

Pre-weaning Sensorial and Motor Development in Mice Transpolygenic for the Critical Region of Trisomy 21

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Trisomy 21 occurs every 1/800 births and is the most frequent genetic cause of mental retardation. Children with trisomy 21 show delayed sensorial and motor development as well as cognitive disorders. We selected a mouse model of trisomy 21 (TRS21): transgenic mice carrying extra copies of a HSA21 region corresponding to the *D21S17-ETS2* region (previously referred to as “Down syndrome critical region 1”). Sensorial and motor development was measured in these partially transgenic mice, from birth to weaning. The four HSA21 regions contributed unequally to sensorial and motor development delay. The more centromeric region (230E8) modified 4 of the development indicators plus the size of the effect, indicated by partial $\eta^2(\eta_p^2)$, reached a median value of 14.5%. The neighboring 141G6 region contributed to 5 developmental differences (η_p^2 median value 14%). The most telomeric region (285E6) only modified one development indicator. An extra copy of an HSA21 fragment (referred to here as the 152F7 region) induced modifications to 14 of the 18 indicators measured with a η^2 median value reaching 20%. The results indicate a noticeable contribution of the 152F7 region to sensorial and motor development. The contribution of this region to cognitive functioning and its neurobiological basis has been already reported. This set of result suggests the location in the *D21S17-ETS2* region of several genes playing crucial role in cognitive and developmental impairment observed in TRS21.

KEY WORDS: DCR-1; Down syndrome; early development; HSA21; MMU16; motor behavior; sensorial development; YAC (Yeast Artificial Chromosome).

Trisomy 21 (TRS21) is associated with a wide set of morphological, physiological, immune and neurological characteristics. The morphology is short and stocky with virtually no neck because of skeletal

abnormalities. The limbs are malformed with short, broad hands with a single transverse palmar crease and a shortened, incurved fifth finger. The facial features of persons with TRS21 typically include oblique eye fissures, epicanthic eye-folds, a flat nasal bridge, the mouth permanently open and the tongue protruding. The incidence of heart defects (atrioventricular septal defect, ventricular septal defect, atrial septal defect or ductus arteriosus) is about 30% in TRS21. Not all TRS21 patients are affected, and of those that are, not all are affected to the same degree of severity (Antonarakis *et al.*, 2004) but mental retardation remains the most striking and permanent feature of TRS21. IQ ranges from 30 to 70 with an average around 50 (Chapman and Hesketh, 2000).

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Well documented psychological profiles of TRS21 subjects (Vicari, 2006) show that not all skills are affected in all persons or to the same extent.

After the pioneering description by Seguin, adopted and adapted by Down, it took more than a century to decipher the etiology of the syndrome. Lejeune *et al.* (1959) were the first to prove that an extra copy of chromosome 21 was associated with the condition referred to as “mongolism” or “Down syndrome” (see Roubertoux and Kerdelhué, 2006, for an historical perspective).

Five years after the full sequencing of the long arm of chromosome 21 (HSA21) by Hattori *et al.* (2000), we know that not all of the 225 genes carried by HSA21 contribute to mental retardation in TRS21. Observations of partial TRS21 cases have suggested that it was probably not the entire HSA21 that was involved in the pattern of traits described in the TRS21 phenotype. The region around 21q22.2, with D21S17 and ETS2 as boundaries, might be involved in most of the phenotypic traits observed in TRS21 and, for this reason, it has been labeled Down Syndrome Critical Region-1. These findings were obtained on the basis of a small number of observations as the prevalence of partial trisomy is less than 1%. Such low prevalence restricts the impact of the critical region concept as the observations were done on tiny samples. The scarcity of cases of partial TRS21 meant it was virtually impossible to discover extra copies of a single gene and to find links between the over-expression of the gene and a phenotypic trait. Mouse models were therefore the obvious choice.

