MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES
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A Year of Unprecedented Progress in Down Syndrome Basic Research

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The years 2006 and 2007 saw the publication of three new and different approaches to prevention or amelioration of Down syndrome effects on the brain and cognition. We describe the animal model systems that were critical to this progress, review these independent breakthrough studies, and discuss the implications for therapeutic approaches suggested by each.
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ANIMAL MODELS OF DOWN SYNDROME

Features of Down syndrome (DS) arise due to overexpression of genes on human chromosome 21 (Hsa21) usually through the triplication of the entire chromosome. Divergence from euploid of the structures and functions that are affected in DS may result from upregulation of Hsa21 genes in adult cells and tissues and/or because of inappropriate cues during development that are perpetuated in descendents of the cells that are affected initially [Potier et al., 2006; Roper and Reeves, 2006]. The central nervous system begins to develop very early in embryogenesis and substantial structural and functional changes are still evident for several years following birth, and to some degree, throughout life. Thus, the brain is especially susceptible to perturbations caused by subtle changes in gene expression. Small structural differences can result in a substantial degree of dysfunction at the level of individual neurons, which will affect the complex circuits involved in cognitive function.

Development cannot be studied in human beings at the level necessary to understand the complex processes that are perturbed by trisomy. In vitro molecular biology and cell culture systems are important tools but do not replicate the developmental processes that are the targets for intervention to ameliorate DS features. Animal models are essential to study the disruption of the developmental *process* caused by trisomy.

The laboratory mouse provides the best-characterized experimental system for studies of mammalian aneuploidy. The first line of evidence supporting the use of mouse models to study DS is genetic. Detailed comparative and physical mapping and now comparative sequence analysis [Hattori et al., 2000; Mural et al., 2002; Toyoda et al., 2002] demonstrate highly conserved linkage with Hsa21 on mouse chromosomes 16 (Mmu16), 17, and 10. Ts65Dn mice are trisomic for nearly half of the mouse equivalents of Hsa21 genes, whereas other models contain subsets of these (Fig. 1). The elevated gene expression due to trisomy is very comparable between these mice and human beings. But will this comparable genetic effect have the same outcome on the features of the mouse, i.e., will analogous phenotypes be produced in the same way?

For many features of DS, there is strong evidence to validate the idea that developmental processes affected by trisomy 21 in humans can be studied in mice. Quantitative phenotype assessments in Ts65Dn mice demonstrate directly comparable dysmorphologies in craniofacial structure, in which the same bones affected in DS are affected in the same way in mice [Richtsmeier et al., 2000, 2002]. Histopathological analysis of the cerebellum in Ts65Dn mice predicted a pattern of cell loss that was subsequently shown to occur in humans with DS [Baxter et al., 2000]. Age-related loss of forebrain cholinergic neurons occurs in Ts65Dn mice and in DS as well as in Alzheimer disease. Deficits in retrograde transport of NGF are responsible for this loss in mice [Cooper et al., 2001] and suggest a therapeutic approach to this problem in humans. Detailed studies of synaptic dysfunction in mouse models point towards specific neuronal pathologies in DS [Belichenko et al., 2004].

Trisomic mouse models also provide a good representation of important aspects of cognitive dysfunction in DS. Work by Nadel and coworkers has demonstrated that functions of the hippocampus are among those most severely affected in DS [Nadel, 2003; Pennington et al., 2003]. A robust learning and memory deficit in tests requiring hippocampal function has been demonstrated in Ts65Dn mice [Escorihuela et al., 1995; Reeves et al., 1995; Holtzman et al., 1996]. Hippocampal dysfunction extends to studies of the electro-

