DOWN SYNDROME: A CURATIVE PROSPECT ?

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Abstract

Experimental work regarding corrective actions on chromosomes and genes, and control of gene products is producing interesting results. It opens the way to advances in dealing with aneuploidies and may lead in the future to important changes in the life of individuals affected with these conditions. A small number of molecules are being investigated in pharmacological research that may have positive effects on cognitive functioning. Studies of the pathological consequences of the amyloid cascade and the TAU pathology in the etiology of Alzheimer disease (AD), more frequent and precocious in persons with Down syndrome (DS), are analyzed. The searches for biological markers of AD incipiens and ways for constrasting its early manifestations are also envisaged.

Key terms: Aneuploidies, Down syndrome, genetic therapy, cognitive pharmacotherapy, Alzheimer disease.

INTRODUCTION

The prevalence rate of intellectual disability in the general population is estimated to be between 1 and 3%. Chromosome abnormalities (numerical as well as structural) are responsible for up to 28% of intellectual disabilities (Kalpala et al., 2017), among which aneuploidies ((i.e., inborn deviations from the normal cohort of 46 chromosomes) are well represented. DS is the most frequent autosomal trisomy (chromosome 21 - C21) occurring naturally in approximately 1 in 700 live births.

C21 is the smallest of human chromosomes. It should bear number 22 as chromosomes are numbered in order of magnitude, and there are 22 pairs of autosomes plus one pair of sex chromosomes, XX for females and XY for males. Human C21, technically called Hsa21[1], harbors around 250 protein-coding genes (the precise number varying depending on genome annotations) and between 165 and 404 non-coding RNA genes (see below).

Overexpression of proteins linked to gene triplication determines a constellation of abnormalities involving heart, nervous system and gastro-intestinal tract. Impaired brain development causing structural anomalies and decreased volumes of frontal and temporal cortices, hippocampus, cerebellum, and brain stem, is typically observed. Anomalies of neural connectivity are the rule. However, almost every aspect of the DS phenotype is subject to an important degree of interindividual variability due to its polygenic nature and interactions with environmental factors (cf. Rondal, 1995; Asim, Kumar, Muthuswamy, Jain, & Agarwal, 2015).

[1] The label Hsa21 will be used in the rest of the article in concordance with the technical literature.

DS appears in several forms. The most common one (95% of cases) is standard trisomy 21 - TR21 - (karyotype 47+21). It is characterized by a triplication of Hsa21 in every cell of the body. In mosaic TR21 (1 to 2% of the cases), only a part of the cells carries one extra Hsa21. The proportion depends on the moment when the triplication of Hsa21 occurs (first, second, or third cell division). In Robertsonian (centric fusion; nonreciprocal) translocations, the participating chromosomes (pairs 13, 14, 15, 21, or 22) break at their centromeres and the long arms (q segments) fuse to form a single large chromosome with a single centromere. The short segments (p) are usually lost.

Robertsonian translocations involving C21 are: C21 with C21, C14, or more rarely C15: formulae 46 t (21;21) + 21, 46 t (14;21) +21, and 46 t (15;21) + 21, respectively (globally 3% of the cases). In isochromosomal or ring chromosome, two long arms of chromosome separate together during egg/sperm development rather than the long and short arm separating together. Partial TRs21 witness only a segment of Hsa21 being triplicated.

DS maps to a region on the long arm of Hsa21 covering an area of 37 to 44 megabytes corresponding to band 21q22 and containing around 225 genes (Lyle et al. (2009). Ait Yahya-Graison et al. (2007) supply a figure of 200-250 genes between 21q21 and 21q22.3. A smaller region in Hsa21, labelled DSCR (Down Syndrome Critical Region), involving bands 21q21.1 and 21q22.2, listing about 50 protein-coding genes and a larger number of non-coding elements, may arguably harbor most of the critical determinants of the phenotype of the condition (Di Cunto & Berto, 2012)..