In sequencing HSA21, Hattori *et al.* (2000) proved that about 80% of HSA21 and MMU16 were syntenic, the remaining HSA21 being syntenic with MMU10 and MMU17. Three main models of murine TRS16 have been developed; these are characterized by the mouse origin of the extra copy of the MMU16 fragments. Mice aneuploid for a single region of chromosome 16, syntenic with human chromosome 21, (Ts65Dn) were developed by Davisson *et al.* (1990, 1993); the extra region encompasses 132 mouse genes from *Mrpl39* to *Znf295* (Baxter *et al.*, 2000). Sago *et al.* (1998) accidentally generated a second aneuploidy on the Ts65Dn background for the region between *Mrpl39* and *Znf295*: a partial trisomy of MMU16 occurred when the *Sod-1* gene was targeted; this included 85 genes from *Sod-1* to *Znf295*, with *Sod-1* not being over-expressed; the new model was labeled Ts1Cje. A third aneuploidy (Ms1Cje) was generated on the same background but covered the

centromeric part of Ts65Dn; Ms1Cje includes the complementary region covered by Ts65Dn; the extra-chromosomal fragment encompasses 46 genes with *App* and *Sod-1* as boundaries. Another group of models for TRS21 was produced by inserting of all or part of HSA21. Shinohara *et al.* (2001) and O'Doherty *et al.*, (2005) incorporated the entire HSA21 into the mouse genome.

With the development of mouse models, it was possible to test the hypothesis of a “critical” region. Olson *et al.* (2004), compared Ts1Cje (including the chromosomal region syntenic with the “critical” region) and Ms1Cje which is too centromeric to include the same region. Morphological abnormalities observed in the bones of the face were not associated with Ts1Cje, but were with Ms1Cje. This finding challenges the validity of the “critical region” concept as an explanation of all the characteristics observed in TRS21. However, there was still the association between the *D21S17-ETS2* fragments and cognitive characteristics found in TRS21 patients. The Ms1Cje fragment does not include the *D21S17-ETS2* region that is syntenic to the “critical region”, but Ts1Cje does include the *D21S17-ETS2* region. Ms1Cje mice do not display cognitive impairment, but Ts1Cje present the same cognitive impairments observed in Ts65Dn mice (Sérégaza *et al.*, 2006).

Smith and Rubin (1997) and Smith *et al.* (1995) provided more direct evidence of *D21S17-ETS2* region involvement in cognitive disorders. They selected four contiguous chromosomal fragments covering the *D21S17-ETS2* region syntenic to the human “critical” region. The fragments were inserted into Yeast Artificial Chromosomes (YACs) and transfected into the mouse genome using standard techniques for producing transgenic mice. These chromosomal fragments cover a region encompassed in the 21q22.2 band and located between human *D21S55* (*D21S55* Site Targeted Sequence—STS) and the *ZNF295* gene, with one gap corresponding to the *SIM2* gene and its neighboring region (see Fig. 1). Smith and Rubin (1997) and Chabert *et al.* (2004) demonstrated that simple learning was not affected in any cases of partial trisomy, but that complex tasks, and specifically the acquisition of new skills, were impaired in several cases of partial trisomy, and, *inter alia*, by an extra copy of the 152F7 fragment. This association was independent of the tests used to assess the learning (Chabert *et al.*, 2004). Laterality, is believed to be implicated in cognition. Hand preference was found atypical in TRS21 persons (Carlier *et al.*, 2006) and in mice carrying triple copies of the

