Research Article

Dosage of the Abcg1-U2af1 Region Modifies Locomotor and Cognitive Deficits Observed in the Tc1 Mouse Model of Down Syndrome

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Abstract

Down syndrome (DS) results from one extra copy of human chromosome 21 and leads to several alterations including intellectual disabilities and locomotor defects. The transchromosomic Tc1 mouse model carrying an extra freely-segregating copy of human chromosome 21 was developed to better characterize the relation between genotype and phenotype in DS. The Tc1 mouse exhibits several locomotor and cognitive deficits related to DS. In this report we analyzed the contribution of the genetic dosage of 13 conserved mouse genes located between Abcg1 and U2af1, in the telomeric part of Hsa21. We used the Ms2Yah model carrying a deletion of the corresponding interval in the mouse genome to rescue gene dosage in the Tc1/Ms2Yah compound mice to determine how the different behavioral phenotypes are affected. We detected subtle changes with the Tc1/Ms2Yah mice performing better than the Tc1 individuals in the reversal paradigm of the Morris water maze. We also found that Tc1/Ms2Yah compound mutants performed better in the rotarod than the Tc1 mice. This data support the impact of genes from the Abcg1-U2af1 region as modifiers of Tc1-dependent memory and locomotor phenotypes. Our results emphasize the complex interactions between triplicated genes inducing DS features.

Introduction

Down syndrome (DS; OMIN #190685) is a multigenic disorder, resulting from three copies of human chromosome 21 (Hsa21) [1]. This condition is a paradigm of human aneuploid disorders with a direct consequence of gene dosage [2–4] and a general perturbation of whole transcriptome [5]. DS represents one-third of cases of intellectual disabilities and cognitive impairment in school-aged children [6–8] and is associated with a wide range of dysmorphologies, such as characteristic faces, skeletal anomalies and brain alterations at the prefrontal cortex, hippocampus and cerebellum levels [9,10]. Clinical features of DS also include
developmental delay, metabolic defects, other symptoms and associated diseases but their overall expressivity and penetrance are highly variable.

Mouse models have been developed in order to better understand the relationship between phenotype and genotype in DS. The long arm of this chromosome (21q) was completely sequenced since 2001 [11], and recent transcription comparisons’ studies report that it contains 696 genes, including at least 235 protein-coding genes and 142 pseudogenes, with a large subset of genes which have a mouse homolog located on regions of synteny carried by mouse chromosomes 16, 17 or 10 [12].

Several models carrying additional copies of regions homologous to Hsa21 were generated and used to decipher the contribution of segments to DS phenotypes [3,10]. Locomotor and learning deficits were found associated with trisomy of several segments located on Mmu16: Ts65Dn [13], Ts1Cje [14]; on Mmu17 Ts(17)1Yey [15] or Ts1Yah [16] and on Mmu10 Ts(10)1Yey [15] and in a single gene model for Dyrk1a [17–20]. A different model was generated in 2005: the Tc1 transchromosomal mouse line carrying an almost complete copy of Hsa21 with human genes expressed in various tissues [21]. Gribble and al. [22] deciphered the sequence of the Hsa21 present in Tc1 cells, and they identified one deletion, six duplications and 25 de novo structural rearrangements presumably due to the gamma irradiation used during the process of creating the mouse line. Nevertheless, the Tc1 mouse line is the unique humanized model for DS, and displays phenotypes affecting short term memory impairment, the hippocampal function [21] and locomotor activities [23,24].

Further analysis started by combining different models to sort out the contribution of subregions to specific DS phenotypes. The Ts65Dn mouse was crossed to the Ms1Rhr to demonstrate that the Down syndrome critical region previously identified in humans was necessary but not sufficient to induce DS cognitive phenotypes [25–27]. The experiment was carried out again for the App-Runx1 deletion crossed in Ts65Dn mice, which rescued post-natal lethality and certain cardiac phenotypes [28]. Similarly, monosomy for the region Cstb-Prmt2 on chromosome Mmu10, named Ms4Yah, was combined with the Tc1 transchromosome to show that the 50 genes orthologous to the Hsa21 region are not involved in Tc1-induced phenotypes [29].

We then further explored the contribution of the Abcg1-U2af1 region, located on mouse chromosome 17, which contains 14 conserved genes, namely Abcg1, Tff3, Tff2, Tff1, Tmprss3, Ubash3a, Rsp1h1, Scl37a1, Pde9a, Wdrr, Ndufv3, Pknox1, Cbs, U2af1, and two additional transcribed units (loc 102631757 and AK019514). The trisomy of Abcg1-U2af1 displayed impairment in working memory, in novel object recognition and overexpression of the conserved genes, except Abcg1 which was inactivated during genetic engineering and U2af1, which is located outside the interval [16]. All the genes from Abcg1-U2af1 genetic interval are trisomic in the Tc1 mouse model except the Ndufv3 gene which is rearranged [22]. The corresponding monosomy Ms2Yah [30] carries a deletion of the 12 conserved genes, plus the last exons of Abcg1, and showed fear conditioning and social recognition defects [30]. To determine whether the region could play a role in several DS phenotypes observed in the Tc1 mouse model, Ms2Yah and Tc1 mouse models were examined for impairments in Open-Field, Morris water maze and rotarod.

