The challenge of discovering preventive therapies for intellectual disability in Down syndrome: could there be a breakthrough?

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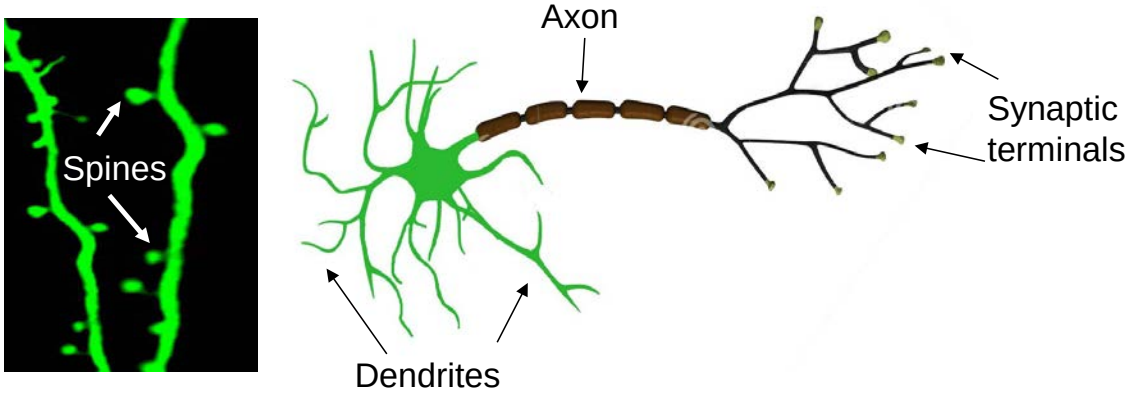
The Committee for Science & Society* of the Trisomy 21 Research Society (T21RS) regularly addresses issues raised by parents and Down syndrome associations through summarizing the state-of-the-art knowledge from a scientific perspective. In this T21RS Science & Society Bulletin professor Renata Bartesaghi and her team (Bologna, Italy) explain how we could possibly prevent or treat intellectual disability in Down syndrome. They studied the effect of treatment with the antidepressant fluoxetine (Prozac) in a mouse model of Down syndrome – with promising results.

One of the most challenging features of Down syndrome (trisomy 21) is the intellectual disability. Diseases of the brain continue to challenge medical, psychological, and social services throughout the world and it is necessary to discover effective interventions. In spite of numerous efforts, the mechanisms whereby a third copy of chromosome 21 leads to the Down syndrome phenotype have not been elucidated and there are at present no therapies to rescue intellectual disability in individuals with Down syndrome.

Milestones in brain development

The basic steps in brain formation consist in the generation of neurons (*neurogenesis*) and glial cells (*gliogenesis*) and their maturation. When neurons mature, they emit thin processes called dendrites (*dendritogenesis*), whose function is to increase the neuronal surface, and a single process called axon (*axonogenesis*), whose function is to transmit signals to target neurons. During their maturation, dendrites emit small protrusions called dendritic spines. Spines are the target of axons from other neurons and therefore represent a crucial structure for the cross talk between neurons. While neurogenesis takes place during fetal life stages in most parts of the brain, neuron maturation continues in the first postnatal period (4, 15, 19). This period is particularly crucial for the establishment of contacts (*synapses*) between neurons.



Developmental brain alterations in Down syndrome

The brain of a child with Down syndrome develops differently from a normal brain: it is reduced in size, and altered in shape. Moreover, the processes of neurogenesis, gliogenesis and dendritogenesis are impaired, starting from prenatal life stages (3, 8). The outcome is a reduction in the number of neurons in the brain, a lower ratio of neurons versus glial cells and a reduction in dendritic length and spine density. From a functional viewpoint, these alterations imply damage to numerous brain functions, including cognitive performance.