Other analyses suggest that there may be several critical regions for different phenotypes and not just one region for all the phenotype. For example, heart defects in DS map to a particular area of 5.2 megabytes within Hsa21 (Barlow et al., 2001; Asim, Kumar, Muthuswamy, Jain, & Agarwal, 2015). Other specific subregions of Hsa21 are associated to hypotonia (Lyle et al., 2009) and to one form of acute leukemia

(megakaryoblastic) representing about 20% of the cases of leukemia in children with DS (Korbel et al., 2009).

It is also possible that the overexpression of a number of genes on Hsa21 in TR21 leads to a genetic imbalance that may deregulate the expression of other genes in other regions of Hsa21 or even in the entire genome (Antonorakis, Lyle, Dermitzakis, Reymond, & Deutsch, 2004). The expression levels of genes located outside of Hsa21 may also be altered as some of the Hsa21 genes have a regulatory role extending beyond this chromosome (Sturgeon, Le, Ahmed, & Gardiner, 2012).

Pelleri et al. (2016) and Pelleri et al. (2019) have published the results of a reanalysis of 132 cases of partial segmental TR21. The main result is that there is one highly restricted region in DSCR, which they dubbed HR (highly restricted)-DSCR, of only 34 kilobases, located in the distal part of the 21q22.13 sub-band, whose duplication is shared by all DS subjects and absent in all non-DS ones. A caveat is that this region contains no known gene. As to the identification of the genetic determinants possibly located in HR-DSCR, the authors speculate that unknown microRNAs (miRNAs) present in this region could be involved in DS pathology with the capability to regulate a large number of protein-coding genes and/or that the HR-DSCR could carry longer-range interactions with other chromosomes.

Further studies are needed to verify whether some elements in putative HR-DSCR are functionally associated to the most common manifestations of DS and play a role in the pathogenesis of the condition.

The cause of standard TR21 is chromosomal nondisjunction mostly during meiosis I in the maternal egg. Paternal nondisjunction occurs during meiosis II in spermatogenesis. Translocations involving Hsa21 may occur de novo during syngamy or be inherited from parental genotypes (in about one quarter of the cases). Although these parents as heterozygous carriers are phenotypically normal (they have no genetic material in excess or deficit; their translocation is said to be equilibrated), they have a 10 % risk

of having a child with DS if the mother carries the translocation and 2.5 % risk if the father is the carrier (Homfray & Farndon, 2014).

The paper analyses ongoing work in genetics, epigenetics, and cognitive pharmacology relevant to the neurobiology of DS. In spite of a large number of conceptual and methodological difficulties existing in these studies, the preliminary character of most of the findings reported, and the biologcal gaps between animal (murine) and human cognition, it would seem that this trend of research holds a potential for improving the neurobiology and possibly some neurobehavioral aspects of people affected with DS in association with environmental interventions.

Cognitive development and functioning in DS persons have been the object of much research worldwide over the last 75 years. There is no need to reassume all or parts of this huge literature in the present context nor propose again a detailed analysis of what is known of the cognitive processes and limitations of people with DS (see several chapters in Nadel's edited book, 1988; Rondal, 2010, for a historical compendium; Chapman & Hesketh, 2000; Abbeduto, Warren, and Conners, 2007; Rondal, 2012; and Rondal & Perera, 2016, for analyses of the cognitive and the language difficulties of DS persons; several chapters in Rondal & Buckley's edited book, 2003; Rondal, 2013, for analyses regarding speech, language and memory development and difficulties in DS persons as well as summaries and discussions of the major principles of behavioral rehabilitation in these matters; and Rondal, Perera, & Spiker's edited book, 2011, for a coverage of various aspects of neurocognitive rehabilitation in children with DS).

GENETIC AND EPIGENETIC APPROACHES

Chromosome and gene correction and reduction of excess protein production contributed by the triplication of a number of genes along the long arm of Hsa21 are the objects of ambitious experimental attempts.

