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Deficiencies in the region syntenic to human 21q22.3 causes cognitive deficits in mice

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Abstract

Copy-number variation in the human genome can be disease-causing or phenotypically neutral. This type of genetic rearrangement associated with human chromosome 21 (Hsa21) underlies partial Monosomy 21 and Trisomy 21. Mental retardation is a major clinical manifestation of partial Monosomy 21. To model this human chromosomal deletion disorder, we have generated novel mouse mutants carrying heterozygous deletions of the 2.3- and 1.1-Mb segments on mouse chromosome 10 (Mmu10) and Mmu17, respectively, which are orthologous to the regions on human 21q22.3, using Cre/*loxP*-mediated chromosome engineering. Alterations of the transcriptional levels of genes within the deleted intervals reflect gene-dosage effects in the mutant mice. The analysis of cognitive behaviors shows that the mutant mice carrying the deletion on either Mmu10 or Mmu17 are impaired in learning and memory. Therefore, these mutants represent mouse models for Monosomy 21-associated mental retardation, which can serve as a powerful tool to study the molecular mechanism underlying the clinical phenotype and should facilitate efforts to identify the haploinsufficient causative genes.

INTRODUCTION

Copy-number variation of chromosome regions exists in mammalian genomes (Adams et al. 2005; Freeman et al. 2006; Sebat et al. 2004; Zhang et al. 2009). Although most copy-number variations do not cause discernible phenotypes, a subset of these alterations are not neutral and are responsible for developmental and/or physiological abnormalities. The copy-number alterations associated with Hsa21 result in two human syndromes: Trisomy 21 (Down syndrome) and partial Monosomy 21. While Trisomy 21 is the most frequent live-born human aneuploidy, *de novo* deletions associated with partial Monosomy 21 occur at a relatively low rate in the human population. Partial Monosomy 21 causes various developmental anomalies, including mental retardation and cardiac defects in patients (Barnicoat et al. 1996; Bartsch et al. 1994; Chettouh et al. 1995; Ehling et al. 2004; Huret et al. 1995; Korenberg et al. 1991; Lyle et al. 2009; Nielsen and Tranebjaerg 1984; Theodoropoulos et al. 1995; Valero et al. 1999; Yamamoto et al. 1979). However, the underlying mechanism of partial Monosomy 21 is unknown.

In an attempt to map the critical genomic regions for the disease phenotypes of partial Monosomy 21, several groups examined relatively large numbers of human partial Monosomy 21 cases (Chettouh et al. 1995; Huret et al. 1995; Katzenstein et al. 2009; Lyle et al. 2009). In these studies, the data based on partial Monosomy 21 patients were used to map genomic regions associated with the disease phenotypes, including mental retardation. Partial Monosomy 21 cases due to interstitial deletions are most desirable for genetic mapping. Unfortunately, some patients with partial Monosomy 21 also carry anomalies involving other human chromosomes, such as segmental trisomies and deletions (Chettouh et al. 1995; Huret et al. 1995; Katzenstein et al. 2009; Lyle et al. 2009). The potential impact of other genomic alterations on phenotypes complicates the interpretation of the genotype-phenotype correlations inferred in some of these studies. While studies of human cases have been useful, causative genes have not been isolated. This is due principally to the small number of patients and to the resulting lack of a complete and informative set of partial Monosomy 21 cases (Lyle et al. 2009).

Given the difficulty in identifying causative genes by relying on patient studies, mouse-based approaches could be employed, which are based on syntenic conservation between the regions on Hsa21 and three regions located on Mmu10, Mmu16 and Mmu17. As gene-rich

genomic segments, the syntenic regions for the proximal and distal parts of human 21q22.3 are located on Mmu17 and Mmu10, respectively (Fig. 1; Supplementary Tables 1, 2) (www.ensembl.org). Deletions of key genes in these regions may cause perturbations of the critical biological processes in various organs, which may in turn lead to abnormal phenotypes associated with partial Monosomy 21, such as cognitive deficits. To test these predictions and to establish a genotype-phenotype relationship, we generated and characterized two mutant mouse strains that carry deletions containing all the orthologs of the Hsa21 genes in the syntenic regions of Mmu10 and Mmu17.