The molecular mechanisms underlying brain alterations in Down syndrome remain elusive

Genes code for proteins involved in different cell functions. While two gene copies produce appropriate levels of proteins, a third copy has adverse effects on proper cell functioning. Intense efforts are currently being made in order to identify the genes whose triplication determines the developmental alterations that characterize the Down syndrome brain. This knowledge is important because, ideally, it will make it possible to specifically target the pathways that lie downstream of these genes, thereby counteracting their negative effects. Yet, the large number of genes in Down syndrome makes the search for the "culprits" very challenging. It is likely that many triplicated genes concur to impair neurogenesis, dendritic development and synaptic organization.

The Ts65Dn mouse model of Down syndrome

Mouse models of various pathologies are useful tools that can be exploited in order to identify molecular mechanisms involved in a given pathology and to devise the effects of therapeutic interventions. Various mouse models of Down syndrome have been created that model the human trisomy 21. Animal models do not reproduce the human disease with all its complexities but rather model specific aspects of the disease. Thus, it is important to observe that no mouse model will be a perfect model of Down syndrome. Even though mice have many similarities to humans, they also present significant differences and no mouse exists that adequately models Down syndrome. The Ts65Dn mouse is the most studied and best characterized model of Down syndrome, in which approximately 55% of human chromosome 21 genes are present in three copies (6). During the past 20 years, various studies have demonstrated numerous common features between Ts65Dn mice and people with Down syndrome, including small brain size, abnormal shape of the skull, congenital heart defects, deficits in learning and memory and early neurodegeneration leading to Alzheimer's-like pathology (20). The Ts65Dn mouse is, at the moment, the model of Down syndrome that is most widely used in animal studies to develop possible therapies for Down syndrome (10).

'Normal' mouse Ts65Dn mouse

The Ts65Dn mouse model of DS

Timing of therapies for Down syndrome

During the last 10 years, various studies have attempted to improve cognitive performance in mouse models of Down syndrome during adult life stages and some of these studies have shown a positive outcome of treatment (16). However, considering that neurogenesis and dendritogenesis alterations are present at the very beginning of brain development, we believe that the period around birth represents a crucial window of opportunity to attempt to rescue overall brain development and, hopefully, its function. With this idea in mind, our group has tested the effects of early treatment with fluoxetine, a widely-used antidepressant (also known with the name Prozac©), in the Ts65Dn mouse model.

Fluoxetine: a potential tool for improving brain development in Down syndrome

Why did we decide to use fluoxetine? Serotonin is a neurotransmitter, i.e. a molecule released by neurons in order to transmit signals to other neurons. During brain development it is fundamental for the proliferation of neural precursor cells (the cells that will give origin to new neurons) and the appropriate development of dendrites (9, 21). The serotonergic system is impaired in Down syndrome, starting from fetal life stages (2, 14, 21). This suggests that impairment of the serotonergic system may contribute to the alterations of brain development that characterize Down syndrome. But the question is: what can we do to counteract this defect? Many neurotransmitters, shortly after having being released, are re-uptaken into the axons. This re-uptake is important because it sets the time of the duration of their action. Since the serotonergic system is impaired in Down syndrome and serotonin is fundamental for neurogenesis and dendritogenesis, we thought that an increase in serotonin availability may ameliorate these processes. An increase in serotonin availability can be obtained by administering the antidepressant fluoxetine, a selective serotonin re-uptake inhibitor (SSRI). Thus, based on all these premises we wondered whether treatment with fluoxetine during early life stage may rescue neurogenesis and dendritogenesis in the Ts65Dn mouse model.