**Materials and Methods**

**Ethics statement**

All animals were treated in compliance with the animal welfare policies of the French Ministry of Agriculture. Yann Herault was granted permission by the French Ministry of Agriculture (law 87 848) under accreditation 67–369. Behavioral experiments were planned in order to
evaluate cognition and motor conditions in these mice as described previously in [29], submitted to the local animal care, use and ethic committee of the IGBMC (Com’Eth), and approved under accreditation number (2012–069) to comply with the new regulation in France. Mice were kept under specific pathogen free conditions with free access to food and water for all the tests. The light cycle was controlled as 12 h light and 12 h dark (lights on at 7AM). The Morris water maze (MWM) was conducted between 9:00 AM and 1:00 PM. All the other tests were done between 9:00 AM and 4:00 PM.

After weaning, male mice were gathered from ten litters and kept as littermates in the same cage with no isolated individual. The different apparatuses used were placed in a dimly lit testing room (approximatively 20 lux). To produce experimental groups, only animals from litters containing a minimum of two male pups were selected. Groups of animals were established for all genotypes on the N2B6C3B genetic background (see below): wt (n = 12), Ms2Yah (n = 11), Tc1 (n = 11), Tc1/Ms2Yah (n = 8). Animals were transferred to the experimental room 30 min before each experimental test. The tests were administered in the following order: open field (week 37), learning and reversal in MWM (weeks 53–56), working memory in MWM (week 62–63), and rotarod (week 72). No invasive procedure was used and the method of euthanasia was CO₂ inhalation.

Mouse lines, breeding and genotyping

The Ms2Yah, official name Del(17Abcg1-Cbs)2Yah, mice were generated on 129/Ola ES cells as described previously [16] and backcrossed on the C57BL/6J genetic background at least to N10 in this study [30]. The Tc1 transchromosomic line has been described previously [21]; These mice were kept on an F1 B6C3B background; the C3B are sighted C3H/HeH, a congenic line for the BALB/c allele at the Pde6b gene in C3H/HeH [31]. The two lines were crossed to generate double mutant and control cohorts on a mixed genetic background B6xB6C3B (N2B6C3B) under Specific Pathogen Free conditions.

For the identification of the Ms2Yah allele and the Tc1 chromosome, genomic DNA was isolated from tail biopsies using the NaCl precipitation technique. The Ms2Yah allele was identified using non quantitative PCR with 2 pairs of primers: one control mapping the end of the wild type (wt) allele form of the U2af1 locus (wt Forward 5′-CCAGCTGAAGATGGGTGTGTCTG-3′ / wt Reverse 5′-AGCCTTCCCTGGGGACCTGAAA-3′) leading to the amplification of a PCR product of 468 bp, and one transgenic pair mapping the junction between the U2af1 insert [16] and the HPRT 3′ vector (Tg Forward 5′-CCAGCTGAAGATGGGTGTGTCTG-3′ / Tg Reverse 5′-AAGCAGCGAGCGCAGCGA-3′) amplifying a product of 272 bp. The Hsa21 present in Tc1 mice was identified by PCR using primers D21S55F (5′_GGTTTGAGGGAACACAAAGCTTAACTCCCA-3′) and D21S55R (5′_ACAGAGCTACACGCCAATAGATGAACT-3′) that are specific for the Hsa21 and control primers specific for the mouse genome (MyoF: 5′_TTACGTCCATCGTGGAGACACAT-3′, MyoR: 5′_TGAGCTGGTGGTATTAGTCTTAT-3′) with specific PCR products of 208 bp and 245 bp, respectively.

Open field

This test measures rodent behavioral responses such as locomotor activity, hyperactivity and exploratory behaviors within a closed space. The test was carried out in a 55 cm diameter round white box and mouse activity was recorded with a video tracking system (Ethovision, Noldus, France) during a single 30 min session. After each mouse trial, the arena was thoroughly cleaned with 50% ethanol, followed by one cleaning with water, and then dried with paper towels to minimize olfactory cues. We quantified the speed and distance traveled during 3 phases (0 to 10 min; 10 to 20 min and 20 to 30 min). We also measured the percentage of
time spent in each arena zone (peripheral, intermediate, central) in the same phases of the session.

Morris water maze spatial memory

Learning protocol. The water maze was a circular pool (150-cm diameter, 60-cm height) filled to a depth of 40 cm with water maintained at 20°C–22°C, made opaque using a white aqueous emulsion (Acusol OP 301 opacifier) and split into 4 quadrants: South-East (SE), North-West (NW), North-East (NE), South-West (SW). The escape platform, made of rough plastic, was submerged 1 cm below the water’s surface.

This experiment was performed to study reference memory through a spatial search strategy that involved finding a hidden platform (6 cm diameter) in a pool. In the reversal mode mice had to learn a new platform position. All the procedures were adapted from Morice et al., 2008 [24] and Duchon et al., 2011 [29]. The spatial memory session consisted of a 6-day (S1 to S6) learning phase with two 90-second trials per day. Each trial started with mice facing the interior wall of the pool and ended when they climbed onto the platform located on the SE quadrant or after a maximum searching time of 90 sec. The starting position was changed pseudo-randomly between trials. Mice were left undisturbed in their home cage for 90-min inter-trial intervals. On the seventh day, mice were given the 60-sec probe test in which the platform had been removed. The distance traveled in each quadrant (NW, NE, SW, SE) was recorded to quantify the time spent in the target quadrant.