MATERIALS AND METHODS

Generation of *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mice using Cre/*loxP*-mediated chromosome engineering

It is required that *Hprt*-deficient mouse embryonic stem (ES) cells be used for Cre/*loxP*-mediated chromosome engineering. Currently, only the ES cell lines isolated from 129 mice carry *Hprt*-null alleles, such as AB2.2 and HM1 (Bradley et al., 1999; Magin et al., 1992). In this study, we used AB2.2 cells isolated from 129SvEv mice. To generate *Df(10)1Yey*, the targeting vector pTV(10)1EP1 (Fig. 2a) was isolated from the 3'*HPRT* vector genomic library (Zheng et al. 1999) using PCR primers 5'-GCTGGCCTCTGA CTGTCTTGTTCC-3' (forward) and 5'-GGGGCCATGTCACTGTCCTGCTA-3' (reverse). The targeting vector pTV(10)1EP2 (Fig. 2a) was isolated from the 5 '*HPRT* vector genomic library using PCR primers 5'-TCTGGAGCCATCTTCATAAAGCTG-3' (forward) and 5'-AACTCACCGGTGATTGTGAAGA-3' (reverse). *LoxP* was targeted by pTV(10)1EP1 and pTV(10)1EP2 to the regions proximal to *Prmt2* and distal to *Pdxk*, respectively (Figs. 1 and 2a). To generate *Df(17)1Yey* in mouse ES cells, the targeting vector pTV(17)1EP1 was generated by inverting the orientation of the mouse genomic insert in MICER clone MHPP309a23 using *Asc*I that flanks the insert (Fig. 2e) (Adams et al. 2004). To generate a gap probe (Probe 17B) for Southern blot analysis, a 2.7-kb *Afl*II-*Afl*II fragment was also removed from the mouse genomic insert in pTV(17)1EP1. We used MICER clone MHPN353c19 as the targeting vector pTV(17)1EP2 (Adams et al. 2004). pTV(17)1EP1 and pTV(17)1EP2 were used for inserting *loxP* into the regions proximal and distal to *Abcg1* and *Rrp1b*, respectively (Figs. 1 and 2e). ES cell culture, gene-targeting and induction of Cre/*loxP*-mediated recombination, Southern blot analysis and injection of ES cells into blastocysts were performed as described previously (Yu et al. 2006).

Fluorescent *in situ* hybridization (FISH) and real-time quantitative PCR

FISH analysis was performed as described previously (Yu et al. 2006). To detect the chromosomal deletion between *Prmt2* and *Pdxk*, BAC clone RP23-175G11 was labeled with digoxigenin and detected with anti-digoxigenin–rhodamine antibody. BAC clone RP23-150E20 was labeled with biotin and detected with fluorescin isothiocyanate-avidin (Fig. 2c, d). To detect the chromosomal deletion between *Abcg1* and *Rrp1b*, BAC clone RP23-304G12 was labeled with digoxigenin and detected with anti-digoxigenin–rhodamine antibody. BAC clone RP23-103F2 was labeled with biotin and detected with fluorescin isothiocyanate-avidin (Fig. 2g–i). Real-time quantitative PCR was carried out as previously reported (Li et al. 2007).

Behavioral tests

The mutant mice and their littermates were maintained at a temperature- and humidity-controlled animal facility. All mice used in the experiments were 2–4 months old. Before behavioral experiments, each mouse was pre-handled for 2 minutes every day for a week. The experimental procedures were approved by the Institutional Animal Care and Use Committee.

Open field observations were carried out in a 40 × 40 × 30 cm Plexiglas box. Each mouse was individually placed in the center of an open field and the following behavioral parameters were recorded during a 5-min test interval: 1) total distance traveled; 2) time spent moving; 3) number of rearing events (vertical activity). Average speed was calculated by dividing the total distance by the time spent moving. Behavioral parameters in the open field were analyzed using HVS Field 2020, an imaging-tracking and analysis system (HVS Image Ltd., Twickenham, Middlesex, UK). The number of rearings was counted manually.

A standard Morris water maze test was carried out in a circular pool (152 cm in diameter) of water at 25 ± 1° C (Clapcote et al. 2005; Clapcote and Roder 2004; D'Hooge et al. 1997; McIlwain et al. 2001). The experimental data were collected and analyzed using HVS Water 2020, an imaging-tracking and analysis system (HVS Image Ltd.). Each mouse had four trials each day. Visible-platform and hidden-platform training trials were carried out on day 1 and days 2–8, respectively. The amount of time spent finding the platform (latency), the distance traveled (path-length) and swimming speed were recorded. On day 9, a probe test was performed in which the platform was removed from the water and each mouse was allowed 60 sec to search the pool. The time spent in each quadrant was measured.

The contextual and cued fear conditioning test was performed as described previously (Clapcote et al. 2005; Lu et al. 1997) using the Fear Conditioning Video Tracking System (Med Associates Inc., St. Albans, VT). The contextual tests were performed 24 hrs as well as 72 hrs after the mice were first exposed to the fear conditioning test chamber.

The foot-shock sensitivity test was performed using the fear conditioning test chamber. A foot-shock was delivered every 10 sec starting at 0.05 mA with a 0.05 mA increment between each shock (Rosa et al. 2007). The minimal level of current needed to elicit flinching or vocalizing was recorded.