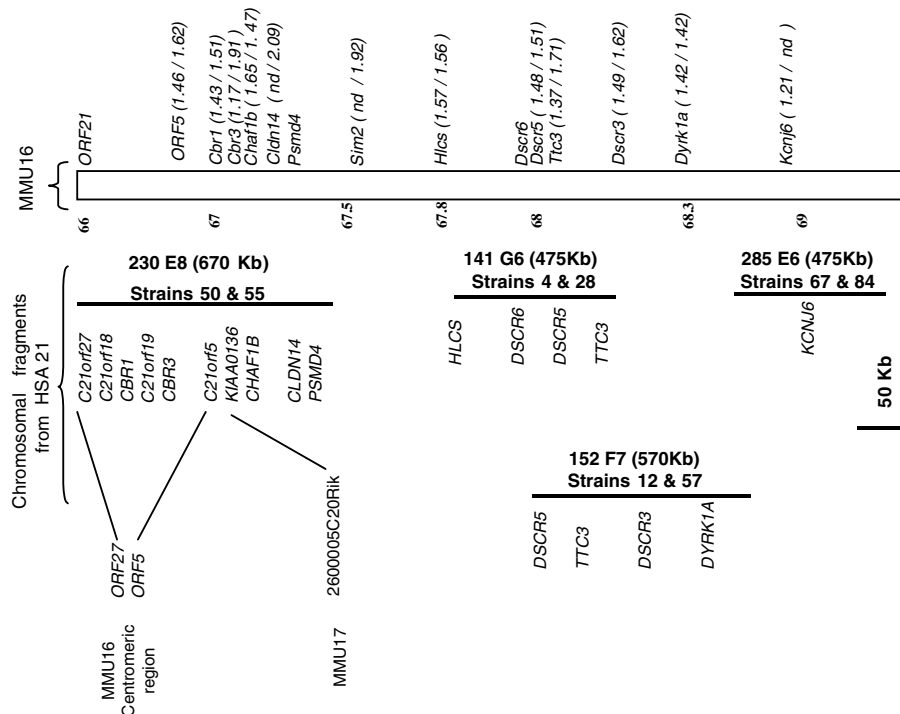


Fig. 1. Map of the syntenic fragment of the *D21S17-ETS2* region on the mouse chromosome 16 (MMU16) and human HSA21 fragments covering the *D21S17-ETS2* region. The distance to the centromere is to the right of MMU16. The names of the murine genes are to the left and followed by the normalized expression value for the gene in the brain and muscle at 30 days of age (in Ts65dn) (from Lyle *et al.*, 2004). nd: not done.

D21S17-ETS2 region (Roubertoux *et al.*, 2005). Mice over-expressing the human *DYRK1A* gene (encompassed in the *D21S17-ETS2* region) show lower learning capacities (Altafaj *et al.*, 2001).

But there is more to cognition than just problem solving. Deficits occurring in the weeks immediately after birth affect the development of cognitive skills. A delay in walking not only affects relationships with others, but also modifies self/body image. It has been suggested that autonomous walking modifies the perception of objects and distances, and makes it easier to develop cognitive skills. Children with TRS21 show delayed motor and sensorial development (Shumway-Cook and Woollacott, 1985; Peuschel *et al.*, 1991; Butler, 1986; Ulrich *et al.*, 1992, 1995; Campos *et al.*, 2000; Clark and Wilson, 2003) noted that independent walking was mastered by TRS21 children one year later than typically developing children, and that prehension was impaired (Kearney and Gentile, 2003). Given the considerable and constant contribution of the *D21S17-ETS2* region to cognition reported, our study of transgenic mice focused on the effects of extra copies of HSA21 covering the *D21S17-ETS2* region and the impact on

sensorial and motor development from birth to weaning. Are these major effects? We addressed the question by computing the size of the effect in our experiment and by comparing the value with data previously obtained on Ts65Dn mice and “one-off” transgenics. Candidate genes among those mapped on the HSA21 fragments were to be considered.

MATERIAL AND METHODS

Transgenic Mice

Yeast Artificial Chromosomes (YACs) containing contiguous HSA21 fragments covering the *D21S17-ETS2* region were transfected into the mouse genome. The transfection of one fragment produced partial trisomy as each of the HSA21 fragments is syntenic with a part of MMU16 (Smith and Rubin, 1997; Smith *et al.*, 1995). Four HSA21 fragments were selected. The matching of murine genes to human genes carried by the *D21S17-ETS2* region is shown in Figure 1. We used two strains for each of the four YACs to detecting any possible insertion effect. Strains 50 and 55 were YAC 230E8 (abbreviated as E8), 4 and 28 were YAC 141G6 (G6), 12 and

57 were YAC 152F7(F7), and 67 and 84 were YAC 285E6 (E6). The integrity of the chromosomal fragments, the number of copies and the expression of the genes had been reported previously (Smith and Rubin, 1997; Smith *et al.*, 1995). One copy of the corresponding HSA21 chromosomal fragment was integrated into all the strains, except strains 28 and 57 where 3 and 2 copies were integrated, respectively; the number of copies carried by strain 84 was unknown. The FVB background 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 derived F₁ male offspring born from C57BL/6J females and transgenic FVB males. The offspring observed in the present experiment were thus born and reared by females with identical C57BL/6J genotypes. This strategy stabilized the pre- and post-natal maternal sources of variation that are known to play a role in pre-weaning development (Le Roy *et al.*, 2001). There were no transgenic strain 4 and 57 females for several generations because the corresponding YACs were inserted into the non-pairing region of the Y-chromosome. Observations were therefore restricted to males only as no transgenic female mice were available from strains 4 and 57. The B6.FVBF₁ males were used as controls.

The sample sizes were: 23B6.FVBF₁ controls; 28E8 mice (16 strain 50 and 12 strain 55); 27G6 (14 strain 28 and 13 strain 4); 49F7 (20 strain 12 and 19 strain 57); 35E6 (17 strain 67 and 18 strain 84). The pups came from 6 to 11 different litters per strain.