Reversal Learning protocol. After the first probe trial, all mice were given a reversal test, in which the hidden platform was moved to a new position (NW,). Mice were trained for 5 days (Reversal Session RS1 to RS5) following the same training procedure and then tested for the second probe trial on the sixth day (13 days after the beginning of the total test). To test mice for long term memory, they were left undisturbed for 20 days before being given a third probe trial.

Rotarod

The Rotarod test was performed to estimate rodent locomotor coordination. The apparatus (Bioseb, France) is made of a rotating bar 5 cm in diameter (hard plastic materiel covered by grey rubber foam) on which mice are placed facing the direction of rotation. The first phase was a learning period composed of one training session with 4 trials per day for 3 days. For each trial mice were placed on a rotating rod, starting from 0 and accelerating to 40 rpm in 5 minutes. We recorded the time spent on the rod and the speed before the fall. The second step of the task occurred on the 4th day. It consisted of 7 trials of 2 minutes, each trial being performed at one selected speed (4, 10, 16, 22, 28, 34 and 40 rpm). This procedure was repeated twice and the time spent on the rod was recorded for each trial.

Statistical analyses

ANOVA was performed to analyze differences between the 4 genotype groups using dedicated commercial Software (Sigmaplot): we applied a two way ANOVA for the Open-Field, Morris Water Maze Probe trials’ and rotarod test phase. we used a two Way repeated measures ANOVA for Learning/Reversal protocols and the learning Rotarod phase. However, for all the ANOVA, the post hoc analysis was done using Tukey’s method. Data are presented as mean ± s.e.m.
Results

The exploratory pattern in the open field is not altered in Tc1/Ms2Yah mice

As described previously the Tc1 mouse displayed a hyperactive exploratory phenotypes in the B6129 [23] but not in the B6C3B background [29]. In order to evaluate exploratory behavior, locomotion deficits and increased anxiety related behavior, we used the open field and we measured horizontal activity during three consecutive 10-minutes intervals (Fig. 1). Habituation was observed for all the genotypes (Two-way ANOVA "time intervals" F(2,111) = 12.524 p < 0.001; Tukey’s post hoc test “0–10 min vs 20–30 min” q = 7.001, p < 0.001) and exploration in the center versus the periphery was similar with no anxiety pattern in the Tc1 group or in Tc1/Ms2Yah (data not shown). The total distances travelled are globally the same after thirty minutes. However, during the first ten minutes, the Tc1/Ms2Yah group are more active compared to control and monosomic individuals (parameter "distance travelled", Two way ANOVA "time intervals 0–10 min", F(2,111) = 12.524 p < 0.001; Tukey’s post hoc method "wt vs Tc1/Ms2Yah" q = 4.663, p = 0.007; “Ms2Yah vs Tc1/Ms2Yah” q = 5.108, p = 0.003 and “Tc1 vs Tc1/Ms2Yah” q = 3.968, p = 0.03). This slight change in the initial exploratory phase in the Tc1/Ms2Yah double-mutants suggested that the Abcg1-U2af1 region might be involved in controlling exploratory behavior. Nevertheless, this behavior was not found in the Tc1 genotype mice, unraveling a hyperactive phenotype in the Tc1 mutant mice on this N2B6C3B background.

Memory impairment in the Morris watermaze of Tc1 mice depends on the number of copies of the Abcg1-U2af1 region

To evaluate the impact of the Abcg1-U2af1 region on learning potential and memory, we performed a Morris water maze test. During the first phase for place learning (Fig. 2A), all four genotypes found the platform with similar efficiency and memorized where the platform was located (parameter “distance travelled”, two way repeated measures ANOVA "learning day", F(1,38) = 64.517 p < 0.001). The distance traveled was reduced between the first and the last day of learning for all genotypes (Two-way repeated measures ANOVA, Tukey’s post hoc test “S1 vs S6”, wild type q = 5.643, p < 0.001; Ms2Yah q = 6.973, p < 0.001; Tc1 q = 7.807, p < 0.001, Ms2Yah vs Tc1/Ms2Yah q = 3.513, p < 0.001). In the probe test (Fig. 2A right panel, 24 hours after the learning session, all mice traveled further in the target quadrant than in the rest of the arena (parameter "% distance travelled" two way ANOVA "quadrant", F(1,160) = 133.77 p < 0.001) and no genotype effect was detected (Tukey’s post hoc method "target quadrant vs non target quadrants", wild type q = 9.322, p < 0.001; Ms2Yah q = 9.301, p < 0.001; Tc1 q = 9.002, p < 0.001, Tc1/Ms2Yah q = 5.678, p < 0.001). The spatial learning and memory of the Tc1 and Tc1/Ms2Yah mice was not affected compared to wild type mice.