Statistical methods

The data from the open field, Morris water maze probe test, contextual and cued fear conditioning tests and foot-shock sensitivity test were subjected to a one-way ANOVA between genotypes. ANOVA did not detect any effects from gender in all the behavioral tests, so the data from males and females were pooled and analyzed together for these experiments. Data from the seven-day training trials of the Morris water maze hidden-platform version were analyzed using a two-way (genotype×day) ANOVA with the genotype as a between-subjects factor and the day as a repeated-measures factor. All values reported in the text and figures were expressed as means ± S.E.M.

RESULTS AND DISCUSSION

The entire Hsa21 syntenic region on Mmu10 spans approximately 2.3 Mb and contains approximately 41 orthologs, with the *Prmt2* and *Pdxk* genes located at the proximal and distal ends, respectively. The entire Hsa21 syntenic region on Mmu17 spans approximately 1.1 Mb and contains approximately 19 orthologs, with the *Abcg1* and *Rrp1b* genes located at the proximal and distal ends, respectively. (Fig. 1; Supplementary Tables 1, 2) (http://www.ensembl.org). To generate a deletion of the syntenic region on Mmu10, *loxP* was targeted to regions proximal to *Prmt2* and distal to *Pdxk* in the genome of AB2.2 mouse ES cells (Fig. 2a). *Df(Prmt2-Pdxk)1Yey*, abbreviated as *Df(10)1Yey*, was generated by transfecting pOG231, a Cre-expression vector, into double-targeted ES cells (Fig. 2a; see Materials and Methods). A similar strategy was used to generate *Df(Abcg1-Rrp1b)1Yey*, abbreviated as *Df(17)1Yey*, by targeting *loxP* to regions proximal to *Abcg1* and distal to *Rrp1b*, in AB2.2 ES cells (Fig. 2e; see Materials and Methods). Chimeras were generated by injecting the mutant ES cells into blastocysts isolated from C57BL/6J females, as described

previously (Bradley 1987). The chimeric males were first mated with C57BL/6J females to assess germline transmission efficiency of the engineered deletions based on coat-color chimerism and Southern blot-based genotyping of the agouti progeny (Fig. 2b, f). The genotypes of the desired mutant mice were also confirmed by FISH analysis (Fig. 2c, d, g–i). The chimeric males with a high efficiency of germline transmission of *Df(10)1Yey* were identified, and they were mated with 129SvEv females to generate *Df(10)1Yey*/+ mice in the 129SvEv strain background. However, the chimeric males generated using the ES cells carrying *Df(17)1Yey* have a very low efficiency of germline transmission, so *Df(17)1Yey*/+ mice from the chimeras were established only in the 129SvEvxC57BL/6JF1 strain background. These *Df(17)1Yey*/+ mice were backcrossed to wild-type 129SvEv mice for seven generations, and the *Df(17)1Yey*/+ mice were then maintained by sibling mating. Both *Df(10)1Yey*/+ mice and *Df(17)1Yey*/+ mice appeared normal, with body weights similar to their wild-type littermates. Heterozygous mutant fetuses at E18.5 and postnatal mice from mating *Df(10)1Yey*/+ males or *Df(17)1Yey*/+ males to wild-type females were present at normal Mendelian ratios, suggesting that no haploinsufficient lethal gene is located in the regions.

We used real-time quantitative PCR to analyze the mRNA levels for five and two genes located within the deleted intervals in *Df(10)1Yey*/+ mice and *Df(17)1Yey*/+ mice, respectively. Our results showed that the deletions led to reduced mRNA levels in the brain for these genes (Table 1), indicating gene-dosage decreases associated with the deletions.

