

Functional Analysis of Genes Implicated in Down Syndrome: 1. Cognitive Abilities in Mice Transpolygenic for Down Syndrome Chromosomal Region-1 (DCR-1)

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Down syndrome occurs every 1/1000 births and is the most frequent genetic cause of mental retardation. The genetic substrate of Down syndrome, an extra chromosome 21, was discovered by Lejeune, half-a-century ago, and the chromosome has been fully sequenced, although the gene(s) implicated in the mental retardation observed with the syndrome are still unknown. Observations of patients with partial trisomy of the 21q22.2 fragment suggest that most of the signs of the syndrome, including mental retardation, could be influenced by the region referred to as the Down Minimal Chromosomal Region-1 (DCR-1) for that reason. Using the extensive synteny between human chromosome 21 and murine chromosome 16, Smith *et al.* (1995, 1997) developed transpolygenic mice with human chromosome 21 fragments covering the DCR-1. Here, we explored cognitive performances in mice over-expressing the genes carried by these fragments with the Morris water-maze and fear-conditioning procedures. The 152F7 transpolygenic mice had lower performance levels, compared to non-transgenic and other transgenic mice on most measurements in the water-maze. In fear-conditioning, all transgenic mice recorded lower performance levels compared to controls in the altered context stage. The 230E8, 141G6 and 285E6 mice failed to learn or react when the sound used as the conditional stimulus was added. These results showed that the 152F7 region played a crucial role in cognitive impairment, supporting the hypothesis of *DYRK-1A* gene involvement. However, the data presented here also suggest that other chromosomal regions within the DCR-1 may be involved in specific cognitive functions.

KEY WORDS: Chromosome 21; cognition; DCR-1; Down syndrome; fear-conditioning; water-maze; YAC.

INTRODUCTION

Down syndrome (DS) occurs in about one birth out of 1000 and remains the major genetic cause of mental retardation (Lejeune, 1990). The syndrome includes a wide set of impairments with highly variable expression. About 60% of perinatal mortality in persons with DS results from cardiac disorders caused by congenital heart conditions (Jackson *et al.*, 1976). Immune disorders, including leukaemia and abnormal levels of T-lymphocytes also have high prevalence in the syndrome (Ugazio *et al.*, 1990). The facial features of persons with DS

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typically include oblique eye fissures, epicanthic eye-folds, a flat nasal bridge, the mouth permanently open and the tongue protruding. The characteristic morphology is short and stocky with virtually no neck because of skeleton abnormalities; the limbs are malformed with short, broad hands with a single transverse palmar crease and a shortened and incurved fifth finger. Mental retardation is variable but remains the most striking feature of the syndrome. After the pioneering description by J.L. Down in 1866, almost one century was needed to decipher the aetiology of the syndrome. Lejeune *et al.*, (1959) were the first to prove that DS was caused by an extra copy of chromosome 21. Poissonnier *et al.* (1976) used chromosome staining methods revealing R and G bands and found a partial 21 trisomy for 21q22.1 and 21q22.2 bands in a person with DS. Partial trisomies have been invaluable in investigating the function of genes carried by chromosome 21, but unfortunately are very rare, accounting for no more than 1% of living trisomic persons. In 10 partial trisomy patients, Delabar *et al.* (1993) suggested that two regions of chromosome 21 were linked with most of the Jackson signs (Jackson *et al.*, 1976), including mental retardation. These regions were located around the D21S55 Site Targeted Sequence (STS) and between D21S55 and the MX1 gene and encompassed the 21q22.2 band. Korenberg *et al.*, (1994) studied a different population and observed that the proximal and distal regions of the 21q arm were also associated with the full DS phenotype. Finally, the implication of the region around 21q22.2 in most of the phenotypic traits used to define DS was recognized, with D21S17 and ETS2 as boundaries. The region was then labelled "the Down syndrome Chromosomal Region-1" (DCR-1). A causal link between the region located between D21S17 and ETS2 to clinical features of DS was confirmed through lattice analysis (Chabert *et al.*, 2001). While chromosome 21 has been sequenced (Hattori *et al.*, 2000) most of the functions of the genes on chromosome 21 and their role in DS are still unknown. The aim of the present paper is to elucidate the function of genes encompassed on DCR-1. As partial trisomies are too rare, mouse models were chosen.

Mouse models for DS have the advantage of synteny between the human chromosome 21 (HSA 21) and the mouse chromosome 16 (MMU 16). Hattori *et al.* (2000) sequenced the human chromosome 21 and demonstrated that about 80% of HSA 21 and MMU 16 were syntenic, the remaining being

syntenic with mouse chromosomes 10 and 17 (Dierssen *et al.*, 2001a, b; Moore *et al.*, 1999).