During the second phase, the reversal phase, all genotypes showed significant global learning between RS1 and RS5 (Fig. 2B; Two way repeated measures ANOVA "trials”, “genotype”, F(1,38) = 33.018, p < 0.001), but the performance of the Tc1 group are reduced compared to wild type and Ms2Yah (Tukey’s post hoc method, Tc1 RS1 vs RS5, ns). This phenotype was rescued in Tc1/Ms2Yah compound mice (Tukey’s post hoc method: q = 3.322, p = 0.024). One day after the last reversal session, a probe trial was performed (Fig. 2B right panel) and we found that all mice remembered the correct position of the platform with greater distance travelled in the target quadrant (parameter "% distance travelled" two way ANOVA "quadrant", F(1,160) = 88.878 p < 0.001, Tukey’s post hoc method "target quadrant vs non target quadrants”, wild type q = 9.383, p < 0.001; Ms2Yah q = 6.830, p < 0.001; Tc1 q = 5.840,
FIGURE 1. Open field locomotor activity of mice. The distance travelled (m) during the exploration of the open-field by mice with different genotypes are shown Tc1/Ms2Yah during the consecutive 0–10, 10–20, 20–30 min intervals. The activity of Tc1/Ms2Yah was increased mainly during the first 10 minutes (parameter “distance travelled”, Two-way ANOVA “0–10 min”, F(2,111) = 12.524 p < 0.001; Tukey’s post hoc method “wt vs Tc1/Ms2Yah” q = 4.683, p = 0.007; “Ms2Yah vs Tc1/Ms2Yah” q = 5.108, p = 0.003 and “Tc1 vs Tc1/Ms2Yah” q = 3.968, p = 0.033 Tc1/Ms2Yah/Tc1/Ms2Yah). Values are means ± s.e.m. doi:10.1371/journal.pone.0115302.g001

p < 0.001, Tc1/Ms2Yah q = 5.088, p < 0.001). None of the groups displayed memory deficits since they described the same performance during the 2 probe trials. However, Tc1 mice needed more time to learn the new task.

Deficits in motor coordination of the Tc1 mice are partially rescued by the loss of Abcg1-U2af1 trisomy

Mutant mice were screened for motor skill using the rotarod test, as described previously [29]. Two patterns were observed during the training phase: wild type and Ms2Yah mice displayed the same capacities to stay on the rod, whereas both Tc1 and Tc1/Ms2Yah mice spent less time on the rod than the wild type control (Fig. 3A; repeated ANOVA “genotype” Tukey’s post hoc analysis; F(3.64) = 25.932. p < 0.001; wild type vs Tc1: q = 11.180, p < 0.001; wild type vs Tc1/Ms2Yah: q = 9.133, p < 0.001; Ms2Yah vs Tc1: q = 7.066, p < 0.001. Ms2Yah vs Tc1/Ms2Yah: q = 5.297, p = 0.004) over the 3 days during the learning phase. Moreover, while the wild type improved their performance during the training period (ANOVA “days”, Tukey’s post hoc method: F(2.64) = 11.477, p < 0.001; wild type day3 vs day1, q = 6.014, p < 0.001), none of the other groups, Ms2Yah, Tc1 and Tc1/Ms2Yah, progressed at the end of the training.

During the test phase (Fig. 3B), we determined the time spent on the rod at a given constant speed, in two sessions per trial and with increasing speed between each trial (4, 10, 16, 22, 28, 34 and 40 rpm) for a maximum of 2 min. As with the training session, a significant difference was observed in performance between the wild type and Ms2Yah versus the Tc1 and Tc1/Ms2Yah genotypes while the speed increased from 4 rpm to 34 rpm (Two way ANOVA “speed” and “genotype”, Tukey’s post hoc method: F(18,224) = 3.308, p < 0.001; wt vs Ms2Yah q = 3.493, p = 0.06; wt vs Tc1 q = 15.000, p < 0.001; wt vs Tc1/Ms2Yah q = 8.389, p < 0.001; Ms2Yah vs Tc1 q = 11.599, p < 0.001; Ms2Yah vs Tc1/Ms2Yah q = 5.219, p < 0.001). An intermediate behavior was detected in Tc1/Ms2Yah mice with better exercise performance than Tc1 but lower than wild type mice (at 10 rpm, wt vs Tc1/Ms2Yah q = 8.923, p < 0.001; Ms2Yah
vs Tc1/Ms2Yah ns; Tc1 vs Tc1/Ms2Yah q = 4.766, p = 0.004 - at 16 rpm, wt vs Tc1/Ms2Yah q = 10.514, p < 0.001; Ms2Yah vs Tc1/Ms2Yah q = 5.502, p < 0.001). In this experiment, Ms2Yah mice showed normal coordination, but could not increase their performance on the rod during the training days; the Abcg1-U2af1 region might play a role in locomotor abilities because, although training appeared equivalent to that of wild type mice, a longer test session revealed that double mutants Tc1/Ms2 could stay longer on the rod than Tc1 mice.

**Discussion**

The present study highlights the contribution of the *Abcg1-U2af1* genetic interval to DS-related features in Tc1 mouse models. We found that reducing the genetic dosage of this region in the
Tc1 mouse models rescued subtle impairments in reversal learning, working memory and did so partially in the rotarod test, but had no impact on hyperactivity or spatial learning.