We used *Df(10)1Yey*/+ mice (n=16; 7 males and 9 females), the wild-type littermates of *Df(10)1Ye*/+ mice (n=16; 5 males and 11 females), *Df(17)1Yey*/+ mice (n=15; 10 males and 5 females) and the wild-type littermates of *Df(17)1Yey*/+ mice (n=15; 10 males and 5 females) for behavioral analysis. Although 129 mice have a reputation as inferior performers in the behavioral test, the 129SvEv substrain has been shown to be an excellent strain background for the Morris water maze test as well as the contextual and cued fear conditioning tests based on the data from Jacqueline Crawley's laboratory (Crawley 2000; Holmes et al. 2002) and one of our own laboratories (Clapcote and Roder, 2004). We first performed a simple assessment of the mutant mice and their wild-type littermates in the open field and found they exhibited normal general activity level, gross locomotor activity and exploration habits ($p>0.05$) (Fig. 3). To examine the effect of *Df(10)1Yey*/+ and *Dp(17)1Yey*/+ on learning and memory, we compared the mutant mice to their wild-type littermates in the Morris water maze tasks. We first compared the mice using the visible platform version of the Morris water maze, which does not test hippocampal function. There were no differences between the mutant mice and their wild-type littermates in latency and path-length in finding the visible-cued platform (Fig. 4). This result indicates that the mutant mice appeared to be normal with respect to vision and motivation in finding the platform. We next compared the mice in the hidden platform version, which is a spatial navigation task that requires the mice to swim in a pool of opaque water until they locate a submerged platform. This type of learning has been considered hippocampus-dependent because of its sensitivity to hippocampal lesions (Sarnyai et al. 2000). Mean values for the mutant mice and their wild-type littermates in the acquisition phase of Morris water maze training in the hidden platform version are shown in Fig. 4. The mean latency for each genotype significantly decreased with training, but both *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mice had a longer average latency than their wild-type littermates. The increased latency by *Df(17)1Yey*/+ mice could be explained by slower swimming speed (Fig. 4g). However, the abnormal latency of the *Df(10)1Yey*/+ mice could be caused by impaired spatial learning since *Df(10)1Yey*/+ mice showed increased swimming speed (Fig. 4a, c). Such possibilities are supported by the observations that *Df(10)1Yey*/+ mice, but not *Df(17)1Yey*/+ mice, took a longer path-length in locating the platform ($p<0.001$). In the probe test conducted on the day after the training period, both *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mice spent a shorter time

in the target quadrant (Northeast, NE) than their wild-type littermates (p=0.0006 and p=0.086, respectively). These results provide conclusive evidence that *Df(10)1Yey*/+ mice are impaired in spatial learning and memory.

We performed additional assessments of the mutant mice's learning and memory based on contextual and cued fear conditioning. Cued fear conditioning tests the amygdalar function, whereas contextual fear conditioning tests both the amygdalar and hippocampal functions (Holland and Bouton 1999; Logue et al. 1997; Phillips and LeDoux 1992). Mice of each genotype had a baseline (test-naïve) freezing level of under 2% prior to the presentation of the audible tone and foot-shock (Fig. 5a, c). There was no difference between the mutant mice and their wild-type littermates in baseline freezing. Both the mutant mice and the wild-type littermates increased their freezing in the 24-hr context tests relative to baseline freezing. However, *Df(17)1Yey*/+ mice froze less than their wild-type littermates ($p<0.01$) (Fig. 5c). In the 72-hr context tests, both *Df(10)1Yey*/+ mice and *Df(17)1Yey*/+ mice exhibited less freezing when compared to their wild-type littermates ($p<0.05$ and $p<0.01$, respectively) (Fig. 5a, c). These results suggest that the mutant mice are impaired in context-associated learning. After altering the context, the freezing levels of all strains prior to the presentation of the audible tone were similar ($p>0.05$) and were significantly lower than their freezing levels earlier when they were exposed to the original context (Fig. 5b, d), suggesting that none of the mouse strains exhibited generalized freezing in all conditions (Balogh et al. 2002). During presentation of the audible tone, all strains increased their freezing relative to the initial freezing in the altered context, and there was no significant difference between the mutant mice and the wild-type littermates in this measure of auditory cue-associated learning (Fig. 5b, d). To facilitate accurate interpretation of the fear conditioning test outcomes, we performed a foot-shock sensitivity test. Our results show that there was no difference in the mean threshold of the current to elicit flinching or vocalizing between the mutant mice and their wild-type littermates (Fig. 6).

The aforementioned phenotypic studies provide conclusive evidence that the heterozygous deletion of the Hsa21 syntenic region on Mmu10 or Mmu17 results in impairment in learning and memory in mice. These results are consistent with data generated from human genetic studies in which patients carrying heterozygous deletions of the human syntenic regions 21q22.3 exhibit the mental retardation phenotype (Ehling et al. 2004; Estabrooks et al. 1990; Katzenstein et al. 2009; Lyle et al. 2009). The smallest deletions in these patients span approximately 7.9 Mb on 21q22.2-q22.3 (Ehling et al., 2004). Therefore, *Df(10)1Yey*/+ mice and *Df(17)1Yey*/+ mice can serve as genetic models for partial Monosomy 21-associated mental retardation.

The prevailing hypothesis on the phenotypic consequences of chromosomal abnormalities is that a specific phenotype is caused by dosage alteration of a specific gene or genes located within the rearranged interval. Many chromosomal abnormalities cause mental retardation-related phenotypes in humans and mice, suggesting the central nervous system is sensitive to dosage alterations of many genes. On the other hand, if the rearranged interval does not contain dosage sensitive genes, deletion or duplication should not lead to mental retardation-related phenotypes. Such cases have been observed in humans and mice (Besson et al. 2007; Gilmore et al. 2001; Zarate et al. 2007). Also, in our preliminary study, the duplication of human chromosome 21 syntenic region on Mmu10 did not cause impairment in the Morris water maze test or the contextual fear conditioning test (Yu et al., unpublished data). Therefore, the mental retardation-related phenotypes observed in *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mice are caused by haploinsufficiencies of specific genes in the regions.