Two families of mouse models have been widely used for investigating genes mapped on DCR-1 on the basis of the extensive synteny between HSA 21 and MMU 16, using mouse trisomy 16 and mice transgenic for human genes. The first family has full or partial trisomy 16. Gropp *et al.* (1975) used Robertsonian translocations and generated aneuploidy in mice, including aneuploidy for MMU 16 (labelled Ts16). The embryos displayed a number of abnormalities found in DS patients (Lacey-Casem and Oster-Granite, 1994), but they died in utero and the presence of other synteny between mouse 16 chromosome and human chromosomes 3, 8, 16 and 22 showed the model to be of limited experimental value. A second mouse model, Ts65Dn mice, developed by Davisson *et al.* (1990, 1993) presents a partial trisomy for a single region of chromosome 16 syntenic with human chromosome 21. This syntenic region encompasses genes from *App* to *Tmprss2* (Baxter *et al.*, 2000). Crnic and Pennington (2000) presented an extensive review of the neurobiological and behavioural similarities between these mice and persons with DS. Ts65Dn mice usually display phenotypes observed in DS patients. The region from *App* to *Tmprss2* has been implicated in hyperactivity (Reeves *et al.*, 1995), learning and memory impairment as tested in radial and water-mazes (Demas *et al.*, 1998; Escorihuela *et al.*, 1998; Holtzman *et al.*, 1996; Reeves *et al.*, 1995) and reduced anxiety was reported in the elevated plus-maze (Cousson-Read and Crnic, 1996; Escorihuela *et al.*, 1995). Alterations were also reported in cAMP production (Dierssen *et al.*, 1997) and two neurotransmission systems (noradrenergic and cholinergic transmission, Dierssen *et al.*, 1997 and Granholm *et al.*, 2000, respectively). The triplicate region of chromosome 16 in Ts65Dn is larger than the syntenic DCR-1, and for this reason, the Ts65Dn cannot be used to disentangle the contribution in DS-related cognitive impairments of the gene carried by DCR-1. A third partial aneuploidy (Ts1Cje mice) was accidentally generated. When the *Sod-1* gene was targeted, a partial trisomy of MMU 16 was induced from *Sod-1* to *Mx-2*, with *Sod-1* not over-expressed; the partial trisomy resulted in impaired cognitive plasticity and motor abilities (Sago *et al.*, 1998). The second family is comprised of transgenic mice over-expressing one gene from HSA 21. Some of these genes are carried by DCR-1 (*DYRK-1A*,

SIM-2, Altafaj *et al.*, 2001; Chrast *et al.*, 2000, respectively), or by the other 21 chromosomal regions (*HPRT*, *SOD-1*, *APP*). Phenotypic examination of mice over-expressing *DYRK-1A* shows impairment of learning, including an effect on cognitive flexibility. *DYRK-1A* mice are more hyperactive and display a reduced emotional response (Altafaj *et al.*, 2001). The results with *SIM-2* did not reveal any major cognitive defects (Chrast *et al.*, 2000). These studies show that the size of the effect on behavioural measures with mice over-expressing one gene is lower than in mice carrying large extra chromosome 16 fragments.

Several limitations are encountered when generating full or partial trisomy 16: MMU16 is not only syntenic with large regions from HSA 21 but with regions outside chromosome 21. Ts65Dn mice seem to be a better model for human trisomy 21 than Ts16 as the triplicate chromosomal region is bounded by *App* and *Mx-2*. However, syntenies for the HSA 21 telomeric region are not on the triplicate MMU 16 region, but on MMU 17 and MMU 10 (Dierssen *et al.*, 2001a). These models do not provide an opportunity for identifying the individual function of the genes and their role in DS. Other limits are encountered with the second group. The hypothesis implicit in the use of transgenic mice with single genes from HSA 21, is that one gene or more may have a substantial effect. Transgenics for individual genes produce less marked effects than those obtained with Ts65Dn. The effect is greater when the triplicate fragment is long. This would suggest that many genes may be implicated in DS with each making a small contribution, or that they may interact with genes located either on HSA 21 or in another chromosomal region. In a recent paper, Busciglio *et al.* (2002) demonstrated that the expression of *APP* was modulated by mutations of mitochondrial DNA.

To avoid such difficulties, Smith *et al.* (1995, 1997) selected short fragments of HSA 21 to produce polytransgenic mice with partial trisomies of the DCR-1. They selected contiguous chromosomal fragments covering the DCR-1. Smith *et al.* (1995) incorporated these fragments of DCR-1 in yeast artificial chromosomes (YACs), each fragment containing between 2 and 10 genes. Mice over-expressing the genes carried by the 152F7 fragment from chromosome 21 had impaired learning when tested in the Morris water-maze (Smith *et al.*, 1997). The deriving of two independent strains from two slightly different chromosomal fragments, with one

of them including the *DYRK-1A* gene, supported the hypothesis of a substantial contribution by the *DYRK-1A* gene to the cognitive impairment associated with DS.