Robustness of Tc1 induced deficits and the influence of the genetic background

In this third study focusing on the behavior phenotype of the Tc1 DS mouse model, we performed the analysis in a new mixed genetic background, i.e. N2B6C3B. Originally, the Tc1 model was studied in the B6129S8 [23,24,32] and more recently transferred in the B6C3B genetic background [28,29,33]. We generated the Ms2Yah, carrying the deletion of the ABCG1-U2AF1 Region and Behavior in Down Syndrome

Figure 3. Locomotor performance through accelerating and continuous speed rotarod tasks. (A) We examined locomotor coordination through three days of learning an acceleration protocol (from 4 to 40 rpm over 5 min). Results are expressed as the time (minutes) that mice remained on the rod before falling (left panel), and the velocity at the time of falling (right panel). Although Ms2Yah did not express any deficit, mice could not improve their performance over the 3 days (figure A, left panel: § p = 0.376). Conversely, Tc1 mice were unable to stay on the rod longer, or improve their time over the 3 days (figure A, left panel: *** p < 0.001 between wt and Tc1 and ### p < 0.001 between wt and Tc1/Ms2Yah groups). (C) The graph displays the time (min) that mice stayed on the rotarod during the test phase when the speed was set at 4, 10, 16, 22, 28, 34 and then 40 rpm, for a maximum of 2 minutes. The performance of the Tc1 group was poor from the beginning of the task (Two-way ANOVA "wt vs Tc1" on speed 4rpm* p < 0.05, 10/16 rpm *** p < 0.001) and 22/28 rpm ** (p < 0.01). Interestingly, the performance of Tc1/Ms2Yah mice was in-between that of Tc1 and controls and the Ms2Yah did not show major differences compared to wt. Values represent means + S.E.M.

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Abcg1-U2af1 region, on the C57BL/6J background. Thus when we crossed B6.Ms2Yah with the Tc1 transchromosomic line kept in the B6C3B genetic background, the Tc1/Ms2Yah compound mice and the relative control groups, wild-type, Ms2Yah and Tc1, were on the N2B6C3B background. Tc1 mice are known to be mosaic, with some cells losing the Hsa21 chromosome [21]; nevertheless, we recapitulated most of the Tc1 phenotypes (Table 1) in the MWM tests (for spatial learning), and in the rotarod tests (learning phase and challenge tests). The hyperactive phenotypes observed in the 129S8 [23], was not found in the B6C3B background [29] but was replicated again in the N2B6C3B background (this study). We hypothesize a contribution of the B6 genetic background which displays a higher level of locomotor activity than 129 and C3H [34–36]. Thus, even if most of the Tc1-induced phenotypes are robust, a few are also affected by the genetic background. The set of disomic genes of the different genetic backgrounds modified the Tc1-induced phenotypes. This study proposes that the variability in the expressivity and penetrance of the features found in DS people depends on genetic interactions between the trisomic genes and the whole genome.

The Abcg1-U2af1 region contributes to learning and memory deficits in the Tc1 DS mouse models

Here we found that reducing the number of copies of the mouse genes located in the Abcg1-U2af1 region, restores some deficits of the Tc1 model in the reversal phase of MWM. The Tc1/Ms2Yah mice learned the location of the platform with similar efficiency to controls while the Tc1 mice needed more sessions to do so. Although all the genotypes finally learned where the platform was located in the probe test, the decrease in the performance of the Tc1 group could be associated with a lack of cognitive flexibility, since learning memory was not altered. This particular phenotype is observed in Down syndrome people [37] and our results suggest that an increase in one or more genes of the Abcg1-U2af1 region contributes to decreased behavioral flexibility.

Tc1 mice have severe deficits in motor skills in different motor coordination tasks such as rotarod, static rod and footprint tests [23]. Rotarod performance analysis in our study confirmed the deficit in the locomotor activity of Tc1 mice, which occurred in the training days and in the test phase. After consecutive days of training, mice usually enhance their performance by staying on the rod longer each day [38,39]. In our experiment, Tc1 and Tc1/Ms2Yah did not improve their performance in the learning phase. The learning mechanisms of locomotor function were altered, and decreasing the number of copies of the Abcg1-U2af1 region could not rescue them.

During the test phase with different increasing rotarod speeds, Tc1 mice displayed strong impairment compared to controls. The Tc1/Ms2Yah showed better performance by staying

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**Table 1. Robustness of the Tc1 induced phenotypes observed in different genetic backgrounds and interference with different rescues.**

| Mouse Models          | Tc1* | Tc1§ | Tc1/ Ms4Yah§ | Tc1§ | Tc1/ Ms2Yah§ | Ms2Yah§ |
|-----------------------|------|------|--------------|------|--------------|---------|
| Background            | B6129| B6C3B| B6C3B        | N2F1 | N2F1         | N2F1    |
| Hyperactivity (open field) | +   | =   | =            | +    | =            | =       |
| MWM spatial learning and memory | =   | =   | =            | =    | =            | =       |
| MWM reversal learning and memory | =   | =   | =            | -    | =            | =       |
| Rotarod learning      | -    | -   | -            | -    | -            | ±       |
| Rotarod test          | -    | -   | -            | -    | ±            | -       |

(+), (=), (-) or (±) respectively indicate a reported effect with an improvement, similar or with an impairment compared to the control littermates. The (±) corresponds to a partial rescue compared to the transchromosomic Tc1 model. Data adapted from [23,24]*, [29]§ and this work.

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longer and at higher speed on the rod than the Tc1 group. We exclude a strong contribution of the genetic background to this phenotype since the C3H fell earlier than the B6 in a similar protocol and the learning phase was not compensated [6]. Thus, even if the Ms2Yah region is not implicated directly in motor learning, at least the over-expression of one or more genes located in the interval definitely modifies locomotor activity.