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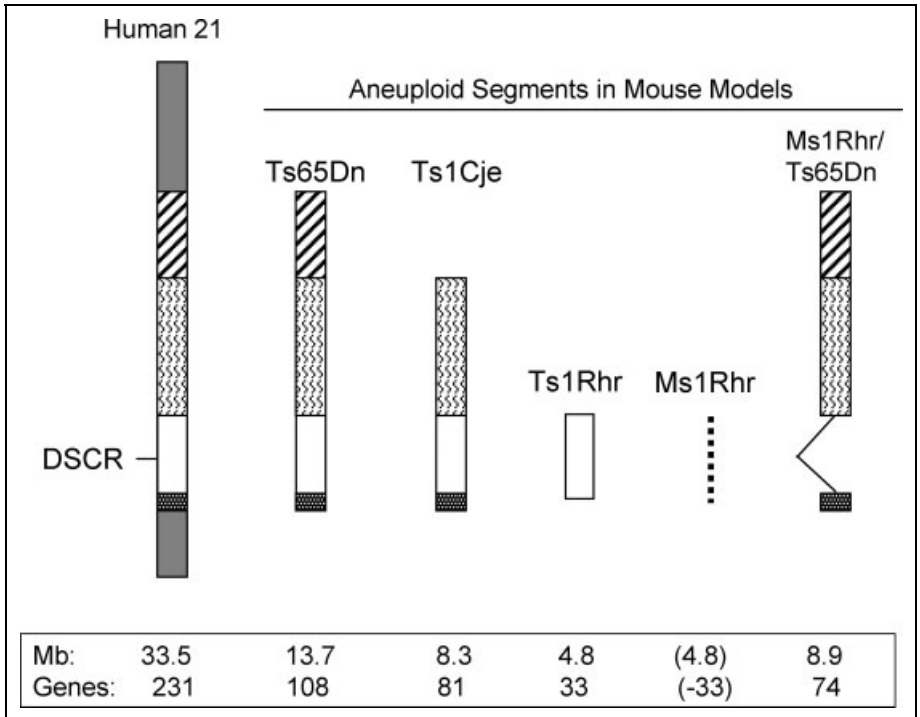

Fig. 1. Aneuploid segments in mouse models used to study Down syndrome. Gene numbers for each segment are based on conserved genes identified by Gardiner et al. [2003].

physiological properties of neuronal circuits in the hippocampus of trisomic mice [Siarey et al., 1997; Kleschevnikov et al., 2004].

Trisomic mouse models have become an essential tool in research into the basic mechanisms of gene dosage effects in DS. The highly parallel outcomes that result when the same evolutionarily conserved genetic programs are perturbed in mice and man validate studies that focus on phenotypes. The three studies discussed here rely on mouse genetic models to explore when, where, and how trisomic development and function deviate from euploid, and each suggests possible therapeutic approaches to overcome these differences.

To illustrate the application of genetic models and introduce tests of hippocampal function in trisomic mice, we review a recent study analyzing the genetic contribution to hippocampal-based cognitive dysfunction measured in the Morris maze deficit [Olson et al., 2007]. We then review the three "breakthrough" studies. The study of Salehi et al. (2006) uses a genetics-based approach to identify a candidate target gene, while the other two studies reach their conclusions by focusing on description of pathogenesis and moderation of phenotypes without looking into the genetic basis for the origins of the phenotype under consideration. All three reach their important conclusions by careful attention to detailed and quantitative analysis of phenotypic outcomes in these models.

THE CONTRIBUTION OF "CRITICAL REGIONS" ON Hsa21 TO PHENOTYPES OF DS

Efforts to identify regions of Hsa21 containing genes that contribute to specific aspects of the DS phenotype have focused on individuals with translocations resulting in trisomy for a subset of Hsa21 genes (translocation DS). Phenotype maps correlating dosage imbalance of specific regions with specific characteristics provide useful information about segments in which to search for genes that perturb specific developmental pathways [Delabar et al., 1993; Korenberg et al., 1994].