We conducted extensive examinations of transgenic mice for contiguous chromosomal fragments covering the DCR-1. Persons with DS have a specific pattern of cognitive skills. Although they succeed in associative learning, they have difficulties when more complex processes are implicated, e.g. managing distal cues in spatial learning, developing new strategies when exposed to novel conditions or remembering acquisitions after a long time. We adapted tests currently used on mice to model the cognitive traits that are impaired in DS and the possible DCR-1 regions that may contribute to these impairments.

MATERIAL AND METHODS

Transgenic Mice

Mice were generated on an FVB inbred background by Smith *et al.* (1995, 1997). To detect a possible insertion effect, Smith *et al.* (1995) developed several strains for each YAC. We used two strains for each of the four YACs. Strains 50 and 55 corresponded to YAC 230E8 (abbreviated here to E8), 4 and 28 to YAC 141G6 (G6), 12 and 57 to YAC 152 F7(F7) and 67 and 84 for YAC 285E6 (E6). The integrity of the transgene, the number of copies and the expression of genes had previously been reported (Smith *et al.* 1995, 1997). All strains integrated one copy of the corresponding HSA 21 chromosomal fragment, except strains 28 and 57 for which 3 and 2 copies were integrated, respectively; the number of copies carried by strain 84 was unknown. The FVB background that was used to micro-inject the transgenic fragments carries a recessive mutation (*rd*) inducing retinal degeneration. To avoid any impact of *rd* affecting visual cues, we used F₁ male offspring born from C57BL/6J females and transgenic FVB males (Smith *et al.*, 1997). Sample sizes were 21 male mice for YAC E8 (8 for strain 50 and 13 for strain 55), 22 for YAC G6 (10 for strain 28 and 12 for strain 4), 21 for YAC F7 (9 for strain 12 and 12 for strain 57), 24 for YAC E6 (13 for strain 67 and 11 for strain 84). We used eleven non-transgenic males as controls; these were F₁ offspring born from C57BL/6J females and non-transgenic FVB males. We maintained the mice with food and water *ad libitum* and a 12/12 light cycle

with lights on at 8 AM. We tested the males aged between 120 and 140 days.

We individually screened the F₁ offspring from each litter using PCR under the following conditions (initial denaturation: 95 °C, 5 minutes; 40 runs with denaturation: 95 °C, 30 seconds; annealing: 52 °C, 90 seconds; extension: 72 °C, 90 seconds). We used the following primers:

5'-AGTTTCTGGAATGACCGT-3', 3'-GCTAGCATTTCCTGGAACAG-5 for E8; 5'-ACCTGGGGACTGTGTGTCTC-3', 3'-TCTCAGTCTTCGGGCACC-5' for G6; 5'-TCCTTCCATGTACTCTGCA-3', 3'-TGCCCTGAAGCACATGTG T-5' for F7 and 5'-CCTTTCTGACCCCAACACAT-3', 3'-GAGAGCACAGTTTTGACACAGG-5' for E6.

Behavioural Measurements

We investigated learning in two situations: the Morris water-maze and fear-conditioning.

The Morris water-maze tested the ability to recognize the location of a submerged resting platform concealed beneath opacified water, an exercise requiring the use of extra-maze visual cues (Morris, 1984). The protocol was adapted from our previous studies (tank 1 m in diameter, water opacified with chalk, and the platform permanently positioned 1.5 cm beneath the surface) (Le Roy *et al.*, 1998; Roubertoux *et al.*, 2003). The water-maze was located in a room devoted solely to this test (room temperature 23 ± 1°C). The configuration of the room provided distal visual cues (water tap on the wall, door, rack, lighting and camera attached to the ceiling). We used the EthoVision system (1.96 version by Noldus, The Netherlands) to track the swim paths on camera. The “probe test” procedure was used to control for the implication of cognitive processes in locating the platform. Reversal and long term-memory measurements were added. To detect any possible contribution of swimming to the learning performance, we examined an independent set of the eight transgenic strains plus controls using the visible platform procedure. Before starting the experiment, we subjected the mice to a shaping session of four trials with 1 minute on the platform before and after each trial. A session consisted in four consecutive trials, with the mouse starting from one of the four quadrant points (N, S, E, and W). The starting quadrant was different for each trial of each session. We defined the order of starting points prior to the experiment. For each trial the mouse had 60 seconds to reach the platform located in

quadrant SW, followed by 60 seconds for resting. The experiment started by training the mice to perform the learning task over 4 days, with two sessions per day and four trials per session. We measured the time to reach the platform. We named this variable “time to reach platform” and expressed it in seconds. For the probe trial test, we removed the platform after the last learning trial, at the end of the second session on the fourth day. We measured the time the mouse took to go across to the position where the platform had been during the previous sessions. The variable was “time to reach virtual platform”. We measured the time the mouse spend swimming outside the quadrant containing the virtual platform. We expressed this new measurement as a percentage of the total time the mouse spent swimming, naming the variable “% time outside quadrant with virtual platform”. Eight days later, we conducted a further learning session in the morning, using the hidden platform protocol. The variable was “long-term memory” and was expressed in seconds. In the afternoon, we moved the platform from quadrant SW to the diametrically opposite quadrant, SE. We measured the time the mouse took to reach the platform in its new location and named the variable “time to reach platform after reversal”. This was the average time needed to reach the platform over the four trials and was expressed in seconds. To check whether the difference in time needed to reach the platform was due to swimming ability, mice were tested using the visible platform protocol with intra-maze visual cues where the platform emerged 5 mm above non-opacified water (Roubertoux *et al.*, 2003). We measured the time required by naïve transgenic male mice from each strain to reach the platform (with five or six per group, depending on the group) and also measured the time for eight non-transgenic controls.