**Searching for candidates in the Abcg1-U2af1 genetic interval**

Identifying genes in the Abcg1-U2af1 region modulating Tc1-induced locomotor phenotypes would certainly improve our understanding of DS and stimulate further therapeutic approaches. As a consequence of the results described here, candidates should be conserved between humans and mice and expressed during the development of the adult brain. Many regions of the brain are involved in motor learning and performance, such as the cerebellum, basal ganglia, and the motor cortex. Thus expression of genes in the brain might help to discriminate candidates for the different phenotypes described here. According to the Allen brain atlas, Abcg1, Tff1, Ubash3a, Pde9a, Ndufv3, Pknox1 and Cbs were found expressed in the adult cerebellum and in the isocortex and are thus candidates. Of particular interest are Pde9a and Cbs, which are both expressed in the central nervous system. Pde9a codes the phosphodiesterase 9a and transforms cAMP and cGMP into their respective monophosphate forms and its inhibition can stimulate neuronal plasticity [40,41]. A delay in neuronal transmission may partially explain the change in learning tasks. Otherwise, the Cbs gene encodes cystathionine-beta-synthase whose deficiency causes homocystinuria (OMIN236200), a metabolic disorder with intellectual disabilities. Cbs is a strong candidate for phenotypes described by both monosomic and trisomic models in the hippocampus [16,30]. In addition, a mouse model overexpressing human CBS displayed an increase magnitude of the long term potentiation in vitro similar to the Ts1Yah electrophysiological phenotypes observed in vivo [42].

Only a few cases of DS have been reported with partial trisomy overlapping the most telomeric part of human chromosome 21 [43,44] and so far none have been described with a trisomy limited to this segment. Conversely several cases with monosomy of the telomeric end have been described with mild phenotypes [45–48]. Similarly, analysis of trisomy and monosomy models for Cstb-Prmt2 showed no phenotypes in open field, in the Morris water Maze, either alone or in combination with the Tc1 transchromosome [15,29,49]. Only in fear conditioning task, the Df(10)Yey/- displayed a deficit in contextual memory [49].

In this report, we confirmed that rescuing the number of copies of Abcg1-U2af1 modulates Tc1-induced phenotypes only slightly, although the region was sufficient alone to induce certain learning and memory deficits [15,16], and as such this genetic interval certainly contributes, along with other regions of Hsa21, to the variability of DS features.

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**Author Contributions**

Conceived and designed the experiments: DM AD YH. Performed the experiments: DM PLP. Analyzed the data: DM YH. Contributed reagents/materials/analysis tools: PLP AD. Wrote the paper: DM AD YH.
References

1. Antonarakis SE, Lyle R, Demitzakis ET, Reymond A, Deutsch S (2004) Chromosome 21 and Down syndrome: From genomics to pathophysiology. Nature Reviews Genetics 5: 725–738. PMID: 15510164

2. Herault Y, Duchon A, Velet E, Marechal D, Brault V (2012) The in vivo Down syndrome genomic library in mouse. Prog Brain Res 197: 169–197. doi:10.1016/B978-0-444-54299-1.00009-1 PMID: 22541293

3. Dierssen M, Herault Y, Estivill X (2009) Aneuploidy: from a physiological mechanism of variance to Down syndrome. Physiol Rev 89: 887–920. doi:10.1152/physrev.00032.2007 PMID: 19584316

4. Korenberg JR, Chen XN, Schipper R, Sun Z, Gonsky R, et al. (1994) Down syndrome phenotypes: the consequences of chromosomal imbalance. Proc Natl Acad Sci U S A 91: 4997–5001. PMID: 8197171

5. Letourneau A, Santoni FA, Bonilla X, Sailani MR, Gonzalez D, et al. (2014) Domains of genome-wide gene expression dysregulation in Down’s syndrome. Nature 508: 345–350. doi:10.1038/nature13200 PMID: 24740065

6. Nadel L (2003) Down’s syndrome: a genetic disorder in biobehavioral perspective. Genes Brain and Behavior 2: 156–166. PMID: 12931789

7. Gibson D, Groeneweg G, Jerry P, Harris A (1988) AGE AND PATTERN OF INTELLECTUAL DECLINE AMONG DOWN SYNDROME AND OTHER MENTALLY-RETARDED ADULTS. International Journal of Rehabilitation Research 11: 47–55. PMID: 2974839

8. Antonarakis SE, Epstein CJ (2006) The challenge of Down syndrome. Trends in Molecular Medicine 12: 473–479. PMID: 16935027

9. Dierssen M, Herault Y, Estivill X (2009) Aneuploidy: from a physiological mechanism of variance to Down syndrome. Physiol Rev 89: 887–920. doi:10.1152/physrev.00032.2007 PMID: 19584316

10. Herault Y, Duchon A, Velet E, Marechal D, Brault V (2012) The in vivo Down syndrome genomic library in mouse. Down Syndrome: from Understanding the Neurobiology to Therapy 197: 169–197. doi: 10.1523/JNEUROSCI.3728-10.2010 PMID: 21068296

11. Hattori M, Fujiyama A, Taylor TD, Watanabe H, Yada T, et al. (2000) The DNA sequence of human chromosome 21. Nature 405: 311–319. PMID: 10830953

12. Sturgeon X, Gardiner KJ (2011) Transcript catalogs of human chromosome 21 and orthologous chimpanzee and mouse regions. Mammalian Genome 22: 261–271. doi:10.1007/s00335-011-9321-y PMID: 21400203