Age of Appearance of Adult Behavior Patterns and Growth Measurements

The age ranges for test periods for the appearance of adult behavior patterns were determined on the basis of previously published data (Le Roy *et al.*, 2001) and were used to set the observation periods for the present experiment. The age (number of days after birth) when a response appeared or disappeared was recorded. The following patterns were observed and grouped into four categories.

The investigation of early post-natal sensorial and motor development monitored 7 patterns.

- (1) *Righting pattern*: when placed on its back, the pup immediately tries to get onto all four paws. We recorded the age when the pup turned over within 20 seconds.

- (2) *Cliff drop aversion*. We placed the pup on the edge of a cliff, forepaws and head over the edge. It turned and crawled away from the cliff.
- (3) *Forepaw grasping pattern*. When the inside of one paw is gently stroked with an object, the paw flexes to grasp the object.
- (4) *Forelimb placing* and (5) *hindlimb placing*. When the dorsum of the forepaw or hindpaw is brought into contact with the edge of an object, the animal raised the paw and placed it on the object.
- (6) *Age of disappearance of rooting pattern*. Bilateral stimulation of the face caused the pups to crawl forwards, pushing the head in a rooting fashion. The response is present at birth and disappears later.
- (7) *Age of disappearance of the crossed extensor pattern*. When pinched, the forelimb stimulated flexed while the opposite hindlimb extended. For (6) and (7), the score was the day when the response disappeared.

We also investigated five sensorial responses screening postural, visual, tactile and auditory systems.

- (8) *Negative geotaxia*. When placed facing downward on a 45° angle slope, the head pointing down the incline, the pup turned to face upwards. The response occurs when the labyrinths are functional.
- (9) *Vibrissae placing pattern*. The pup was suspended by the tail and lowered towards the tip of a pencil. When the vibrissae touched the pencil the pup raised its head and performed a placing response with the extended forelimb. The response appears when the tactile system is functional.
- (10) *Age at eyelid opening*: the score is the age in days when the pup's eyes opened.
- (11) For *Visual placing* as for vibrissae placing, we suspended the pup by the tail and lowered it towards the tip of a pencil, without touching the vibrissae. The maturity of the visual system is shown by the pup raising its head and performing a placing response with a forelimb extended. We recorded the number of days when the response occurred, after visual placing.
- (12) *Startle response*. A composite sound (including 10–30 kHz frequencies) was emitted 4 cm above the head of the pup and the startle response was observed visually.

We measured the age of appearance of six motor behavior responses, including walking patterns.

(13) and (14)

Bar holding pattern. The forepaws are placed on a round wooden bar (7 mm in diameter). The ability to hang for 10 seconds using the forepaws appears before the pup can also put its hindpaw on the bar for a longer period of stability. The age of appearance of the two behavior responses were scored independently.

(15) and (16)

Vertical clinging and climbing. The pup was held against a vertical metal grid (wire: 0.6 mm in diameter, mesh: 6 mm wide). We scored: clinging for 10 seconds and climbing after clinging.

We also measured the age when two walking patterns occurred.

(17) *Adult paw position.* The criterion for the response was reached when the pup put all four paws flat on the ground.

(18) *Adult walking pattern.* During the first days of the pup's life, the steps produce a pivoting walk, later developing into linear displacement. We recorded the age when linear displacement replaced pivoting.

Body weight was individually measured, rounded off to the nearest centigram, at 1, 5, 10, 15 and 20 days of age.

Biparous females were isolated approximately 3 days before parturition and were maintained with food and water *ad libitum* under a 12/12 light cycle with lights on at 8 am. We inspected visually the cages at 07:30 am and 08:30 pm. Pups born during the night were selected. The day when the birth was discovered was recorded as day 0; the error on estimated time of birth was therefore ± 6.5 hours. Litters with less than 5 pups were discarded; larger litters were culled to 6. On day 1, each pup was marked on the tail, with India ink, and re-marked if necessary. All the male pups in each litter were observed. Genotyping was conducted after the behavioral observations and according to the protocol previously described (Chabert *et al.*, 2004). Pups were allocated to the corresponding genotype *a posteriori*. The behavioral observations were thus made blind.