The apparatus for fear-conditioning (Ugo Basile, Comerio, Varese, Italy) consisted of a black plastic conditioning chamber (30 × 8 × 15 cm) with a grid floor (wire mesh 2 mm in diameter, spaced 1 cm apart) and a transparent Plexiglas lid. A computer was linked up and emitted a sound (70 dB, 670 Hz), either paired or unpaired to an electric shock to the paws (0.4 mA, for 2 seconds). On day one, we allowed the mouse to explore the chamber for 2 minutes, then a sound was emitted for 30 seconds and used as a conditioned stimulus. The unconditioned stimulus (the electric shock) came 2 seconds after the sound. The paired stimuli were given again, 100 seconds later. A further 30 seconds

later, we returned the mouse to its home cage. Twenty-four hours later we placed the mouse in the same conditioning chamber for 3 minutes and every 10 seconds recorded the freezing episodes (no movement other than breathing, Paylor *et al.*, 1994; Wehner *et al.*, 1997). The variable was the “number of freezing episodes in identical context”. We returned then the mouse to its home cage. One hour later, we placed the mouse in a modified conditioning chamber: the grid floor had been covered with transparent Plexiglas and the chamber was half the previous size, being divided in half by a metal wall (8 × 15 cm). We counted the number of freezing episodes in the new conditions over a 3 minute period, naming the variable “number of freezing episodes in altered context”. At the beginning of the next 3 minute period, the conditioned (sound) stimulus was emitted for 30 seconds. We recorded freezing episodes, as previously and named this last variable “number of freezing episodes in altered context plus sound”.

Statistical Analysis

For the different analyses, we transformed raw data to fulfil normal distribution and homoscedasticity (Bartlett’s test) conditions. We performed inter-strain comparisons within YAC comparisons (Student’s *t*-test) to ensure there was no insertion effect, testing each variable. For the “time to reach platform” variable, we conducted a two-way ANOVA (YACs plus controls—5 levels, sessions being

treated as repeated measures—8 levels). Partial comparisons between YACs on the one hand, and between YACs and controls on the other, were done using the same design with two levels for YACs. For the other variables, a one-way ANOVA was used (YACs plus controls—5 levels) and partial comparisons were done using the Student’s *t*-test.

RESULTS

Inter-strain intra-YAC comparisons produced one comparison in 28 reaching a significant difference (strain 50 versus 55) *time to reach virtual platform* ($p < 0.03$). Given the probability of obtaining a significant difference when performing multiple comparisons, we considered the difference was due to chance. Subsequent analyses pooled the two strains of the same YAC.

Morris Water-Maze

Results for the *time to reach platform* are shown in Figure 1. YACs, sessions and interaction differed significantly ($F = 12.03, p < 0.001$; $F = 26.5, p < 0.001$; $F = 7.58, p < 0.001$, respectively). Partial comparisons showed no difference between E8, G6, E6 and controls, but F7 differed from both the other transgenics and non-transgenics ($0.012 < p < 0.008$).

Figure 2 shows the results for the variable *time to reach virtual platform*. An overall strain effect appeared ($F = 17.19, p < 0.001$). Partial

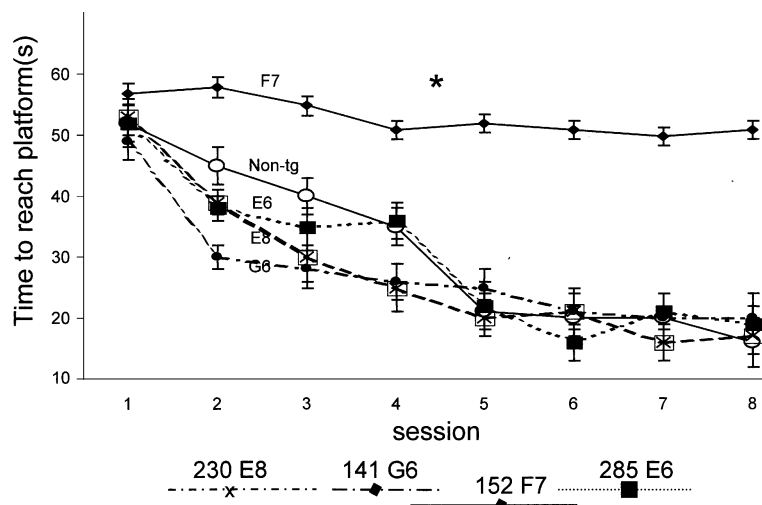


Fig. 1. Time to reach the hidden platform (means ± SEM) in Morris water-maze. Approximate locations of the YACs on chromosome 21 are indicated below the X-axis. Open circle indicates non-transgenic controls. * marks a significant difference compared to controls.