Chromosome correction

At least three techniques have been reported as having met success in removing one extra Hsa21 in trisomic cells.

Takahashi et al. (2007) demonstrated that induced pluripotent stem cells (iPSCs) can be generated from adult human dermal fibroblasts through genetic engineering using particular transcription factors. Pluripotent stem cells are cells capable of self-renewing and differentiating into a limited set of specialized cells in the body such as blood, liver, heart, or brain cells, but not all types of cells as this is the case for embryonic or so-called omnipotent or multipotent stem cells originating from the inner mass of the embryonic blastocyst.

On this basis, Li et al. (2012) generated iPSCs from fibroblasts obtained from adults with DS. They then introduced a *TKNEO* fusion transgene carried by a modified adenovirus at the locus 21q21.3 of the gene *APP* (amyloid-bêta precursor protein) into one copy of Hsa21. This gene was chosen as the target locus due to its location on Hsa21 and its high expression in iPSCs. The operation resulted in spontaneous loss of an entire copy of Hsa21 in a large majority of clones while point mutations, epigenetic silencing, and *TKNEO* deletions occurred at much lower frequencies in the five experiments undertaken. No damage to other chromosomes was observed.

Disomic cells proliferated faster in a coculture than their trisomic counterparts doubling their population on average in about 37 ± 0.7 hr

against 45 ± .09 hr for trisomic counterparts. The authors suggest that iPSCs offer a promising way to study human trisomy because they can be derived from the somatic cells of individuals with trisomy and that their approach could also be used to eliminate unwanted trisomies arising frequently in stem cell cultures.

Jiang et al. (2013) took advantage of a natural phenomenon to correct TR21. Nature has evolved a mechanism to compensate for the difference in dosage of X-linked gene copies between mammalian females and males. In humans, the formula for the sex chromosomes is XY for males and XX for females. However, the Y chromosome is much smaller than its X counterpart. It contains only a few dozen genes against close to 3000 for the X. Natural X dosage reduction in females is driven by a particular large non-coding ribonucleic acid (RNA), named *XIST* (for X-inactive specific transcript) produced exclusively from the inactive X chromosome. This RNA inactivates the DNA (deoxyribonucleic acid) of this chromosome through methylation and chromatin modification turning it into a Barr body.

Jiang et al. reprogrammed fibroblasts obtained from male individuals with DS into iPSCs. They inserted a transgene *XIST* at locus 21q22 of the gene *DYRK1A* in one of the three Hsas21. This silenced this chromosome in 85% of the clones treated. Silencing of a dozen genes on the inactivated Hsa21 was confirmed. No alterations of the other chromosomes were observed. A few sub-clones remained in the 245 colonies of cells treated showing either one Hsa21 fused with the *XIST* RNA, two Hsas21 in the same state, or the three Hsas21 fused with the *XIST* RNA.

As in the experiment of Li et al. (2012), disomic cells exhibited a capacity for in vitro-proliferation above their trisomic counterparts.

Another RNA in mammalian females, antagonist of *XIST* and named *TSIX* (anagram for *XIST*), has been identified. *XIST* and *TSIX* neutralize each other on the X chromosome that remains active, whereas the expression of *TSIX* is stopped on the X chromosome to be inactivated.

In the experiment of Jiang et al. (2013), the fibroblasts were obtained from male persons with DS. It is not known what would happen in the same situation using fibroblasts from a female person with DS. Applying the above *XIST* dosage compensation technique, one could end up with some cells in the colonies treated exhibiting one Hsa21 and one X chromosome inactivated, and others where one Hsa21 and the two X chromosomes would be inactivated.