The resolution and interpretation of the "critical regions" identified by phenotype maps is severely limited by several factors. Notably, there is no collection of human beings who are trisomic for only the critical region, whereas assessment of variable human phenotypes requires assessment of many individuals to draw meaningful conclusions. To address this issue directly, Olson et al. [2004] used chromosome engineering of Mmu16 to create mice with a duplication or a deletion of the so-called DS critical region (DSCR) (Fig. 1) and asked whether the 33 DSCR genes were necessary and/or sufficient to produce DS features attributed to genes in that region. Several DSCR features have parallels that have been documented in trisomic Ts65Dn mice including short stature; anomalies of the craniofacial skeleton and skull that result in flat facies, brachycephaly and protruding tongue (small mandible); and (with caveats) "mental retardation of the DS type," specifically impaired hippocampal function. Ts1Rhr mice, which are trisomic only for the DSCR, showed none of the features of the craniofacial skeleton attributed to the DSCR. Ms1Rhr/Ts65Dn mice are trisomic for all the genes triplicated in Ts65Dn *except* those in the DSCR (Fig. 1). These mice had craniofacial features similar to Ts65Dn. Thus, trisomy for the DSCR is not sufficient and for the most part is not necessary to produce these features of trisomy.

The picture is more complex when the question of mental retardation of the DS type is addressed [Olson et al., 2007]. Disruption of hippocampal functions in DS can be modeled in the performance of Ts65Dn mice in the Morris water maze (MWM) paradigm. In MWM, a mouse placed in a swimming tank is trained to navigate to a platform based on visual cues in the room (Fig. 2a), a visiospatial integration task that is dependent on hippocampal function. Ts65Dn mice are significantly impaired in the MWM test, whereas Ts1Rhr mice that are trisomic for only the DSCR genes performed the same as euploid in both phases of the test [Olson et al., 2007]. Thus, trisomy for the DSCR is not *sufficient* to produce the kind of hippocampal disruption seen in Ts65Dn mice and in DS, contrary to the prediction of the DSCR hypothesis. Next, Ts65Dn mice were crossed to Ms1Rhr mice, returning the DSCR genes to the normal two copies in a mouse that was trisomic for all of the other genes triplicated in Ts65Dn. These Ms1Rhr/Ts65Dn mice performed like euploid (Fig. 2b), showing that a gene (or genes) from this segment is *necessary* to produce this specific hippocampal deficit, and acts in combination with other genes from Mmu16 (and Hsa21) to do so.

$GABA_A$ ANTAGONISTS AND LONG-TERM CORRECTION OF LEARNING AND MEMORY [FERNANDEZ ET AL., 2007]

The previous experiments describe functional tests at the organismal level that demonstrate the effects of trisomy. The deficit in MWM performance in Ts65Dn mice is paralleled by a change in the way that neurons com-

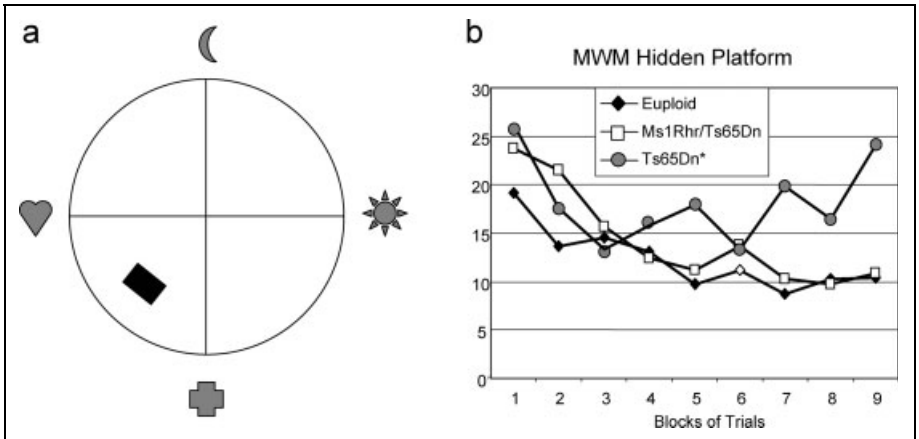

Fig. 2. Testing hippocampal function in mice. (a) The Morris water maze tests the ability of a mouse to navigate to a platform (black rectangle) based on visual cues placed around the room. (b) Ts65Dn mice perform poorly in the MWM (final trials 7, 8, and 9), but when the so-called DSCR is genetically "subtracted" in Ms1Rhr/Ts65Dn mice, they perform like euploid [adapted from Olson et al., 2007].