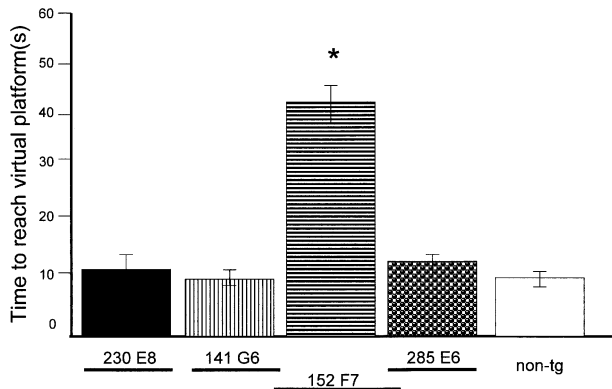


Fig. 2. Time to reach the virtual platform (position where the platform was before being removed); Morris water-maze (means \pm SEM). Approximate locations of the YACs on chromosome 21 are indicated below the columns. * marks a significant difference compared to controls.

comparisons show that F7 spent more time reaching the position where the platform was previously located, in quadrant SW, compared to the other strains, including the non-transgenic mice.

The YACs and controls differed significantly for % time outside virtual platform quadrant ($F = 8.49$, $p < 0.005$). The F7 strain explored the three quadrants where the platform had never been located less than the other strains (Fig. 3).

A global ANOVA showed a significant difference in the five groups for long-term memory ($F = 11.71$, $p < 0.001$), F7 and E6 did not differ from one another but both differed from E8, G6 and the non-transgenics as shown in Figure 4.

The one-way ANOVA showed a main effect on strains for the time to reach platform after reversal ($F = 8.92$, $p < 0.001$). F7 mice did not find the platform in its new location (Fig. 5).

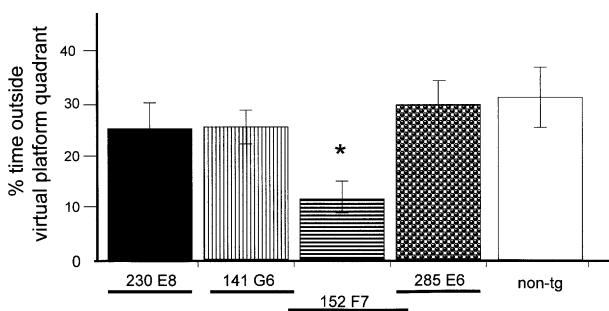


Fig. 3. Percentage of time outside the quadrant where the platform was before being removed; Morris water-maze (means \pm SEM). Approximate locations of the YACs on chromosome 21 are indicated below the columns. * indicates significant difference compared to controls.

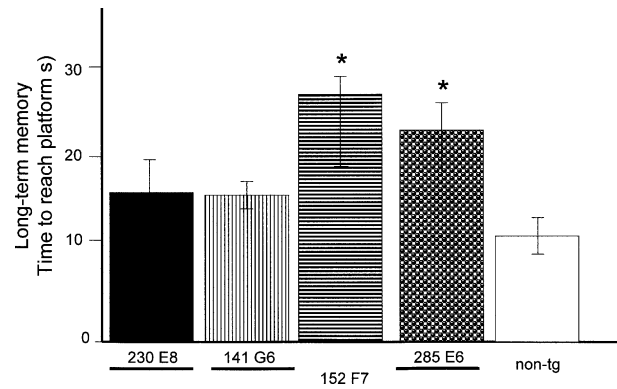


Fig. 4. Long-term memory. Time to reach the platform 8 days after the last learning session in the Morris water-maze (means \pm SEM). Approximate locations of the YACs on chromosome 21 are indicated below the columns. * indicates significant difference compared to controls.

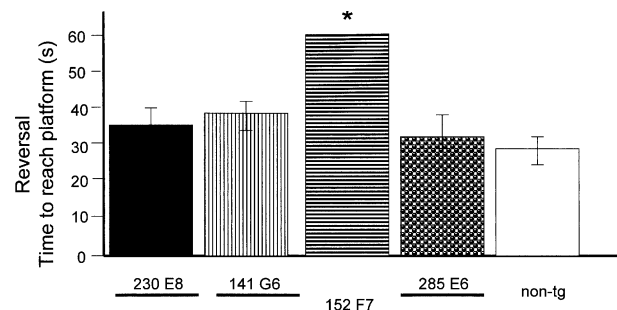


Fig. 5. Reversal. Time to reach the platform when it was moved to the diametrically opposite quadrant in the Morris water-maze (means \pm SEM). Approximate locations of the YACs on chromosome 21 are indicated below the columns. * indicates significant difference compared to controls.