Natural silencing of one chromosome X in females is never complete and the choice of the genes remaining active is random. However, Jiang et al. (2013) reported that the global expressivity of the two active Hsas21 in their clones was reduced by 20, 15, and 19%, respectively, in the three clones tested, which is close to the 22% usually observed in disomic iPSCs that lack the third Hsa21 altogether. This suggests that the *XIST* RNA inserted in the extra Hsa21 covers key regions of this chromosome actually preventing transcription factors from reading the sequence of the nucleic acids. *XIST* appears to induce a robust dosage compensation of most Hsa genes overexpressed in TR21.

Amano et al. (2015) normalized the karyotypes in a culture of mouse embryonic stem cells engineered to become aneuploid or polyploid, using a biologic made of a mammalian-specific gene, *ZSCAN4* (zinc finger - see below - and scan domain) containing 4 transcription factors regularly expressed in pre-implantation embryos and occasionally in stem cells., encoded for delivery in a synthetic messenger RNA (mRNA) and Sendai virus vector.

They then tested this biologic on iPSCs generated from non-immortalized[2] fibroblasts obtained from DS individuals and carrying

[2] An immortalised cell line is a population of cells that have evaded normal senecence and therefore can keep undergoing division for prolonged periods of time, at least in vitro. The mutations necessary for immortality can occur naturally or be induced for experimental purpose

standard TR21. Within a few weeks, chromosome examination showed the emergence of up to 24% and then 40% of cells with a normal karyotype. These findings were confirmed by whole-exome sequencing. Similar results were obtained for cells with TR18, known as Edwards syndrome. The authors suggest that introduction of human *ZSCAN4*-mRNAs into cells may have the ability to remove chromosome extracopies without affecting the remainder of the genome. They speculate that a *ZSCAN4*-mediated mechanism detects unpaired chromosomes during cell division (meiosis or mitosis) and detaches them from the rest of the replication apparatus. Amano et al. (2015) are hopeful that human *ZSCAN4* biologics can be developed into natural chromosome therapy for treating cells directly.

An unexpected result has been reported by Inoue et al. (2019). They established independent immortal iPSC lines derived from amniotic fluid obtained from a fetus with DS associated with polyhydroaminios at 29 weeks of gestation for the purpose of reducing amniotic fluid. Karyotypic analyses confirmed that all iPSCs contained Hsa21 trisomy in all the lines. They then continuously cultivated the iPSCs lines for 70 weeks. At that time, normal Hsa21 diploids (formula 47, XX) were observed in 20% of the cells. The experiment was repeated several times to make sure that trisomy rescue was not due to Hsa21 mosaicism. Expression counts based on gene chip analyses performed on the diploid and the TR21 iPSCs indicated that the expression levels of the genes for *DYRK1A*, *SOD1*, *ETS2*, *APP*, and *DSCR1* (see below for a definition of their functions) were decreased to two-thirds in diploid iPSCs Hsa21 compared to TR21 iPSCs. This implies that the revertant cells had regained normal gene expression. A sample of the two types of iPSCs cells was differentiated into neural stem cells (NSCs) as to morphology and neural marker expression. It was observed that the

and serve in biotechnology. Immortalised cells should not be confounded with natural stem cells. The latter can also divide indefinitely but they are a normal constituent of the development of a multicellular organism.

expression levels of genes *APP* and *DSCR1* in TR21 NSCs were higher than in diploid NSCs.

The authors attribute the observed spontaneous reversion from trisomic cells to disomy without genetic manipulation, chemical treatment, or exposure to radiation, to mitotic chromosome nondisjunction during iPSCs long-time cultivation. Trisomic rescue may be tissue-dependent (Epstein, 2001). This could mean that the tissue environment of aneuploid cells is a key variable in chromosome regulation.

Although the way towards clinical applications of the above technologies may still be incommensurably long, the results obtained so far suggest the feasability of normalizing operations on the trisomic cells at least in vitro.

Acting on genes

The genetic "scissor" CRISPR-Cas9 can perform precise cutting on the DNA and RNA ribbons. It is possible to snip out portions of a chromosome or see what happens when a particular gene is removed. Enzymes that have the ability to catalyze larger DNA or RNA molecules are employed. Zinc finger nucleases (used by Jiang et al., in the work mentioned above) are a class of engineered DNA binding proteins exploiting particular properties of the zinc ion. The particular form that it takes at the contact of the DNA molecule motivates its finger name.