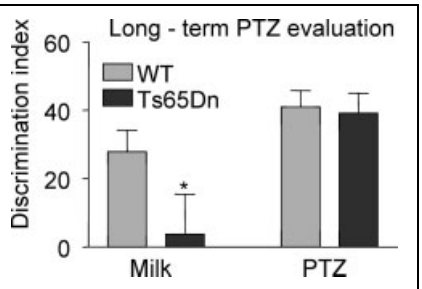

Fig. 3. Ts65Dn mice are impaired relative to euploid in the hippocampal-based object recognition task ("Milk"), but treatment with PTZ allows them to perform normally, even several months after the end of treatment [adapted from Fernandez et al., 2007].

municate. These changes in synaptic plasticity are measured by sensitive electrophysiological tests as changes in long-term potentiation (LTP) and long-term depression (LTD), changes that are robust in Ts65Dn mice [Sago et al., 1998; Siarey et al., 1999, 2006; Kleschevnikov et al., 2004]. In fact, Ts65Dn mice can show LTP in the dentate gyrus but are normally inhibited from inducing it due to excessive inhibitory input [Kleschevnikov et al., 2004]. Studies that examined excitatory and inhibitory inputs to various parts of the hippocampus found that inhibitory inputs were somewhat more prevalent and more active in trisomic than in euploid mice [Hanson et al., 2007]. These inhibitory neurons are characterized by their use of a neurotransmitter called GABA. Use of $GABA_A$ receptor antagonist in *in vitro* studies results in enhancement of the development of normal LTP in the dentate gyrus of Ts65Dn brain slices.

These observations led Dr. Craig Garner and coworkers to examine the possibility that administration of non-competitive $GABA_A$ receptor antagonists could have a positive effect in mice [Fernandez et al., 2007]. We considered three antagonists of GABA, compounds that counter the effects of this neurotransmitter by blocking its receptor. The first compound is called picrotoxin (PTX) and is widely used in experimental paradigms to induce seizures. (Indeed, all three of these molecules can cause seizures, discussed further below.) PTX has a very small window between the amount required to show a positive effect in LTP experiments and the amount required to induce seizures, and thus is an unlikely candidate as a drug. However, its effects and mode of action are very well studied and thus the results here can be related to a wealth of additional findings. The second compound is bilobalide (BB), one of several compounds extracted from the leaves of the ginkgo biloba tree. A variety of effects, many conflicting, have been reported for this compound, but the most remarkable is as a noncompetitive antagonist of $GABA_A$ receptors. The third compound, pentylenetetrazole (PTZ), is also a noncompetitive $GABA_A$ receptor antagonist. It was administered to patients for many years as a circulatory and respiratory stimulant [Levy, 1953]. It has also been used ineffectively, at higher doses that cause convulsion, for the treatment of psychiatric disorders such as schizophrenia and at low nonepilepitic doses as a procognitive drug for the treatment of senility in geriatric patients. Given that it was ineffective for the treatment of either of these disorders and is potentially harmful at high doses, approval for PTZ was revoked by the FDA in 1982. The fact remains that large numbers of people were treated with this compound for years, and although the drug showed little efficacy, it also showed few side effects in most people at low doses. Given this history, of the three compounds tested in this study, PTZ is the most intriguing to consider as a drug.