No difference was found either between YACs or between YACs and non-transgenic mice ($F < 1$) for the time to reach the visible platform (data not shown).

In fear-conditioning, the number of freezing episodes in identical context did not differ either between YACs or between YACs and non-transgenic mice on the day after the mice were exposed to the paired stimuli or when the context was the same as shown in Figure 6 ($F < 1$).

A significant YAC-related group effect occurred for the number of freezing episodes in altered context ($F = 32.58$, $p < 0.001$). Figure 7 shows two groups, one (E6 and controls) with a lower score than the other (E8, G6, F7) ($0.01 < p < 0.001$) (Fig. 7). Non-transgenics and E6

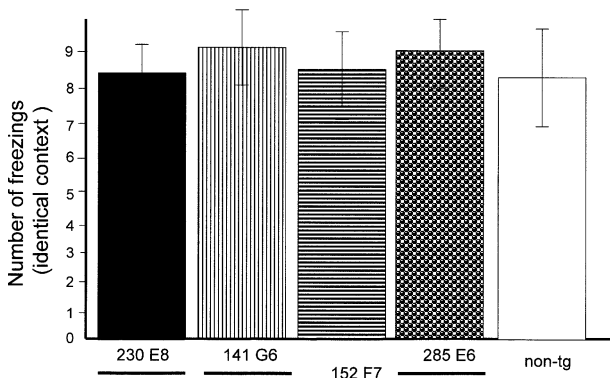


Fig. 6. Number of freezing episodes with identical context in fear-conditioning (means \pm SEM). Approximate locations of the YACs on chromosome 21 are indicated below the columns.

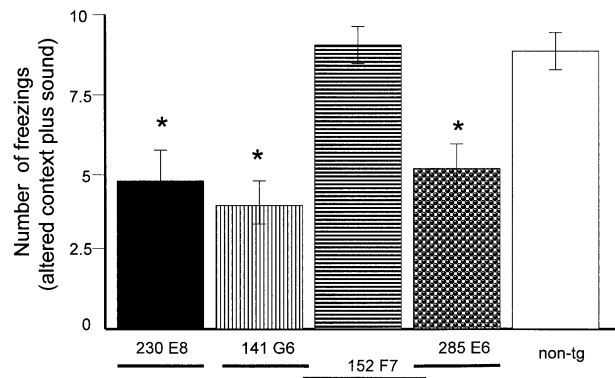


Fig. 8. Number of freezing episodes in altered context plus conditioned stimulus in fear conditioning (means \pm SEM, transformed values). Approximate locations of the YACs on chromosome 21 are indicated below the columns. * indicates significant difference compared to controls.

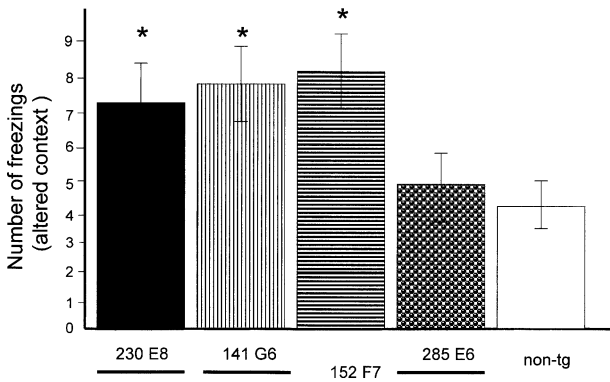


Fig. 7. Number of freezing episodes with altered context in fear-conditioning (means \pm SEM). Approximate locations of the YACs on chromosome 21 are indicated below the columns. * indicates significant difference compared to controls.

noticed that the conditioning chamber had changed and did not generalize the stimulus.

The strains differed for the *number of freezing episodes in altered context plus conditioned sound* ($F=9.50$, $p < 0.001$) and partial comparisons showed that F7 and controls differed from E8, G6 and E6 ($0.04 < p < 0.0001$). Only F7 and controls reacted to the sound (Fig. 8).

DISCUSSION

Two questions arise with modelling DS in mice. How does the behaviour of transgenic mice fit the cognitive phenotypes observed in persons with DS? What correlations can be found between behaviour patterns and MMU 16 genes homologous for HSA 21?

DS cognitive processes have been relatively well documented (Brown *et al.*, 2003; Clark and Wilson, 2003; Krinsky-McHale *et al.*, 2002; Laws, 2002; Pennington *et al.*, 2003). Cognitive impairment is the salient feature of the syndrome, with IQ ranging from 30 to 70 and averaging around 50 (Chapman and Hesketh, 2000). All studies indicate, however, that not all skills are affected in all persons or to the same extent. Briefly, and limiting the characteristics to those we modelled in mice, there is a general consensus characterizing persons with DS: they have normal performance level in simple tasks, difficulties with spatial memory, poor long-term memory performances and difficulties acquiring new skills.