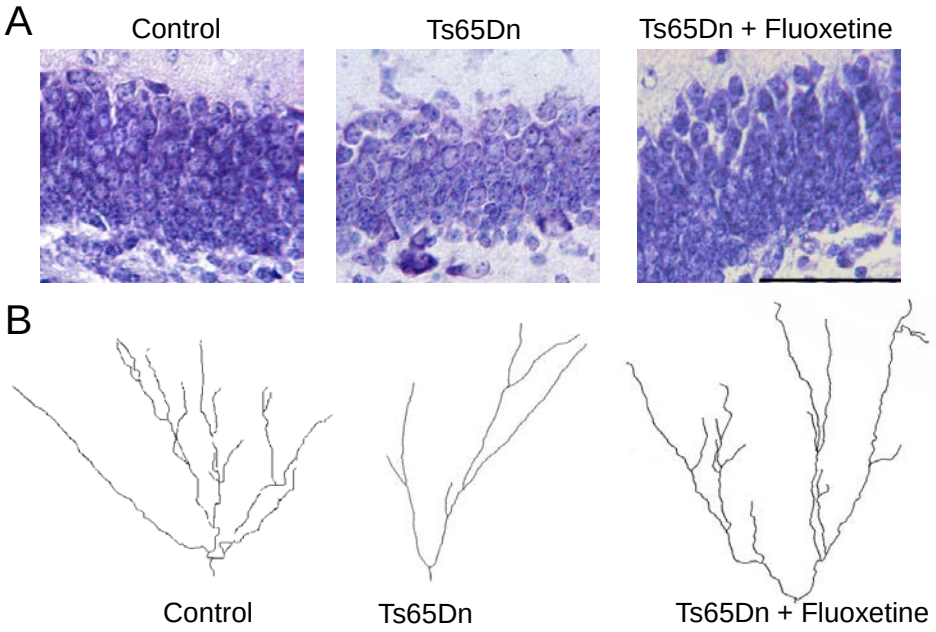
Effects of early treatment with fluoxetine in the Ts65Dn mouse

We started our study with neonatal therapy (5). We treated mice from postnatal (P) day 3 (P3) to postnatal day 15 (P15) because, in rodents, this is the period of maximum neurogenesis in the hippocampus, a region that plays a crucial role in learning and memory. We examined the outcome of treatment at P15, at one month and at 3 months after the treatment stopped. We found that in Ts65Dn mice aged 15 days neurogenesis was fully restored. In mice aged 45 days, that is at one

month after the treatment stopped, hippocampal neurogenesis was still restored as were also total number of neurons, dendritic and synaptic architecture and hippocampus-dependent memory (5, 12, 18). When the mice were three months of age these positive effects were still present (17).

Based on these encouraging results, we decided to treat pregnant Ts65Dn female mice with fluoxetine starting from embryonic day 10 to delivery (11). The pups were examined at two days of age (P2) or at 45 days of age (P45) to examine short-term and long-term effects of embryonic treatment, respectively. First of all, we evaluated the number of proliferating precursor cells in the brain of mice at two days of age: untreated Ts65Dn mice showed a severe neurogenesis reduction and low number of cells throughout the brain. Importantly, embryonic treatment restored the number of proliferating cells, as well as full restoration of the total amount of cells.

Next, we studied the long-term effects of embryonic treatment in mice aged 45 days. We found that the number of proliferating cells in the two major formation sites of new neurons was still higher in treated than in untreated trisomic mice. Indeed, the number of neurons in the hippocampus was still larger in treated Ts65Dn mice, indicating that rescue of cell production outlasted treatment cessation. Moreover, the number of branches and total dendritic length underwent a large increase. The counterpart of this effect was restoration of synapse development. Finally, embryonically-treated Ts65Dn mice aged 45 days showed a recovery of behavioral performance: poor memory performance was fully rescued.



Prenatal treatment with fluoxetine restores the number of neurons (A) and dendritic development (B).

Early therapy with fluoxetine reverts trisomy-linked brain alterations: a cautious hope for children

Down syndrome-linked neurodevelopmental alterations were thought to be irreversible. Our study demonstrates that it is possible, at least in the mouse model, to pharmacologically fully rescue brain development. Based on our data in a mouse model, fluoxetine may be a good candidate to improve intellectual disability in Down syndrome. However, there are some unanswered questions regarding the use of fluoxetine for Down syndrome:

1) It remains to be established whether this drug has a beneficial effect on brain development in humans with Down syndrome similarly to the mouse model. Clinical trials are needed in order to obtain an answer to this question.
2) Fluoxetine is in clinical trial in children as a treatment for various behavioral disturbances (1, 7, 13). In view of the effects of fluoxetine on hippocampal neurogenesis and dendritogenesis, a clinical trial in children with Down syndrome may be attempted in order to improve these processes and, hopefully, memory functions. Possible side effects, however, cannot be completely ruled out.
3) The use of fluoxetine during pregnancy poses various caveats, which should be taken into account and considered very carefully.