In contrast to patient-based studies, which are limited by the availability of partial Monosomy 21 cases carrying informative chromosomal deletions, the establishment of

desired mouse models will make it feasible to perform in-depth genetic analysis of the syntenic genomic regions. Recently, Besson *et al*. reported that a 0.5-Mb heterozygous deletion between *Prmt2* and *Col6a1* in the Hsa21 syntenic region on Mmu10 did not cause impairment in learning and memory in Ms1Yah mice (Fig. 1; Supplementary Table 1) (Besson et al. 2007). Since the *Prmt2-Col6a1* region is located within the deleted interval of *Df(10)1Yey*, 13 Hsa21 gene orthologs deleted in Ms1Yah mice can be excluded as the causative genes for the mutant phenotype observed in *Df(10)1Yey*/+ mice. Therefore, the causative genes for partial Monosomy 21-associated mental retardation are among 28 and 19 Hsa21 gene orthologs located on Mmu10 and Mmu17, respectively. Eighteen of these candidate genes have been mutagenized by using a gene-targeting approach (http://www.informatics.jax.org) but none of these targeted mutations has been reported to give a heterozygous cognitive phenotype related to mental retardation. To search for the causative genes, we could first generate and analyze subdeletions of the associated genomic regions using Cre/*loxP*-mediated chromosome engineering, which would be facilitated by ready-made targeting vectors from the Mouse Insertional and Chromosome Engineering Resource (MICER) (Adams et al. 2004). This effort should significantly further narrow down the critical genomic regions and reduce the number of candidate genes. The identities of the causative genes for Monosomy 21-associated mental retardation could be established by analyzing knockout mice carrying null alleles of these candidate genes. The null alleles generated from ongoing public knockout mouse projects, including those from the International Gene Trapping Consortium and from BAC-recombineering-based gene targeting pipeline, will provide timely-reagents for such a genetic analysis effect (Austin et al. 2004;Chan et al. 2007;Testa et al. 2004;Wurst 2005).

For mutational analysis of the mouse genome, heterozygous deletions with defined endpoints are important reagents because they facilitate rapid genetic mapping and maintenance of randomly generated mutations, such as those generated during ethylnitrosourea mutagenesis screens (Rinchik 2000; Rinchik and Russell 1990). Therefore, as viable heterozygous deletion mutants, *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mice can also be used for functional analysis of the deleted genomic intervals.

Engineered mouse mutants have been instrumental in fruitful molecular genetic studies of several major human chromosomal deletion disorders, including DiGeorge syndrome (Lindsay et al. 1999; Lindsay et al. 2001; Merscher et al. 2001), Prader-Willi syndrome (Tsai et al. 1999) and Smith-Magenis syndrome (Walz et al. 2003). Similarly, *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mutants developed from this study will play a critical role in genetic dissection of Monosomy 21-associated mental retardation, which in turn may lead to unraveling of the molecular mechanism underlying the clinical phenotype of this disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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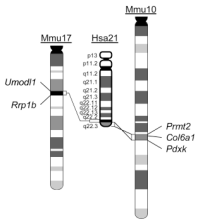

Fig. 1.
Hsa21 and the syntenic regions on Mmu10 and Mmu17. The endpoints of engineered chromosomal deletions in the mouse mutants are indicated.