The ability to perform simple tasks is illustrated by two studies. Caycho *et al.* (1991) demonstrated that persons with DS succeeded in associative learning. Nadel (1996) indicated that DS children were able to find hidden toys employing proximal visual cues. Our results fit with this observation in two tasks. Transgenic mice did not differ from controls for simple associative learning. Transgenic as well as non-transgenic mice succeeded in the water-maze when tested on the visible platform version (data not shown) and the day after mice were exposed to the paired stimuli and with the context unchanged (Fig. 6), the number of freezing episodes was the same for both transgenic and non-transgenic mice. The two studies showed that in the visible platform version the time required to reach the platform was the same for transgenic and non-transgenic mice. In the same way as persons with DS, the mice that over-express the genes carried by

DCR-1 exhibit normal associative learning ability and learn efficiently when proximal visual cues are available.

Persons with DS have difficulties with more complex cognitive tasks. In one experiment quoted above (Nadel, 1996), DS children and controls were tested in a situation where children had to find a hidden object by using spatial cues (the visual cues were not relevant). Similarly, the hidden platform version of the Morris water-maze forces the mouse to use spatial memory. The mouse has to integrate different spatial cues to succeed when starting from different points. One group of transgenics, F7, took more time than both other transgenics and controls to reach the hidden platform (Fig. 1). No difference was observed between the other transgenics (E6, E8 and G6) or with the controls. The learning curve of E6, E8 and G6 and control mice reached a plateau at session five, going through to eight, and the overall learning curve for F7 was flatter than for all the others. This suggests an association between the chromosomal region carried by the YAC transfected to F7 mice and impaired use of spatial cues. The findings of Smith *et al.* (1997) and the present results converge, indicating that the over-expression of genes carried by F7 and E8 affects the cognitive processes involved in the treatment of spatial information.

Persons with DS have difficulties acquiring new skills (Hodapp *et al.*, 1999; Raz *et al.*, 1995), mostly caused by the persistent use of old strategies to solve new problems (Hodapp *et al.*, 1999; Raz *et al.*, 1995; Wishart, 1993). Several of our measures addressed the ability of transgenic mice to acquire new skills. Under the reversal condition (Fig. 5), F7 mice were unable to detect the new location of the platform in the diametrically opposite quadrant. Under the probe test conditions, the F7 mice did not discover the exact location of the platform but did spend more time in quadrant SW at the end of the eighth trial (data not shown). After reaching the virtual platform, the controls and other transgenic mice explored the other quadrants, whereas F7 mice kept on exploring quadrant SW (Fig. 2). This persistent exploration of the SW quadrant, together with the inability to succeed in the reversal test, suggest that F7 mice lack the behavioural plasticity needed to succeed when the rules are changed in the water-maze. The ability to inhibit the generalization of the stimulus reflects the degree of behavioural plasticity. In the altered context version of the fear-conditioning task, suc-

cess depends on the ability to inhibit freezing when the context has changed and to adjust behaviour to the new conditions. Three groups of transgenic mice failed to inhibit freezing in the altered context: E8, G6 and F7 mice recorded more freezing episodes, while E6 behaved the same way as controls (Fig. 7). Paradoxical results occurred with a more complex task; all the mice, except the controls and F7, had fewer freezing episodes when the conditional stimuli, the sound, was emitted in the altered context. While the behavioural aspect of the task may seem simple, the neuronal bases are complex. Contextual fear-conditioning involves paired hippocampal and amygdalian functions, whereas the learning of the association between the conditional stimulus and the unconditional aversive stimulus only involves amygdalian function (Crnic and Pennington, 2000). Persons with DS have abnormal cerebellar morphology with reduced grey matter and small cerebellar size (Raz *et al.*, 1995). The involvement of the cerebellar pathways, which has been demonstrated in the classical conditioning process (Carey and Lisberger, 2002), cannot be excluded here.

Long-term memory deficits characterize persons with DS (Brown *et al.*, 2003; Clark and Wilson, 2003; Hodapp *et al.*, 1999; Raz *et al.*, 1995). We measured long-term memory performance, eight days after the last learning session. The F7 and E6 mice spent more time reaching the platform at the recall session (Fig. 4), showing they did not accurately remember the location of the platform.

Taken together these results confirm previous results by Smith *et al.* (1997). They show that the F7 region in triplicate is associated with impaired spatial memory. The present findings also demonstrate that mice over-expressing the genes carried by the F7 fragment have lower performance levels both in the ability to develop new strategies and in consolidating acquisitions. All the equivalent human characteristics of this impairment have been reported in persons with DS. These characteristics seem to be task-dependent in mice, because the examination of the mice transgenic for the other DCR-1 fragments shows difficulties in fear-conditioning: E8, G6 and F7 had reduced inhibition of the generalization of the stimulus. E8, G6 and E6 reacted less than F7 and non-transgenics when two types of cue co-occurred, indicating that the fragments of DCR-1 did not contribute equally to the cognitive defects and that this contribution is task-dependent.