One may envisage removing or inactivating triplicated genes located on Hsa21. Partial corrections may already have a significant effect. This is suggested by the milder phenotype characteristic of those cells of TR21 mosaicism that have a normal karyotype.

Efforts are being made to identify the particular set of genes whose overexpression most determine the brain alterations observed in DS persons.

The problem is complex, however. Several genes may concur to impair neurological development and genes on Hsa21 may have an impact on other genes in the genome.

A number of genes on Hsa21 show dosage effect in TR21. This means that their expression is increased (more or less 50% in general) in the cells or tissues of DS persons. This increase can be measured in terms of biological activity (enzymatic, for example), quantity of proteins produced, or mi-RNAs. Ait Yahia-Graison et al; (2007) counted 120 genes expressed in lymphoblastoid cells derived from 10 DS persons (3 women and 7 men) and 11 controlled individuals (4 women and 7 men). Twenty-two percent of these genes were overexpressed in DS cells in correspondence with the gene-dosage effect and 7% were amplified beyond this level. Fifteen percent were highly variable among individuals (which may account at least partially for the phenotypic variability observed in this syndrome). Fifty-seven genes were found to be compensated by decreased or increased transcription, which the authors consider an indication of genetic robustness.

The overexpressed genes in TR21 include:: *APP* (amyloid-beta precursor protein), *SOD1* (superoxide dismutase-1), *DYRK1A* (dual specificity tyrosine Y- regulation kinase 1A), *EURL* (bêta-catenin signaling modulator), *CBS* (cystathionine-bêta synthase), *OLIG1* and *OLIG2* (oligodendrocyte transcription), *IFNAR* (alpha-interferon receptor), *CBR1* and *CBR2* (carbonyle reductase), *S100B* (glial function in neurons), *ERG* (regulator of hemato-immune cells), *DSCR1* (inhibitor of calcineurin-mediated signaling), *RCAN1* (calcineurin regulator), and *ETS2* (encoding a transcription factor).

However, there exists important variations in gene expression in the condition. As already suggested by Lejeune (1990), it is unlikely that all genes on Hsa21 would produce equal marked biological harm when triplicated because this would probably have lethal consequences.

As suggested in DS, DS-derived iPSCs, and DS mouse models, overexpression of genes *DYRK1A*, *APP*, *EURL*, involved in various cell functions and structural aspects of neurogenesis, *OLIG1/2*, responsible for myelinating cells and oligodendrocyte differentiation - a type of neuroglia, i.e., cells providing support and protection for the neurons, in the central nervous system, *ERG*, and *RCAN1*, affecting the central nervous system, is probably among the most noxious mechanisms in T21 brain etiopathology (Delabar et al., 1993; Stagni, Giacomini, Emili, Guidi, & Bartesaghi, 2018).

For example, *DYRK1A* transgenic mice (see below) exhibit neurogenesis alterations and brain and behavioral abnormalities comparable to those of human beings with T21 (Tejedor & Hammerle, 2010). In particular, Thomazeau et al. (2014) found that overexpression of *DYRK1A* increases the number of spines on oblique dendrites of pyramidal neurons in the prefrontal brain of adult mice trangenic for the gene *DYRK1A* (see below for additional information on Thomazeau et al.'s research). Li et al. (2016) observed that perturbation of *EURL* mRNA levels in mice C57BL/6 impair progenitor proliferation and neuronal differentiation, and reduce the dendritic spine densities of cortical neurons. These authors objectified similar features in tissue samples from human fetuses between 16-19 weeks of gestation. Chakrabarti et al. (2010) established that overexpression of *OLIG1* and *OLIG2* in the forebrain of mice Ts65Dn (see below) leads to defective neurogenesis (see below for information on other aspects of Chakrabarti et al.'s study).