Statistical Analysis

For the different analyses, we processed raw data to achieve normal distribution and homoscedasticity (Bartlett's test) conditions. We performed between strain comparisons within the transgenic

fragment to detect any possible insertion effect for each variable. As the insertion effect was undesirable, we maximized the probability of detecting it by using pair comparisons (Student's *t-test*). Data from the two strains were pooled within each HSA21 fragment when no insertion effect appeared and then one-way ANOVA was conducted (HSA21 fragments plus controls—5 levels); partial comparisons were done using the Student's *t-test*. Repeated measures ANOVA were done for body weight. We chose the threshold of 0.05. The size of the effect (η_p^2) was computed according to Cohen (1973).

RESULTS

Between-strain/within chromosomal fragment comparisons were performed to detect any possible insertion effect on developmental measurements. We detected one significant difference for *Cliff drop aversion* between strains 4 and 28 carrying the 141G6 chromosomal fragment and this variable was therefore rejected. For the other measurements, the two strains carrying the same chromosomal fragment were pooled and the mean performance, corresponding to each chromosomal fragment (E8, G6, F7 and E6), was compared to the non-transgenic group (Figs. 2–6). The size of the effect seen in this comparison is reported in Table I.

DISCUSSION

We compared sensorial and motor development in four groups of mice carrying segmental trisomies covering the *D21S17-ETS2* region, also known as the Down syndrome chromosomal region 1 (DSCR1). An extra copy of each of the four fragments of HSA21 was associated with modifications in the rate of development but, the four chromosomal fragments did not have the same effect on development.

How Does the Phenotype of Transgenic or Targeted Mice Fit the Phenotypic Traits Observed in Humans?

TRS21 persons are short and stocky, with general growth retardation affecting birth length and final height, body mass index, head circumference and limb length (Myrelid *et al.* 2002); this begins during pre-natal development (Mazzone *et al.*, 2004). Reduced body mass growth was not observed from birth to weaning for the partial transgenics 230E8, 141G6 and 285E6, when compared to the diploid mice. The 152F7 trisomics were significantly lighter

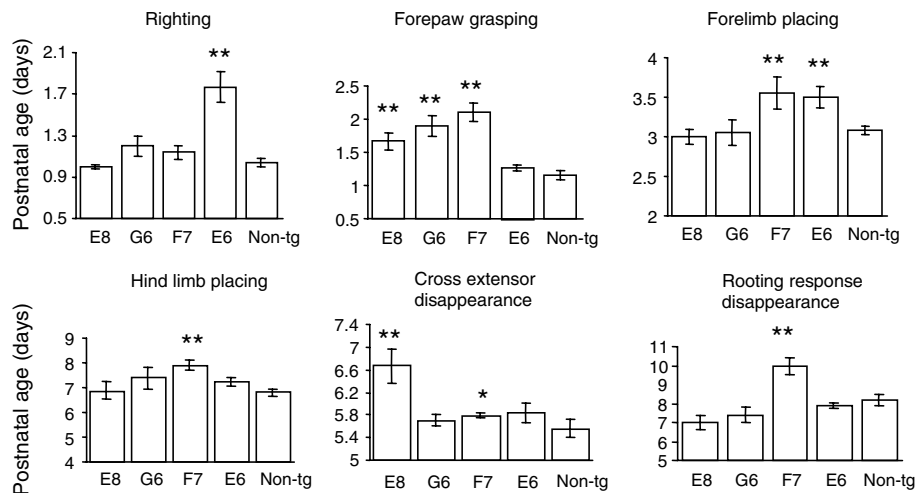


Fig. 2. Early patterns of adult motor behavior. Age of appearance in partial trisomic mice (E8 for 230E8, G6 for 141G6, F7 for 152F7 and E6 for 285E6) compared to non-transgenic diploid mice (Non-tg). Mean \pm SEM. *t*-value: * p < 0.05; ** p < 0.01.

from birth to day 20, and differed from both the diploids and the other transgenics. The smaller body mass of 152F7 persisted after the pre-weaning period and cannot be attributed to maternal effects (pre- or post-natal) as the five groups of mice had the same C57BL/6J mothers. The size of the effect, comparing the 152F7 trisomics and the diploid mice, is 11%. This is high but not as high as the 18% that we deduced from data published by Holtzman *et al.* (1996) comparing Ts65Dn trisomic and diploid mice. This difference in effect suggests that the

D21S17-ETS2 region contributes to the smaller body weight in TRS21, *via* (a) gene(s) mapped on the F7 fragment, but that genes outside this region also contribute to the lower weight.