13. Reeves RH, Irving NG, Moran TH, Wohn A, Kitt C, et al. (1995) A MOUSE MODEL FOR DOWN-SYNDROME EXHIBITS LEARNING AND BEHAVIOR DEFICITS. Nature Genetics 11: 177–184. PMID: 7550346

14. Sago H, Carlson EJ, Smith DJ, Kilbridge J, Rubin EM, et al. (1998) Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. Proceedings of the National Academy of Sciences of the United States of America 95: 6256–6261. PMID: 9600952

15. Yu T, Liu CH, Belichenko P, Clapcote SJ, Li SM, et al. (2010) Effects of individual segmental trisomies of human chromosome 21 syntenic regions on hippocampal long-term potentiation and cognitive behaviors in mice. Brain Research 1366: 162–171. doi:10.1016/j.brainres.2010.09.107 PMID: 20932954

16. Lopes Pereira P, Magnol L, Sahún I, Brault V, Duchon A, et al. (2009) A new mouse model for the trisomy of the Abcg1-U2af1 region reveals the complexity of the combinatorial genetic code of down syndrome. Hum Mol Genet 18: 4756–4769. doi: 10.1093/hmg/ddp438 PMID: 19783846

17. Fotaki V, de Lagran MM, Estivill X, Arbones M, Diersson M (2004) Haploinsufficiency of Dyrk1A in mice leads to specific alterations in the development and regulation of motor activity. Behavioral Neurosci ence 118: 815–821. PMID: 15301607

18. de Lagran MM, Altafaj X, Gallego X, Martí E, Estivill X, et al. (2004) Motor phenotypic alterations in TgDyrk1a transgenic mice implicate Dyrk1A in Down syndrome motor dysfunction. Neurobiology of Disease 15: 132–142. PMID: 14751778

19. Dierssen M, Altafaj X, Guimera J, Arbones M, Estivill X, et al. (1999) Transgenic mice overexpressing the rat minibrain gene (Dyrk1a): implications for Down syndrome. Cytogenetics and Cell Genetics 86: 11–12.

20. Altafaj X, Dierssen M, Baamonde C, Martí E, Visa J, et al. (2001) Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down’s syndrome. Human Molecular Genetics 10: 1915–1923. PMID: 11555628

21. O’Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, et al. (2005) An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. Science 309: 2033–2037. PMID: 16179473
22. Gribble SM, Wiseman FK, Clayton S, Prigmore E, Langley E, et al. (2013) Massively Parallel Sequencing Reveals the Complex Structure of an Irradiated Human Chromosome on a Mouse Background in the Tc1 Model of Down Syndrome. Plos One 8. doi: 10.1371/journal.pone.0082806 PMID: 24482673

23. Galante M, Jani H, Vanes L, Daniel H, Fisher EMC, et al. (2009) Impairments in motor coordination without major changes in cerebellar plasticity in the Tc1 mouse model of Down syndrome. Human Molecular Genetics 18: 1449–1463. doi: 10.1093/hmg/ddp055 PMID: 19181682

24. Morice E, Andreæ LC, Cooke SF, Vanes L, Fisher EMC, et al. (2008) Preservation of long-term memory and synaptic plasticity despite short-term impairments in the Tc1 mouse model of Down syndrome. Learn Mem 15: 492–500. doi: 10.1101/lm.969608 PMID: 18626093

25. Olson LE, Richtsmeier JT, Leszl J, Reeves RH (2004) A chromosome 21 critical region does not cause specific down syndrome phenotypes. Science 306: 687–690. PMID: 15499018

26. Olson LE, Roper RJ, Sengstaken CL, Peterson EA, Aquino V, et al. (2007) Trisomy for the Down syndrome ‘critical region’ is necessary but not sufficient for brain phenotypes of trisomic mice. Human Molecular Genetics 16: 774–782. PMID: 17339268

27. Belichenko NP, Belichenko PV, Kleschevnikov AM, Salehi A, Reeves RH, et al. (2009) The “Down Syndrome Critical Region” Is Sufficient in the Mouse Model to Confer Behavioral, Neurophysiological, andSynaptic Phenotypes Characteristic of Down Syndrome. Journal of Neuroscience 29: 5938–5948. doi: 10.1523/JNEUROSCI.1547-09.2009 PMID: 19420260

28. Raveau M, Lignon JM, Nalesso V, Duchon A, Groner Y, et al. (2012) The App-Runx1 Region Is Critical for Birth Defects and Electrocardiographic Dysfunctions Observed in a Down Syndrome Mouse Model. Plos Genetics 8. doi: 10.1371/journal.pgen.10020473

29. Duchon A, Poithon S, Braut V, Sharp AJ, Tybulewicz VLJ, et al. (2011) The telomeric part of the human chromosome 21 from Cstb to Prmt2 is not necessary for the locomotor and short-term memory deficits observed in the Tc1 mouse model of Down syndrome. Behavioural Brain Research 217: 271–281. doi: 10.1016/j.bbr.2010.10.023 PMID: 21047530

30. Sahún I, Marechal D, Lopes Pereira P, Nalesso V, Gruart A, et al. (2014) Cognition and Hippocampal Plasticity in the Mouse Is Altered by Monosomy of a Genomic Region Implicated in Down Syndrome. Genetica.