To test these compounds, the investigators turned to the Ts65Dn mouse model since hippocampal impairments in these mice parallel those in DS. They used two nonstressful tests of hippocampal function. The first is called "Object recognition." The mouse is placed in a cage with two objects for 15 min, during which time it will spend about half the time examining each object. The next day, the mouse is placed back in the cage with one of the same objects and a second, new object. Euploid (normal) mice will remember the old object and spend most of their time examining the new one, but trisomic mice show no evidence that they recognize the first object. The second test is an "Alternating T maze," a long platform with two arms. In multiple trials, a mouse with normal hippocampal function comes to the "T," remembers the arm that it investigated in the preceding trial, and goes down the other arm. Mice in which the hippocampus is impaired, including Ts65Dn mice, choose the arms randomly, suggesting that they do not remember the preceding trial.

In the first series of experiments, Ts65Dn mice given BB or PTX at doses far below those used to induce seizures showed marked improvement compared to saline-treated trisomic mice, performing the same as their euploid littermates in both the object recognition and T-maze tests. The authors observed that if animals were injected with PTX for 2 weeks followed by saline for 2 weeks, they performed just as well as animals that had just finished the drug treatment.

Based on these results, a series of exposures to PTZ was designed. This drug can be given orally, so euploid and trisomic mice were trained to drink chocolate milk with or without the drug daily for 3 weeks. Two to three months after the animals stopped getting the drug, they were tested in the object recognition test. Trisomic mice that got PTZ performed as well as euploid (Fig. 3). This long-term improvement was also evident in a sensitive electrophysiological test of LTP in the dentate gyrus, a measure of synaptic plasticity that is normally lacking in Ts65Dn mice. Trisomic mice that had received PTZ had significantly improved LTP relative to saline-treated Ts65Dn mice [Fernandez et al., 2007].

Serious questions remain to be answered before clinical trials can be considered. The first is the phenomenon known as kindling, in which repeated medium doses of chemicals—including all three of those used in these experiments (20–30 mg/kg)—make animals more susceptible to seizures. It remains to be seen whether the far lower doses used here (1–3 mg/kg), which fail to elicit seizures in euploid rats and mice, will increase seizure risk in trisomic mice. A related question is whether there is a genetic predisposition to seizures that would make the use of this drug more dangerous in some people than in others and/or whether young children are more susceptible than young adults. A second important goal is the elucidation of the mechanisms by which short-term treatment of noncompetitive $GABA_A$ receptor antagonists leads to a long-term improvement in function. This is not only important for the direct consideration of safety of these drugs, but is important to choosing (or designing) safer and more effective treatment strategies in the future. Even given these cautions, this study represents an extremely exciting breakthrough on which to focus research efforts in the near term.

Conclusion Study 1

Decreased hippocampal performance in mouse models of DS is linked to an imbalance in the weighting of excitatory and inhibitory circuits. At present, the underlying etiology of this imbalance is unknown. Administration of low, nonepileptic doses of noncompetitive $GABA_A$ antagonists can restore normal cognitive function in Ts65Dn mice. This information suggests a new and exciting avenue for the possible treatment of cognitive dysfunction in Down syndrome. While serious questions remain, the questions are well defined and there is every reason to think they can be answered. Whether PTZ ends up as a drug of choice, these experiments indicate that drugs directed toward GABA pathways that presumably lower inhibitory inputs in the trisomic hippocampus hold great promise for improvement of cognition in DS.

DEGENERATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS [SALEHI ET AL., 2006]

The second major breakthrough of the past year was established by Dr. William Mobley's group at Stanford [Salehi et al., 2006]. The basis for this discovery is described in depth elsewhere in this issue and is reviewed briefly here to put it in context with the other two studies. Mobley and coworkers have used mouse models extensively to understand how trisomy causes changes in structure and function of neuronal synapses, including some key observations about inhibitory input to the hippocampus as discussed earlier [Kleschevnikov et al., 2004]. They have also studied the loss of a specific population of neurons that communicates between the basal forebrain and the hippocampus. These basal forebrain cholinergic neurons (BFCN) utilize acetylcholine as a neurotransmitter and are seen to degenerate in Alzheimer disease (AD), in DS, and in Ts65Dn mice. (In fact, the study by Salehi et al. presents evidence suggesting that rather than degenerating, these neurons actually become quiescent, turning off expression of the marker commonly used to identify them.) This BFCN quiescence is believed to contribute to the age-related decline in cognitive function that is seen in many individuals with DS.