A number of longitudinal studies have focused on the motor development of TRS21 patients and have shown heterogeneous development during infancy (Mazzone *et al.*, 2004). TRS21 children aged 2 have limited gestural repertoires (Iverson *et al.*, 2003), and TRS21 children have less harmonious development of their postural synergies, compared to typically developing persons (Latash *et al.*, 2005a, b). All these studies report delayed development in TRS21 persons. We therefore expected delayed emergence of adult sensorial and motor skills in our mice carrying extra copies of the *D21S17-ETS2* critical region. Our observations show that age of appearance of sensorial and motor adult patterns was delayed in the four groups of partial transgenic mice, compared to diploids, with one exception: *eyelid opening age* in E8 mice. E8, G6 and E6 mice registered a smaller number of cases of delayed emergence of adult patterns; but the delays were substantial in F7 with 12 of the 17 adult patterns tested appearing later than in diploid mice; the size of the effect for 8 of these differences can be considered as high (>20%). It was difficult to compare these values with Ts65Dn data as Holtzman *et al.* (1996) did not provide information on the method of calculation, but the conclusion reached was similar with only a small number of sensorial measurements affected in the Ts65Dn, and also in our mice, while the age of

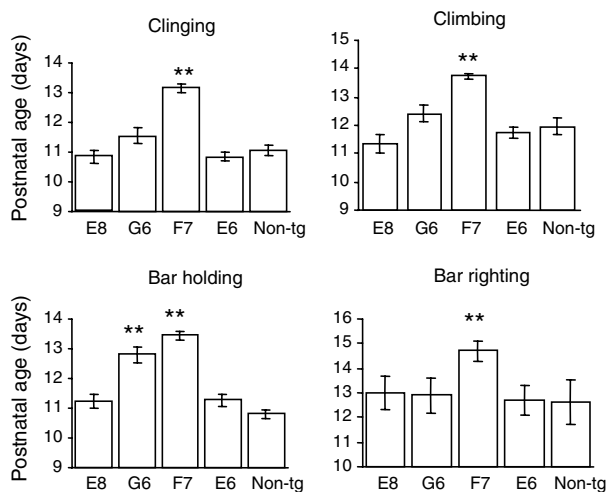


Fig. 3. Patterns of adult motor behavior occurring day 10. Age of appearance in partial trisomic mice (E8 for 230E8, G6 for 141G6, F7 for 152F7 and E6 for 285E6) compared to non-transgenic diploid mice (Non-tg). Mean \pm SEM. *t*-value: * p < 0.05; ** p < 0.01.

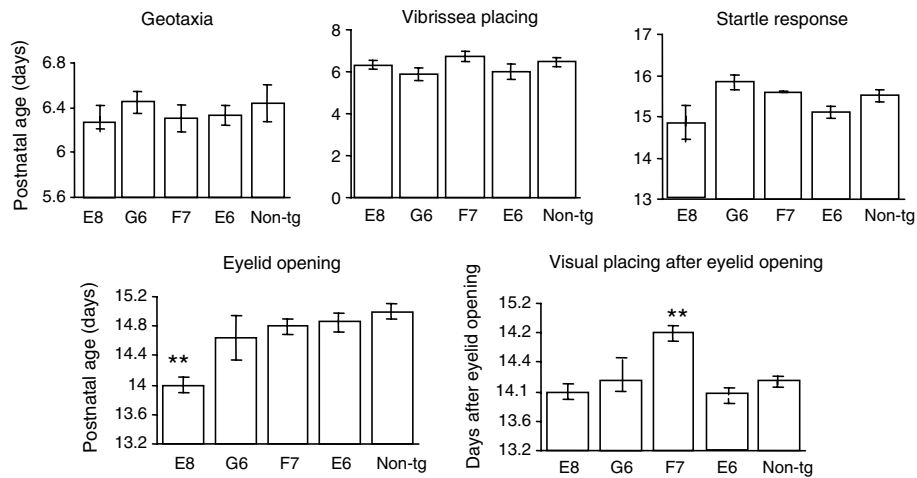


Fig. 4. Sensorial responses. Age of appearance in partial trisomic mice (E8 for 230E8, G6 for 141G6, F7 for 152F7 and E6 for 285E6) compared to non-transgenic diploid mice (Non-tg). Mean \pm SEM. *t*-value: * $p < 0.05$; ** $p < 0.01$.