Manley and Anderson (2019) observed that *OLIG2* gene dosage alters cerebral cortical interneuron development and contributes to cognitive disability in mice. Ishihara et al. (2019) observed that *ERG* gene triplication contributes to the dysregulation of the homeostatic proportion of the populations of immune cells in the embryonic brain and decreases prenatal cortical neurogenesis in a mouse model.

There is a large number of RNAs, some coding proteins and others non-coding. Hsa21 harbors four major types of micro RNAs (miRNA-99a, miRNA-125b, miRNA-155, and miRNA-802). They are short non-coding RNAs mediating post-transcriptional gene silencing whose overproduction is involved in the etiopathology of T21 (Salemi et al., 2018). Selective inactivation of all or at least some of these RNAs could be an efficient strategy for improving the DS phenotype (Aboudafir, 2017). Other RNAs can be used to silence other genes (see Fillat et al., 2014, below, for an example).

Wang et al. (2013) documented an association between T21 and abnormally low levels of proteins SNX27 in cells. This protein protects neurons from excess quantities of the neurotransmitter glutamate. Increased production of miRNA-155, linked to the triplication of several genes on Hsa21, is correlated with a reduced amount of SNX27.

Wang et al. (2013) then used mice genetically modified to produce fewer proteins SNX27 (mice named SNX27+/-). These mice showed a learning and memory handicap associated with reduced synaptic recycling of glutamate in spite of a globally normal neuroanatomy.

Synapses operate in association with specific neurotransmitters. They are produced pre-synaptically and recuperated post-synaptically. Wang et al. (2013) engineered a therapy of replacement at the level of the hippocampus (a brain structure involved in memory) for restoring normal levels of protein SNX27 and post-synaptic glutamate recycling. This therapy determined better learning and memory in the treated mice.

Murine models are useful tools in genetic research and engineering. However, TR21 in humans is orders-of-magnitude more complex than the corresponding mouse models., especially regarding cognitive functions. This should make people wary of too speedy generalizations from lower to higher cognitive levels.

Mice Ts65Dn and Ts1Cje, for example, are genetically modified to correspond as much as possible to the triplication of Hsa21 in humans (cf. Aboudafir, 2017, for a differential analysis of various mouse models). Ts65Dn partially mimic the DS human condition, including developmental delay and memory deficit. Ts1Cje mice show spatial learning deficits and craniofacial alterations. Genes in mice orthologous to Hsa21 are distributed on Mmu chromosomes 10 (39 genes), 16 (112 genes), and 17 (19 genes). In these regions, the orthologous genes are syntenically conserved, i.e., with the same blocks of order on the chromosomes. Ts65Dn mice have genes corresponding approximately to 60% of the genes harbored by Hsa21 (Rueda, Flórez, & Martinez-Cue, 2012).

This means that these models, although extremely valuable for the study of genotypic/phenotypic features of DS, are incomplete at best. Mice with full T21, i.e., with all their genes ortholog to those on Hsa21 triplicated, can be created by complex crossing of genetic lines (for example, Yu et al., 2010). But these mice are difficult to produce, expansive, and short living.

Transgenic mice are mice whose genomes have been modified by the transfer of a gene or a chromosome from another species.

Fillat et al. (2014) employed an adenovirus-associated AAV2/1-shDYRK1A as vector to carry a miRNA into the hippocampus of the experimental mice Ts65Dn for reducing the expressivity of gene *DYRK1A* and in the control group a virus with a sequence that does not interfere with the gene *DYRK1A* (AAV2/1-scDyrk1A(SC). Separately, a lentivirus LY-anti-miR155-802 was used to increase the expression of another gene, *MECP2*, located on the X chromosome at the locus Xq28. In both cases a statistically significant improvement (at the $p < .01$ level) was observed in the learning ability of the treated mice on the Morris aquatic labyrinth against the euploid mice. In this task, the mouse has to learn to locate a hidden plateform through its visuospatial perceptual ability and memory.