Like many neurons, the BFCN require specific signals to remain active and alive. Several years ago, Mobley and coworkers [Cooper et al., 2001] used Ts65Dn trisomic mice to demonstrate that neurons do not properly transport an important signal, nerve growth factor (NGF), from the nerve terminals in the hippocampus back to the neuronal cell body. It was known that this retrograde transport of NGF was a signal that maintains BFCN, and so the absence of this transport in the trisomic neurons was considered likely to be the cause of their demise.

At this point, the incomplete but very powerful set of mouse models for DS and also for AD played a prominent role. Ts1Cje mice [Sago et al., 1998] are trisomic for about 80% of the genes triplicated in Ts65Dn (Fig. 1). When Mobley's group compared BFCN-related neuropathology in euploid, Ts65Dn, and Ts1Cje mice, they found that retrograde transport of NGF was much closer to that of euploid mice in the Ts1Cje mice (Fig. 4a). Accordingly, attention was focused on the genes that are present in three copies in Ts65Dn but not Ts1Cje. Several of these have known functions in the CNS, including *Gabpa, Grik1, App,* and *Sod1.* Of these, *APP* plays a major role in AD, as shown by the fact that a cleavage product of the *APP* protein is a major constituent of the neuritic plaques that characterize the histopathology of this disease. Further, mutations in *APP* are associated with inherited forms of AD with very early age of onset.

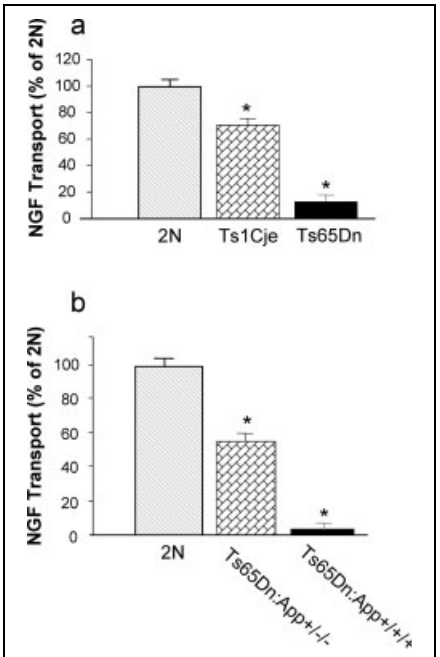

Fig. 4. BFCN must transport NGF produced in the hippocampus back to the cell body in the basal forebrain to remain active. (a) Retrograde transport of NGF is greatly impaired in Ts65Dn mice, but affected to a lesser degree in Ts1Cje mice. (b) Genetic "subtraction" of App from the trisomic gene set in Ts65Dn substantially improves retrograde NGF transport (Ts65Dn:App+/+/− vs. Ts65Dn:App+/+/+); *indicates a significant difference [adapted from Salehi et al., 2006].

To determine whether dosage (3 copies vs. 2 copies) of *App* contributed to the NGF retrograde transport deficit in Ts65Dn mice, a genetic cross was made between Ts65Dn, which have three copies of *App* (Ts65Dn:App+/+/+), and mice with only one copy of *App* (App+/−) and the offspring were analyzed. The severe reduction in transport of Ts65Dn mice was substantially improved in the trisomic mice that had two instead of three copies of *App* (Ts65Dn:App+/+/−, Fig. 4b). Furthermore, mice that were euploid except for extra copies of the APP gene were affected. Careful experiments established that an APP cleavage product from the COOH-terminal end of the protein was responsible for the retrograde transport deficit, possibly through interaction at the level of the early endosomes that carry NGF.