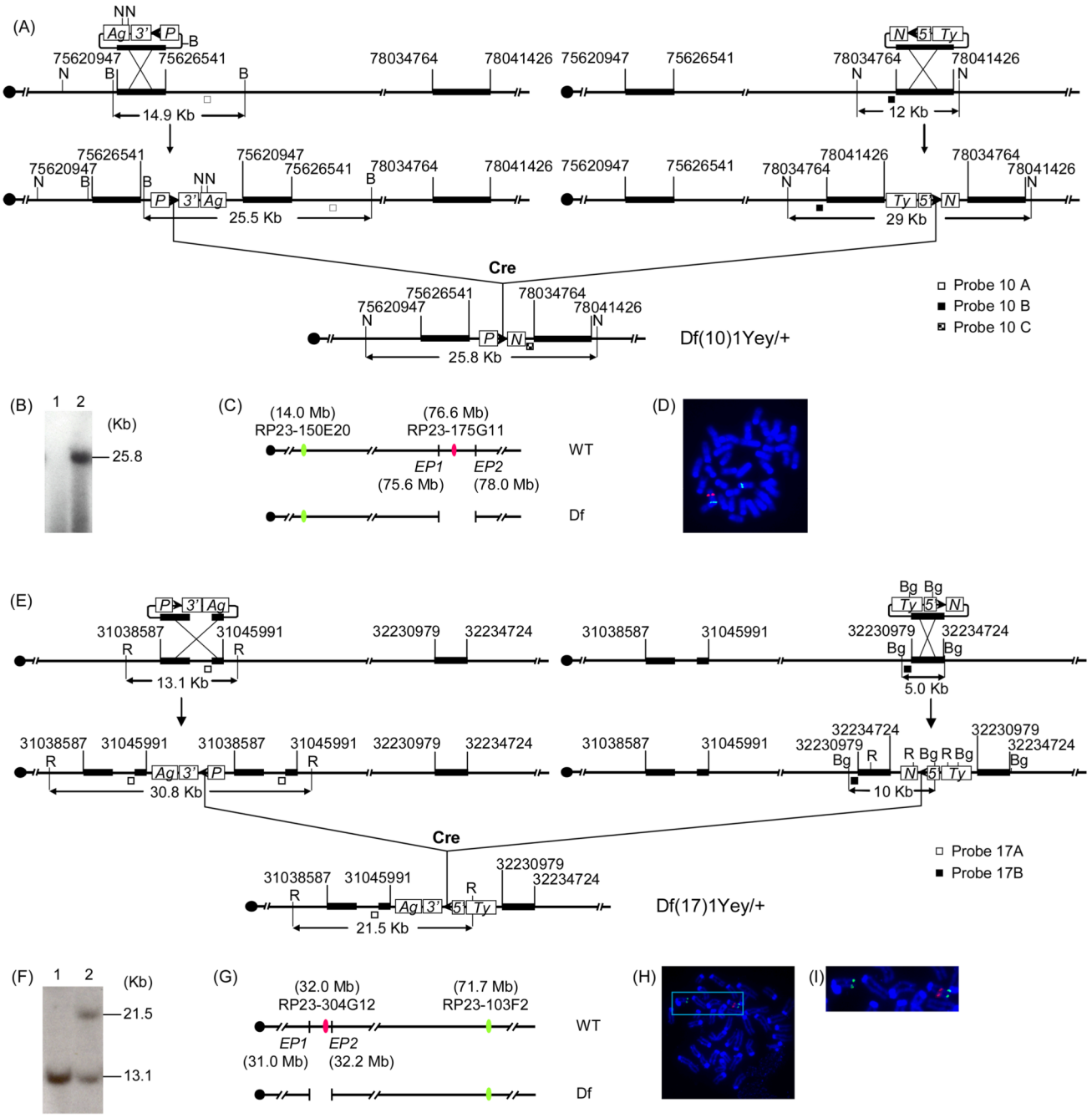

Fig. 2.

Development of *Df(10)1Yey*/+ and *Df(17)1Yey*/+ mice using Cre/*loxP*-mediated chromosome engineering. (**a**) Strategy to generate *Df(10)1Yey*. B, *Bam*HI; N, *Nhe*I; 5', *5'HPRT* fragment; 3', *3'HPRT* fragment; *N*, neomycin-resistance gene; *P*, puromycin-resistance gene; *Ty*, Tyrosinase transgene; *Ag*, Agouti transgene; arrowhead, *loxP* site. (**b**) Southern blot analysis of *Nhe*I-digested mouse tail DNA using Probe 10C. Lane 1, the wild-type mouse; lane 2, *Df(10)1Yey*/+ mouse. (**c**) Schematic representation of the genomic locations of BAC probes for FISH analysis; EP1 and EP2, endpoint 1 and endpoint 2, respectively. (**d**) FISH analysis of metaphase chromosomes prepared from embryonic fibroblasts carrying *Df(10)1Yey*/+. (**e**) Strategy to generate *Df(17)1Yey*. R, *Eco*RI; Bg, *Bgl*II.

(**f**) Southern blot analysis of *EcoR*I-digested mouse tail DNA using Probe 17A. Lane 1, the wild-type mouse; lane 2, *Df(17)1Yey*/+ mouse. (**g**) Schematic representation of the genomic locations of BAC probes for FISH analysis; EP1 and EP2, endpoint 1 and endpoint 2, respectively. (**h**) FISH analysis of metaphase chromosomes prepared from embryonic fibroblasts carrying *Df(17)1Yey*/+. (**i**) Higher magnification of the area boxed in (**h**).