Can these findings and results obtained with other models of trisomy 21 be used to formulate hypotheses on the function of the DCR-1 genes?

The three models of partial trisomy, Ts65Dn, Ts1Cje and mice transpolygenic for DCR-1, all present cognitive defects, but carry different copies of chromosome 16 or 21 (see Crnic and Pennington, 2000; Dierssen *et al.*, 2001b). In Ts65Dn, the extra chromosomal region includes genes from *App* to *Mx-1*. The *Sod-1* gene, the third copy of which was inactivated, and *Mx-1* form the boundaries of the triplicate copy in Ts1Cje. Because *App* is more centromeric than the *Sod-1* gene, the triplicate fragment in Ts1Cje is shorter than the Ts65Dn's one. Ts1Cje therefore carries a smaller number of genes than Ts65Dn (deduced from Hattori *et al.*, 2000). Ts65Dn mice show impaired acquisition on every test (Escorihuela *et al.*, 1998; Holtzman *et al.*, 1996; Reeves *et al.*, 1995 for the water-maze, Demas *et al.*, 1998, for the radial-maze) except for simple associative tests (Crnic and Pennington, 2000; Dierssen *et al.*, 2001b; Tassone *et al.*, 1999). Ts1Cje mice, which have three copies of DCR-1, were also poor learners (Sago *et al.*, 1998). Difficulties were also observed with Ts65Dn mice which were unable to successfully complete inhibiting behaviour or shift to other strategies (Crnic and Pennington, 2000). Ts65Dn mice tested with fear-conditioning did react to contextual conditioning but did not differentiate the altered context from the initial context (Crnic and Kelly, 1998). The impairment of long-term memory tested on Ts65Dn in the radial-maze was reported by Demas *et al.* (1998) and with Ts1Cje in the water-maze (Sago *et al.*, 1998, 2000). Common cognitive phenotypes were observed in Ts65Dn and Ts1Cje. The size of the effect is higher in Ts65Dn than in Ts1Cje, suggesting that the region between *App* and *Sod-1*, which is not present in Ts1Cje, may also contribute to the cognitive impairment observed in DS. The HSA 21 region covered by the four YACs is smaller, measuring only 2 Mb. The boundaries and length of each YAC have been recorded by Chumakov *et al.* (1992) and Smith *et al.* (1995, 1997). The HSA 21 map (Hattori *et al.*, 2000) showed that the DCR-1 region included 24 genes or predicted genes. Ts65Dn, Ts1Cje and the set of four YACs share three copies of this region. Mice with YACs covering DCR-1 show the same cognitive impairments as Ts65Dn and Ts1Cje, which suggests that the region from the centromere to the beginning of DCR-1 is involved in the cognitive dysfunction which characterises DS.

F7 mice encompass four human 21 chromosome genes from the centromeric to the telomeric location: *DSCRC*, *TTC3*, *DSCRA* and *DYRK-1A*. The *TTC3* and *DSCRA* and *DYRK-1A* genes are expressed in nerve tissues in the mouse embryo (Ngimbous *et al.*, in preparation Please update). *DYRK-1A* gene, which is a homologue minibrain gene (Guimera *et al.*, 1996; Song *et al.*, 1996; Tejedor *et al.*, 1995), is associated with reduced mushroom bodies and learning deficits in drosophila (Heisenberg *et al.*, 1985). The hypothesis that *Dyrk-1A* played a key role was supported by the comparison of mice with the F7 region either carrying or not carrying *DYRK-1A* (Smith *et al.*, 1997) and by the development of mice transgenic for this gene. Mice over-expressing *DYRK-1A* (Altafaj *et al.*, 2001) display learning deficits similar to the deficits observed in F7. As *TTC3* and *DSCRA* genes are also expressed in the embryonic mouse brain, their contribution to learning deficits in F7 mice cannot be excluded. The present findings also support the hypothesis that non-F7 DCR-1 fragments contribute to the cognitive defects commonly observed in patients with DS. Spatial memory, long-term memory and inhibition of a generalized response to a conditioned stimulus play central roles in cognitive processes and could generate cascades of impaired cognitive defects resulting in a dramatic general deficit. The question is whether the different fragments covering DCR-1 contribute in a cumulative way to the cognitive impairment observed in Ts65Dn and Ts1Cje. The size of the effects are smaller in mice over-expressing a single gene, compared to mice carrying large fragments of DCR-1; and the effects are greater in mice with large triplicate fragments of chromosome 16. This proportional relationship between the magnitude of the effects and the size of the triplicate region suggests that genes located on DCR-1 and outside DCR-1 interact in the different cognitive defects reported in patients with DS. Mice combining the different YACs are currently being developed and should lead the way for testing the interaction hypothesis.

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