Conclusion, Study 2

Restoration of NGF transport and survival of BFCN might counteract, to some degree, the age-related cognitive decline seen frequently in DS. The

pharmaceutical industry is not likely to make a large investment in DS because the number of affected individuals is too small to return a profit in their economic model. In that light, the association of APP C-terminal fragments with the pathology that is shared in AD and DS is fortuitous, because extensive efforts are already focused on APP processing as a possible intervention for AD. The indications in this study of APP interference with retrograde transport via early endosomes suggest that reducing levels of APP could have a positive effect in reducing BFCN degeneration, with beneficial consequences in DS and AD.

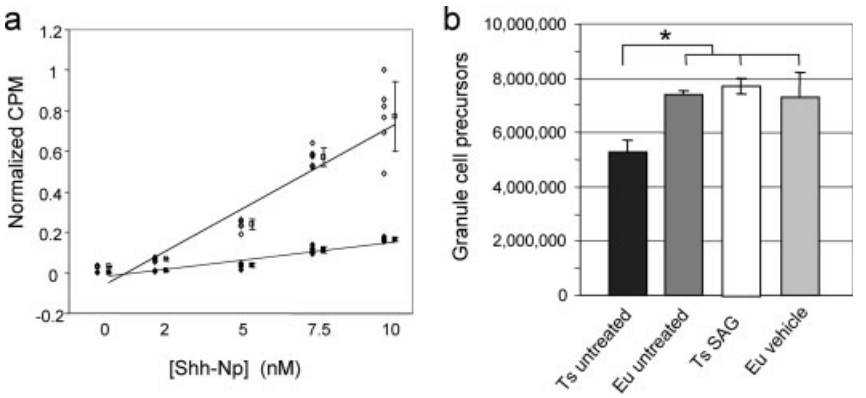

Fig. 5. Growth of the Ts65Dn brain is reduced because trisomic mice do not respond properly to the SHH growth factor. (a) Purified GCP from both trisomic and euploid mice respond to increasing concentrations of SHH, but trisomic GCP respond less. A representative experiment is shown. (b) A single treatment at P0 with SAG is sufficient to restore the number of GCP in the cerebellum 1 week later; *indicates a significant difference [adapted from Roper et al., 2006].

REVERSAL OF A GROWTH FACTOR RESPONSE DEFICIT AFFECTING BRAIN DEVELOPMENT [ROPER ET AL., 2006]

The third breakthrough study comes from Dr. Reeve's laboratory [Roper et al., 2006]. It appears likely to be farther removed from potential clinical application, but may have more wide-reaching effects regarding the broad range of features that characterize DS. This study also demonstrates the ability to reverse one very specific aspect of the phenotype using a potential drug. In the course of fundamental research on the development of the trisomic brain, these studies identified a deficit in response to a growth factor that affects many different tissues at different stages of development. We went on to demonstrate that administration of an experimental compound could return a specific aspect of the developmental process to normal in the cerebellum. As in the preceding studies, these findings relied on genetic mouse models of DS.

The experiments started several years ago with efforts to compare brain development in trisomic Ts65Dn mice to that in human beings with DS. The cerebellum is significantly reduced in DS, even relative to the overall smaller size of the brain [Crome et al., 1966; Aylward et al., 1997]. Unlike many features of DS which may or may not be present in an individual with trisomy 21, a small cerebellum appears to occur in everyone with this condition. Thus, it was expected that a mouse with a similar genetic constitution should display corresponding effects on development of the cerebellum.

Baxter et al. [2000] used high resolution 3d MRI to show that while the brain was the same size as euploid in Ts65Dn mice, the cerebellum was disproportionately small. (The fact that the brain was not reduced overall in Ts65Dn was not terribly surprising as much of the reduction seen in DS occurs in the cerebral cortex, which represents a much larger proportion of the brain in human beings than in other mammals.) Given the reduced Ts65Dn cerebellum, histological approaches were used to determine where the reduction occurred. Both the granule layer and molecular layer were affected. Unexpectedly, Baxter et al. demonstrated that not only was the volume occupied by granule cells and Purkinje cells reduced, but in addition there was a reduction in the cell density of both types of neurons, further reducing the total number of cerebellar neurons. This phenotype had never been reported in DS. Using age-matched brain specimens from individuals with two or three copies of Hsa21, the same reduction of granule cell density was seen in DS.