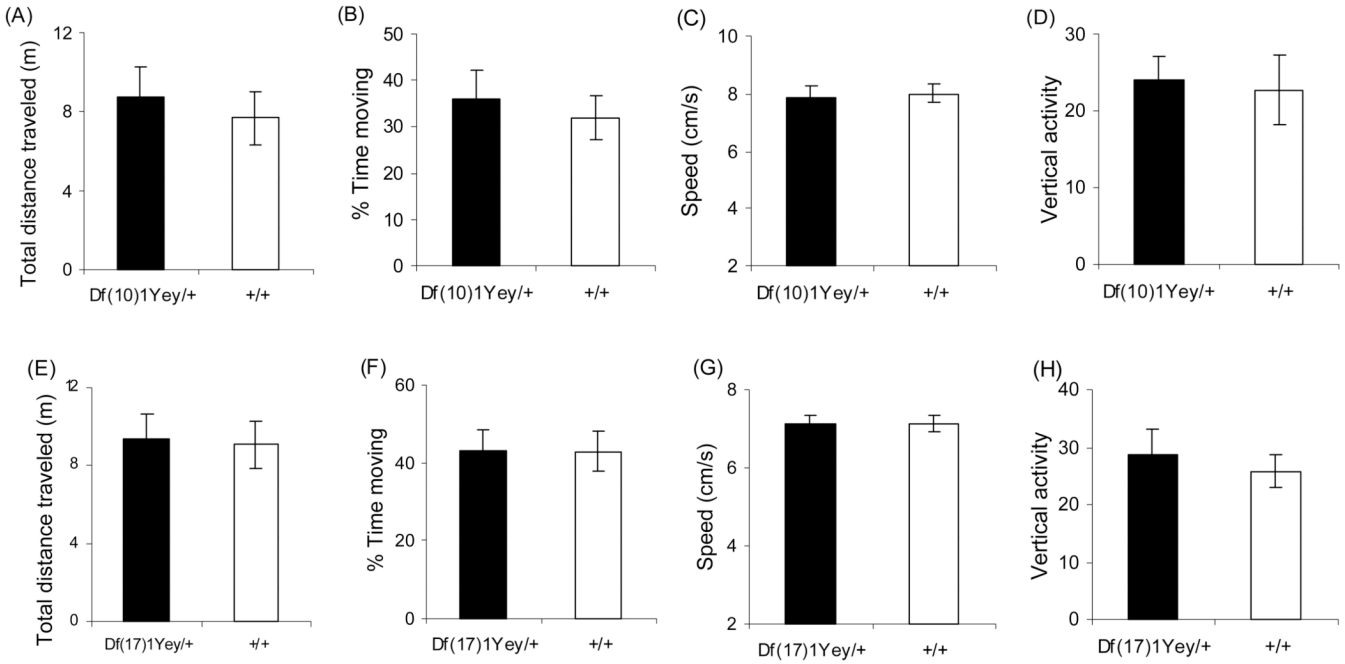

Fig. 3.

Open field observation. *Df(10)1Yey*/+ mice (n=16) and *Df(17)1Yey*/+ mice (n=15) as well as their wild-type littermates with equal sample sizes were assessed by (**a, e**) total distance traveled (m, meter), (**b, f**) percentage of time spent moving, (**c, g**) average moving speed (cm/s, centimeter/second) and (**d, h**) vertical activity (number of rearings).

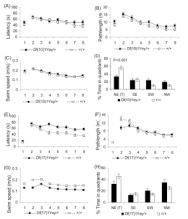

Fig. 4.

Behavioral analysis using Morris water maze. *Df(10)1Yey*/+ mice (n=16) (**a–d**) and *Df(17)1Yey*/+ mice (n=15) (**e–h**) as well as their wild-type littermates with equal sample sizes were examined, as described in Materials and Methods. (**a, e**) Latency to locate the platform (s, second), (**b, f**) path-length to locate the platform (m, meter) and (**c, g**) swimming speed (m/s, meter/second) during the learning trials of Morris water maze test. (**d, h**) The relative amount of time spent in different quadrants in the probe test on the second day after the end of the learning trials.