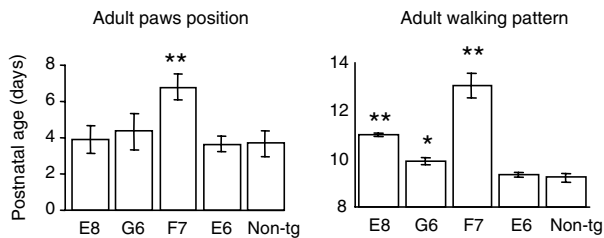


Fig. 5. Age of appearance of adult walking patterns in partial trisomic mice (E8 for 230E8, G6 for 141G6, F7 for 152F7 and E6 for 285E6) compared to non-transgenic diploid mice (Non-tg). Mean \pm SEM. *t*-value: * $p < 0.05$; ** $p < 0.01$.

appearance of adult walking patterns was delayed in both studies. The same result was obtained with mice over-expressing the *DYRK1A* gene, carried by F7 and therefore by the Ts65Dn mice. The size of the effect was larger with the *DYRK1A* transgenic mice than with F7 mice which carry extra copies of 4 genes. This finding supports the hypothesis of an epistatic effect between *Dyrk1a* and the other F7 genes.

Candidate Genes and Age of Appearance of Adult Sensorial and Motor Patterns

The genes carried by the triplicated fragment of MMU16 in Ts65Dn mice do not produce the same gene dosage effects. Lyle *et al.* (2004) noted that not all the genes that are present in three copies are over-expressed. Some of the HSA21 genes with three copies do not show more expression than would be expected with two copied genes (Antonarakis *et al.*, 2004). Gitton *et al.* (2002), Kahlem *et al.* (2004) and

Kahlem (2006) reported that differences in expression of the triplicated genes of HSA21 were tissue-dependent. Reymond *et al.* (2002) and Lyle *et al.* (2004) reported that the level of expression was also age-dependent, varying from embryonic to aerial life, in mice.

To identify genes modulating sensorial and motor development in our model of transgenic mice,

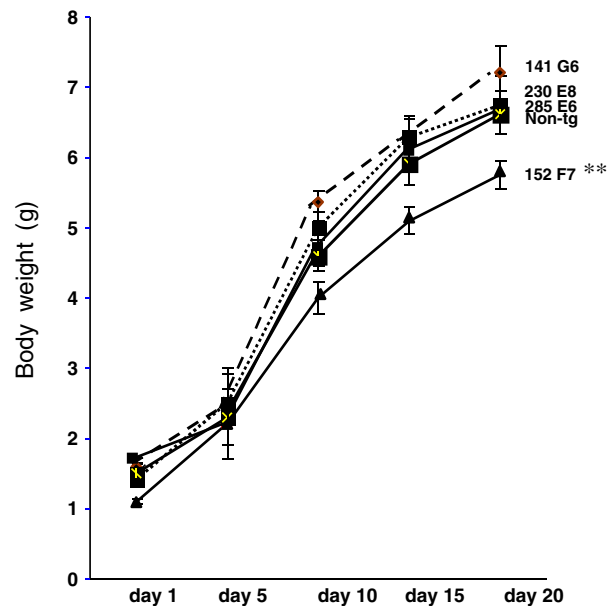


Fig. 6. Body weight development in partial trisomic mice (E8 for 230E8, G6 for 141G6, F7 for 152F7 and E6 for 285E6) compared to non-transgenic diploid mice (Non-tg). Mean \pm SEM. *F* value: ** $p < 0.01$.

Table I. Effect Size on Sensorial and Motor Development Measurements for the Comparisons between Partial Trisomic Mice Carrying HSA21 Fragments and Diploid Mice. (E8 for 230E8, G6 for 141G6, F7 for 152F7 and E6 for 285E6)

Early development indicators	η_p^2			
	E8	G6	F7	E6
Righting pattern			14	
Forepaw grasping pattern	11	14	21	
Forelimb placing			22	11
Hindlimb placing			15	
Age of disappearance of rooting pattern			19	
Age of disappearance of crossed extensor pattern	18		8	
Negative geotaxia				
Vibrissae placing pattern				
Age at eyelid opening	7			
Visual placing			15	
Startle response				
Bar holding pattern		20	25	
Bar righting			26	
Vertical clinging		12*	20	
Vertical climbing		14*	22	
Adult paw position			27	
Adult walking pattern	26	17	31	
Body weight			11	

*Indicate differences that did not reached the 0.05 threshold.

proof is needed of an association between genes and phenotype, but that alone is not sufficient. The level of expression must be determined. Figure 1 shows the level of expression of the genes in the *D21S17-ETS2* region. We selected the levels of expression measured at weaning (30 days of age) in the brain and muscle using data published by Antonarakis's team (Lyle *et al.*, 2004). The normalized expression value for a gene with two copies being 1.00, the expected normalized value for a gene with three copies is 1.50.