The ability of a mouse model to correctly predict a new phenotype of a human condition is a strong indication that the developmental processes are the same in the two species. Accordingly, we next asked at what point the pattern of development diverged between trisomic and euploid mice [Roper et al., 2006]. Again using histological approaches, we found that every parameter that could be measured 1 week after birth (postnatal day 6, P6) was reduced in Ts65Dn mice, but these differences were not evident at P0. Very precise statistically validated sampling of cerebellum revealed that while the number of granule cell precursors (GCP) that give rise to granule cell neurons was the same in Ts65Dn and euploid littermates, the number of GCP undergoing cell division was significantly reduced in trisomic mice.

Collectively, these findings identified a specific effect of trisomy on brain growth and defined the developmental timing (P0), the affected cell type (GCP), and the disrupted process (cell division). Studies from a number of laboratories had established that the growth factor, sonic hedgehog (SHH), is the major signal to induce cell division of GCP. Crosses between Ts65Dn and mice with a reporter of SHH pathway activity suggested that this pathway was somewhat downregulated in Ts65Dn mice. Accordingly, we isolated GCP and cultured them in the presence of increasing amounts of duly-lipidated SHH (Fig. 5a). This analysis showed two important things. First, trisomic GCP responded less at every concentration of SHH, demonstrating a cell autonomous deficit in response to this important growth factor. Second, trisomic GCP did respond, suggesting that a small increase in SHH might elevate proliferation rates of trisomic GCP to the same levels as euploid.

To test this, we injected a small molecule that activates the SHH pathway (SHH agonist, SAG) into trisomic pups the day they were born and assessed the number of cells in the cerebellum 6 days later. The single administration restored the number of GCP (Fig. 5b) and the volume of the cerebellum to euploid levels. Further, the number of dividing GCP was also restored to euploid level, suggesting that the ameliorative effect of this single treatment would continue through cerebellar development [Roper et al., 2006].

Conclusion, Study 3

SHH is important in brain development from very early stages. In addition, this potent morphogen and growth factor affects the proliferation, migration, differentiation, and survival of many populations of cells beginning early in development and continuing throughout life. Thus, any stimulation of this pathway must be precisely targeted, both in which cells are exposed and when during development this stimulation takes place.

On the other hand, only those cells with the appropriate receptors and transducers of the SHH signal can respond; those without the receptor are impervious to its affects. If all trisomic cells show the same attenuated response to SHH, a more general application of a drug targeted to this specific response deficit could have wide-reaching ameliorative effects. SHH is important in the formation of a substantial part of the brain. In addition, it affects all structures derived from neural crest, including the craniofacial skeleton, enteric ganglia, and components of the outflow tract of the heart. This morphogen is primarily responsible for establishment of patterning anterior–posterior polarity in the hand. Thus, an attenuated SHH response could contribute to multiple aspects of the DS phenotype. However, substantial additional fundamental discovery will be required to determine whether SHH is beneficial in the longer term and what aspects of development may specifically benefit from this type of approach.

OVERALL CONCLUSIONS

The occurrence of three breakthrough studies in 1 year is a harbinger of great progress to come in DS and a call to the wider scientific community to consider what other areas of research might benefit from the perspectives provided by defined genetic effects in trisomy. Collectively, these studies represent a synthesis of approaches based in development, structure, function, and age-related changes that must be the basis for any consideration in this complex disorder. These multi-faceted considerations can only be addressed using mouse models that reflect the developmental and physiological processes affected in DS, and that provide tools essential for understanding the genetic basis of the divergence between trisomic and euploid individuals. A common element of these approaches is that all were designed with consideration of the possibilities for pharmaceutical (or other) amelioration. Combined with ongoing clinical trials, e.g., of antioxidants and cholinesterase inhibitors, the prospects for improving cognitive function in DS in the foreseeable future appear to be strongly positive. ■

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