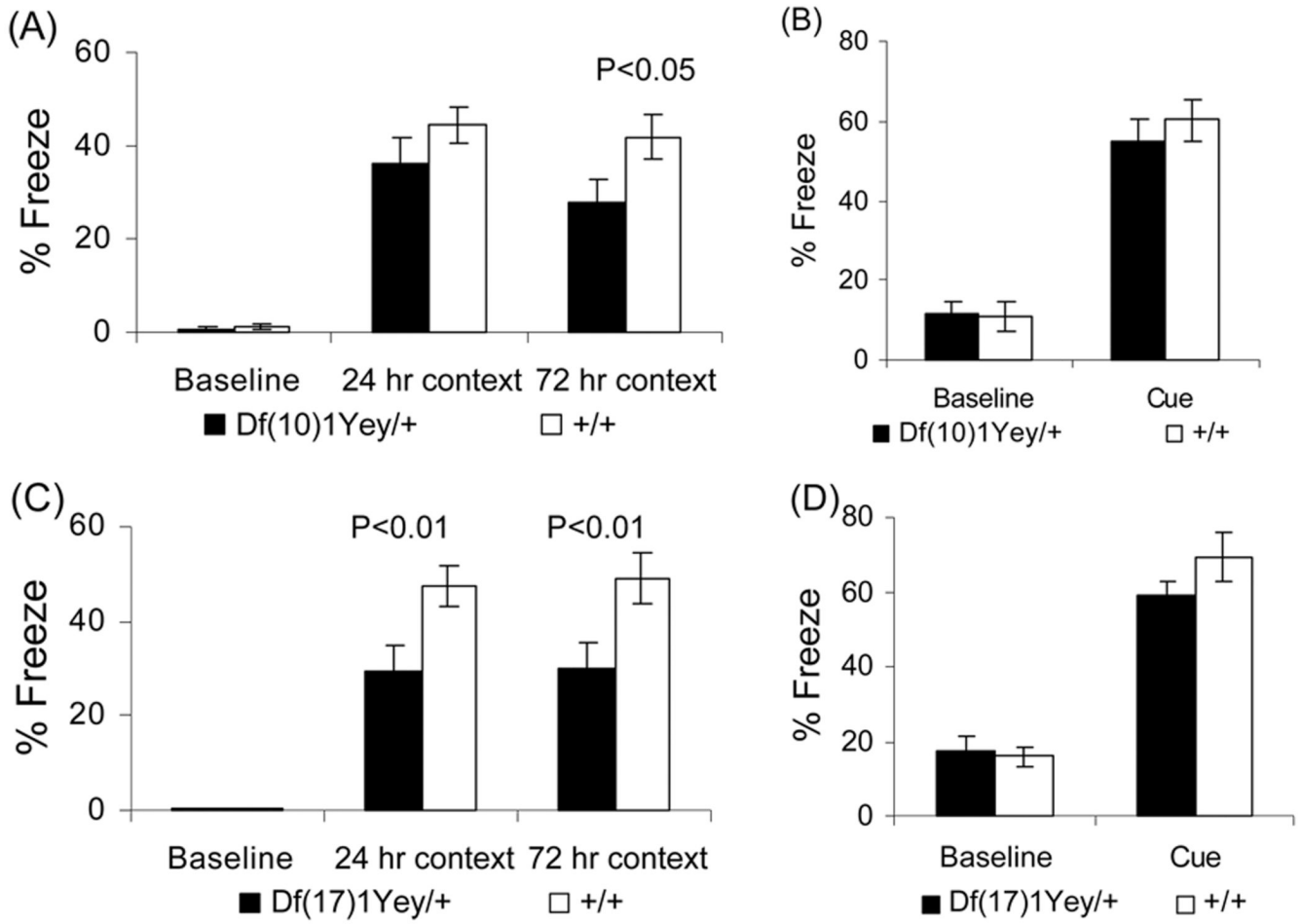

Fig. 5.

Behavioral analysis using contextual and cued fear conditioning. *Df(10)1Yey*/+ mice (n=16) (**a, b**) and *Df(17)1Yey*/+ mice (n=15) (**c, d**) as well as their wild-type littermates with equal sample sizes were examined as described in Materials and Methods. (**a, c**) The percentage of time spent freezing before the foot-shock (baseline) as well as during the 24- and 72-hr contextual tests is shown. (**b, d**) The percentage of time spent freezing in the altered chamber before the auditory tone cue was delivered (baseline) as well as during the cue delivery is shown.

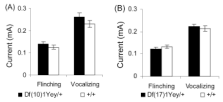

Fig. 6.

Analysis of sensitivity to electric foot-shock. The minimal levels of currents (mA) needed to elicit a response, either flinching or vocalizing, from *Df(10)1Yey*/+ mice (n=16) and *Df(17)1Yey*/+ mice (n=15) as well as their wild-type littermates with equal sample sizes are shown.

Table 1

Normalized relative values (RQ) of expression in the brains*

	Gene name	RQ ± S.E.M.
Df(10)1Yey/+ over +/+	*Adar2*	0.48±0.02
	Dnmt3l	0.52±0.06
	Itgb2	0.52±0.01
	S100b	0.48±0.01
	Trpc7	0.53±0.02
Df(17)1Yey/+ over +/+	*Cbs*	0.44 ± 0.01
	Pknox1	0.62 ± 0.03

* The values represent the means of triplicated samples. *Gapdh* was used as an internal control and is disomic in all strains.