In 230E8 mice, three development indicators were delayed and one was accelerated. Several of the ten genes carried by the fragment can be involved in neuronal or muscular development affecting the behavioral measurements used to assess the mice during pre-weaning development. *CLDN14* (Claudin tight junction protein) plays a role in myelin sheaths in the brain (Morita *et al.*, 1999) and may have crucial role to play in the development of neuronal transmission. The normalized expression value of *CLDN14* in the brain is high at 1 month (2.09). *CBR1* and *CBR3* (carbonyl reductase 1 and 3) modulate NADPH activity, and NADH is involved in neuronal functioning. *CBR1* is over-expressed at 1 month in the brain and muscle (1.43 and 1.51, respectively). *CBR3* is expressed later in the brain, at 1 month, but its expression is very high in muscular tissue.

Three development indicators were delayed in 141G6 mice. Four genes are carried by 141G6, the

function of two of them—*DSCR6* and *DSCR5* (Down Syndrome chromosomal region protein 5 and 3)—is unknown, but *DSCR5* may be a potential candidate as it is over-expressed in both the brain and muscle (1.48 and 1.51). The *HLC6* gene (holocarboxylase synthetase, modulating the frequency, rate and/or extent of the cell cycle), is over-expressed in brain and muscle at 1 month of age (1.57 and 1.56), and may be involved in neuronal development *via* its function in the cell cycle. *TTC3* (tetratricopeptide repeat domain 3) is expressed more in muscle (1.71) than in the brain (1.37).

The 152F7 triplicated region is associated with 14 delayed development indicators. The fragment encompasses four genes; 2 are common to 141G6 (*DSCR5* and *TTC3*) as 152F7 and 141G6 overlap. The function of *DSCR3* is unknown, but as it is over-expressed in the brain (1.49), it may be implicated in neuronal development. The *DYRK1A* gene (dual-specificity tyrosine-regulated kinase) is linked with delayed sensorial and motor development (Altafaj *et al.*, 2001) and affects early neuronal morphology (Fotaki *et al.*, 2002); it is over-expressed (1.42) in both the brain and muscle. Given the overlapping of 141G6 and 152F7 and given the minor impact of the extra copies of the four genes carried by 141G6, we can deduce that the extra copies of the genes carried by 152F7 only may be involved in sensorial and motor development.

A triple copy of 285E6 delayed two development indicators. The *Kcnj6* gene (potassium inwardly-rectifying channel, subfamily J, member 6) is the sole gene carried by the 285E6 fragment. *Kcnj6* is slightly over-expressed in brain and this may explain its minor impact on sensorial and motor development.

Syndromic and Non-syndromic Delayed Development

In a previously published study, we mapped Quantitative Trait Loci (QTL) (Le Roy *et al.*, 2001) linked to the indicators used to explore development in trisomic mice, focusing on the *D21S17-ETS2* region. The behavioral examination was conducted under identical conditions and by the same person (P.L.R.). The genetic pool was partially different as we used intercrosses between C57BL/6J and NZB/BINJ (Le Roy *et al.*, 2001) and the present study had F₁ intercrosses between C57BL/6J and FVB. We did not detect any links between developmental measurements and markers located on MMU16, but triple copies of chromosomal syntenic fragments covering the *D21S17-ETS2* region revealed the contribution of MMU16 or HSA21 genes. This finding is similar to results previously reported on laterality in mice (Roubertoux *et al.*, 2005). We mapped a major QTL linked with laterality on chromosome 4 (Roubertoux *et al.*, 2005), but detected an effect of the *D21S17-ETS2* region on HSA21 modifying paw preference (Roubertoux *et al.*, 2005). All these results support the distinction made between syndromic and non-syndromic traits. The same trait can be observed in association with one or even several syndromes, and also independently of any syndrome. The same trait can be modulated by different genes in patients presenting a syndrome and in typically developing persons.

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