampus and prefrontal cortex [47]. Although subtle cytoarchitectonic changes have been reported in frontal cortical neurons [48], Disc1 L100P mice lack the reduced number of parvalbumin-positive interneurons observed in Disc1 transgenic models [12,18].

Sociability and social novelty preference tests used in the present study are used frequently to investigate disrupted social interactions in mouse models of schizophrenia [34]. The impoverished social interaction in Disc1 mice is believed to be a relevant quantitative trait of social withdrawal in schizophrenia [14]. Consistent with Clapcote et al. [24], we found that the three L100P genotypes did not differ in sociability or social novelty preference. Intriguingly, sociability was markedly impaired in conditional transgenic lines with either constitutive expression of [14] or inducible expression of a mutant DISCI C-terminal fragment during the early postnatal
We hypothesize that transgenic mice over-expressing human truncated DISC1 protein exhibit a dominant-negative effect that may lead to diminished binding between the DISC1 interacting proteins and relevant domains of DISC1 protein, thus altering the behavioral phenotype [15]. Based on these results, the relevance of Disc1 L100P as a mouse model of schizophrenia should be re-evaluated.

A recent review stressed that ENU-mutagenized mice are useful for establishing novel models of complex human diseases, including neuropsychiatric diseases [49]. A range of ENU-generated mutations based on a phenotype-driven approach has an advantage over a complete loss-of-function mutation in recapturing the pleiotropic nature of human neuropsychiatric diseases and the subtlety of manifestation [49]. Thus, both disease-causative and other unexpected variants should be made publicly available [28,29]. This phenotype-driven approach requires high-throughput behavioral screening to link mutation with function. One promising candidate screening methodology is the identification of outliers by neuroimaging. For example, only a few studies on developmental mouse models of psychiatric illness, including those focusing on DN-DISC1 models, have highlighted enlargement of the lateral ventricle without a marked change in overall brain size [16-18,50]. Phenotype screening based on standardized behavioral tests [31,51] combined with neuroimaging [50] to detect enlargement of the lateral ventricle may be an efficacious approach for the identification of robust schizophrenia models.

Several limitations of this study should be considered. First, we did not conduct a mouse comprehensive battery of behavioral tests including cognitive tasks and PPI but only tests of locomotor activity in a novel environment, psychostimulant-induced behavior and sociability in the three-chamber test. Therefore, our results do not clarify effects of the L100P amino acid substitution in exon 2 on other behavioral phenotypes related to psychiatric disorders including schizophrenia. Second, handling, experimental protocols, rearing environment and other environmental factors may influence behavioral test results and contribute to variability across studies [51,52]. Third, L100P mice used in the current study were not genotyped for all ENU-induced mutations found in the exome and vicinity of the G1 genome, some of which may have been transmitted to progeny despite numerous backcrosses [24]. Given that 117 ENU-induced mutations in the G1 mouse identified with the whole-exome resequencing, it is plausible that 50-fold excess or more than 5,000 of ENU-induced mutations should exist in the entire G1 genome. Although detailed genetic information on models relevant to human disease should be freely available, routine implementation of whole-genome sequencing awaits measures for improved cost-effectiveness. However, as most of the ENU-induced mutations in G1 map to chromosomes other than chromosome 8 (containing Disc1), it is likely that they are transmitted to progeny to some extent regardless of the breeding process used to select mice harboring Disc1 L100P. Although it may not be possible to investigate whether genetic background or other ENU-induced mutations have any effects on behavior because of a generalised lack of information on genotype differences among any of their mouse lines or offspring, further behavioral and genetic studies are warranted. For example, generation and breeding of another mouse line harboring Disc1 L100P, which can be accomplished by in vitro fertilization by the cryopreserved sperm of the G1 progeny owned by RIKEN BRC, may facilitate full elucidation of a relationship between genetic components, including Disc1 L100P mutation per se, and behavioral phenotypes, related with schizophrenia in this mouse line.

**Conclusion**

The present study using behavioral genetic approaches provides an insight into the role of Disc1 L100P and other single nucleotide variants in behavioral phenotypes associated with psychiatric disorders such as schizophrenia. Our present findings suggest that causal attribution of the discrepancy in behavioral phenotypes of the Disc1 L100P mutant mouse line existing among different research groups, including our own, needs to be cautiously investigated in further studies by taking into account the effect(s) of other ENU-induced mutations and/or SNPs from DBA/2 J. Further behavioral genetic analyses are needed to elucidate the cause of behavioral variance associated with Disc1 L100P strain. Using a polygenic animal model including ENU mutagenesis provides an efficacious approach to explore the relationship of variants to behavioral phenotypes associated with polygenic and multifactorial disorders such as psychiatric disorders.

**Additional files**

Additional file 1: Figure S1. Pedigree of Disc1 < Rgsc1390 > on RIKEN BRC.

Additional file 2: Table S1. Characteristics of mouse strain SNPs in Disc1<Rgsc1390>.

**Competing interests**

Kazuumi Akiyama has received consulting honoraria from Taisho Toyama Pharmaceutical Co., Ltd. This consultancy is totally compliant with the regulation regarding the Conflict of Interest of Dokkyo Medical University School of Medicine, and had no further role in the study design, the collection, analysis and interpretation of data, the writing of the report, or the decision to submit the paper for publication. None of the remaining authors declare any conflicts of interest.
Authors’ contributions
YA was involved in the design of the study, performed the behavioral experiment, and drafted the manuscript. RF and YG were involved in establishing of the original DisC1 L100P line and discovery of ENU-induced mutations in the exome of the original G1. IM and SW were involved in genotyping of a panel of SNPs in the entire genome of L100P/L100P mice. KM and AW were involved in the maintenance of L100P/L100P line that was deposited from the research group (Clapcote et al., 2007) into the EAD at RIKEN. KA was involved in editing the manuscript. All authors read and approved final manuscript.

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