Conclusions

In conclusion, our studies demonstrate that it is possible to pharmacologically prevent trisomy-linked brain defects in a model of Down syndrome and that the positive effects of treatment largely outlast treatment cessation. We do not know whether fluoxetine or other drugs have similarly radical effects in the Down syndrome brain. At the moment, fluoxetine might represent a useful treatment in children with Down syndrome, provided that well-designed clinical trials demonstrate its effectiveness and the lack of side effects. We deem it extremely important now to expedite the discovery of additional therapies practicable in humans, in order to identify the best treatment/s in terms of efficacy and paucity of side effects. Importantly, early therapies for Down syndrome may have a very positive impact on intellectual disability. This achievement would give children with Down syndrome the opportunity to lead a normal and autonomous life, alleviate the psychological burden on their families and solve a public health problem. Preclinical researchers throughout the world are working intensely to achieve this goal.

** The Committee for Science & Society consists of Peter Paul De Deyn (chairman, Belgium), Juan Fortea (Spain), Sebastián Videla (Spain), Alain Dekker (The Netherlands), Lotta Granholm (USA, Sweden) and Cindy Lemere (USA).*

Bibliography

1. Alcami Pertejo M, Peral Guerra M, Gilaberte I (2000). Actas Esp Psiquiatr.28(6):353-6.
2. Bar-Peled O, Gross-Isseroff R, Ben-Hur H, *et al.* (1991). Neurosci Lett.127(2):173-6.
3. Bartesaghi R, Guidi S, Ciani E (2011). Rev Neurosci.22(4):419-55.
4. Berger-Sweeney J, Hohmann CF (1997). Behav Brain Res.86(2):121-42.
5. Bianchi P, Ciani E, Guidi S, *et al.* (2010). J Neurosci.30(26):8769-79.
6. Davisson MT, Schmidt C, Reeves RH, *et al.* (1993). Prog Clin Biol Res.384:117-33.
7. DeLong GR, Ritch CR, Burch S (2002). Dev Med Child Neurol.44(10):652-9.
8. Dierssen M (2012). Nat Rev Neurosci.13(12):844-58.
9. Faber KM, Haring JH (1999). Brain Res Dev Brain Res.114(2):245-52.
10. Gardiner KJ (2015). Drug Des Devel Ther.9:103-25.
11. Guidi S, Stagni F, Bianchi P, *et al.* (2014). Brain.137(Pt 2):380-401.
12. Guidi S, Stagni F, Bianchi P, *et al.* (2013) Brain Pathol.23(2):129-43.
13. Hollander E, Phillips A, Chaplin W, *et al.* (2005). Neuropsychopharmacology.30(3):582-9.
14. Risser D, Lubec G, Cairns N, Herrera-Marschitz M (1997). Life Sci.60(15):1231-7.
15. Spalding KL, Bergmann O, Alkass K, *et al.* (2013). Cell.153(6):1219-27.
16. Stagni F, Giacomini A, Guidi S, *et al.* (2015). Front Behav Neurosci.9:265.
17. Stagni F, Giacomini A, Guidi S, *et al.* (2015). Neurobiol Dis.74C:204-18.
18. Stagni F, Magistretti J, Guidi S, *et al.* (2013). PLoS One.8(4):e61689.
19. Stiles J, Jernigan TL (2010). Neuropsychol Rev.20:327-48.
20. Vacano GN, Duval N, Patterson D (2012). Curr Gerontol Geriatr Res.2012:717315.
21. Whitaker-Azmitia PM (2001). Brain Res Bull.56(5):479-85.