31. Hoelter SM, Dalke C, Kallnik M, Becker L, Horsch M, et al. (2008) “Sighted C3H” mice—a tool for analysing the influence of vision on mouse behaviour? Frontiers in Bioscience 13: 5810–5823. PMID: 18508624

32. Dunlevy L, Bennett M, Slender A, Lana-Elola E, Tybulewicz VL, et al. (2010) Down’s syndrome-like cardiac developmental defects in embryos of the transchromosomal Tc1 mouse. Cardiovasc Res 88: 287–295. doi: 10.1093/cvr/cvq193 PMID: 20558441

33. Duchon A, Raveau M, Chevalier C, Nalesso V, Sharp AJ, et al. (2011) Identification of the translocation breakpoints in the Ts65Dn and Ts1Cje mouse lines: relevance for modeling down syndrome. Mamm Genome.

34. Gubner NR, Wilhelm CJ, Phillips TJ, Mitchell SH (2010) Strain Differences in Behavioral Inhibition in a Go/No-go Task Demonstrated Using 15 Inbred Mouse Strains. Alcoholism-Clinical and Experimental Research 34: 1353–1362. doi: 10.1111/j.1530-0277.2010.01219.x PMID: 20491731

35. Bolivar VJ, Caldarone BJ, Reilly AA, Flaherty L (2000) Habituation of activity in an open field: A survey of inbred strains and F-1 hybrids. Behavior Genetics 30: 285–293. PMID: 11206083

36. Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, et al. (2007) Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behav Brain Res 176: 4–20. doi: 10.1016/j.bbr.2010.10.023 PMID: 21047530

37. Campbell C, Landry O, Russo N, Flores H, Jacques S, et al. (2013) Cognitive flexibility among individuals with Down syndrome: assessing the influence of verbal and nonverbal abilities. Am J Intellect Dev Disabil 118: 193–200. doi: 10.1352/1944-7558-118.3.193 PMID: 23734614

38. Jones BJ, Roberts DJ (1968) QUANTITATIVE MEASUREMENT OF MOTOR INCOORDINATION IN NAIVE MICE USING AN ACCELERATING ROTAROD. Journal of Pharmacy and Pharmacology 20: 302–8. PMID: 4384609

39. Rustay NR, Wahlsten D, Crabbe JC (2003) Influence of task parameters on rotarod performance and sensitivity to ethanol in mice. Behavioural Brain Research 141: 237–249. PMID: 12742261

40. Kroker KS, Rast G, Giovannini R, Marti A, Dorner-Ciossek C, et al. (2012) Inhibition of acetylcholinesterase and phosphodiesterase-9A has differential effects on hippocampal early and late LTP. Neuropharmacology 62: 1964–1974. doi: 10.1016/j.neuropharm.2011.12.021 PMID: 22245562

41. Hutson PH, Finger EN, Magliaro BC, Smith SM, Converso A, et al. (2011) The selective phosphodiesterase 9 (PDE9) inhibitor PF-04447943 ([1H]-[3S,4S]-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) enhances synaptic
plasticity and cognitive function in rodents. Neuropharmacology 61: 665–676. doi: 10.1016/j.neuropharm.2011.05.009 PMID: 21619887

42. Regnier V, Billard JM, Gupta S, Potier B, Woerner S, et al. (2012) Brain Phenotype of Transgenic Mice Overexpressing Cystathionine beta-Synthase. Plos One 7. doi: 10.1371/journal.pone.0051204 PMID: 23349657

43. Lyle R, Béna F, Gagos S, Gehrig C, Lopez G, et al. (2009) Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. Eur J Hum Genet 17: 454–466. doi: 10.1038/ejhg.2008.214 PMID: 19002211

44. Korbel J, Tirosh-Wagner T, Urban A, Chen X, Kasowski M, et al. (2009) The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. Proc Natl Acad Sci U S A 106: 12031–12036. doi: 10.1073/pnas.0813248106 PMID: 19597142

45. Hannachi H, Mougou-Zerelli S, BenAbdallah I, Mama N, Hamdi I, et al. (2011) Clinical and molecular characterization of a combined 17p13.3 microdeletion with partial monosomy 21q21.3 in a 26-year-old man. Cytogenet Genome Res 135: 102–110. doi: 10.1159/000330880 PMID: 21876345

46. Melis D, Genesio R, Cappuccio G, MariaGinocchio V, Casa RD, et al. (2011) Mental retardation, congenital heart malformation, and myelodysplasia in a patient with a complex chromosomal rearrangement involving the critical region 21q22. Am J Med Genet A 155A: 1697–1705. doi: 10.1002/ajmg.a.33976 PMID: 21671372

47. Roberson ED, Wohler ES, Hoover-Fong JE, Lisi E, Stevens EL, et al. (2011) Genomic analysis of partial 21q monosomies with variable phenotypes. Eur J Hum Genet 19: 235–238. doi: 10.1038/ejhg.2010.150 PMID: 20823914

48. Lyle R, Bena F, Gagos S, Gehrig C, Lopez G, et al. (2009) Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. European Journal of Human Genetics 17: 454–466. doi: 10.1038/ejhg.2008.214 PMID: 19002211

49. Yu T, Clapcote S, Li Z, Liu C, Pao A, et al. (2010) Deficiencies in the region syntenic to human 21q22.3 cause cognitive deficits in mice. Mamm Genome 21: 258–267. doi: 10.1007/s00335-010-9262-x PMID: 20512340