This research appears to confirm that modifications of genes located on other chromosomes than the 21st, for example a downregulation of *MECP2*, may also have a role in the DS phenotype. It also suggests a biological way to address the reduced or suppressed function of gene *MECP2* causally involved in the etiology of Rett syndrome, a degenerative condition in female children and adults characterized by serious difficulties in language, motor behavior, and intellectual capacity.

Mouse chimeras are used in experimental neurology to investigate cell development. They are obtained by injection of targeted embryonic stem cells into blastocysts (early embryonic stage) resulting in animals that have two (or more) populations of genetically distinct cells coming from different zygotes.

Xu et al. (2019) observed that iPSCs derived from DS persons overproduce *OLIG2* ventral forebrain neural progenitors, which favors excess production of sub-classes of GABAergic interneurons (neurons organizing circuits between efferent and afferent neuronal bodies). Transferred in neuronal chimeric mice, this causes impaired memory recognition. Short hairpin RNAs (shRNAs) were used to reverse abnormal *OLIG2* expression. This had the effect of reducing interneuron production in DS iPSCs and chimeric mouse brain, which in turn improved behavioral deficits in the latter. The implication drawn by the authors is that altered *OLIG2* expression may underlie neurodevelopmental abnormalities and cognitive defects in persons with DS.

Chakrabarti et al. (2010) removed one allele of each triplicated gene within the genome of Ts65Dn mice by breeding Ts65Dn females with *OLIG1/2* double heterozygous males to normalize the dosage of genes *OLIG1* and *OLIG2* in the forebrain of the resulting pups. Returning Ts65Dn animals to disomy for *OLIG1* and *OLIG2* genes had the effect of normalizing neurogenesis. This restituted a normal balance between excitatory and inhibitory neurons in the central nervous system alleviating

the defect in synaptic plasticity due to the overinhibition phenotype suspected to be one of the underlying causes of the cognitive deficit in Ts65Dn mice.

Ishihara et al. (2019) restored the observed perturbed proportions of immune cells in Ts1Cje mice embryo brains through a genetic manipulation rendering these mice disomic for the *ERG* gene (but otherwise trisomic on a Ts1Cje background). The neurogenesis defects observed in Ts1Cje were reduced in the *ERG* modified mice embryos. The authors concluded that their findings suggest that *ERG* gene triplication contributes to a dysregulation of the proportions of immune cells in the DS embryo, which perturbates prenatal cortical neurogenesis.

Acting on genes may not be without risk, however. The immunological systems have evolved to contrast viral aggression. It is imperative to make sure that reprogrammed cells are not confounded with infected ones and become targets for destruction.

Gene correction may become technically possible in DS in the middle term, including prenatally. Obviously, the case being, it should be envisaged with extreme caution as major risks exist for mothers and fetuses, given the toxicity of the viruses, possible adverse immunological reactions, and the risk of tumor generation (Caplan & Wilson, 2000).

Acting on gene products

Besides modifying genes, it is also possible to operate on the proteins and enzymes encoded by these genes.

Nakano-Kobayashi et al. (2017) reported that the oral administration of a growth inducer identified in their screen of neural stem cells (NSCs),

named ALGERNON (for altered generation of neurons)[3], which they claim has inhibitory activity against the expression of the gene *DYRK1A*, rescues NSC proliferation and increases the number of newborn neurons in Ts65Dn mice derived neurospheres and in human NSCs derived from human fibroblasts with DS. When administered to pregnant Ts65Dn dams, the medication induced improved cortical formation and prevented the development of abnormal behaviors in the Ts65Dn mouse offsprings. The authors suggest that ALGERNON prenatal therapy may have the capacity of preventing structural and developmental aspects of the DS neurogenic phenotype.

A catechin molecule named epigallocathechin 3 - gallate (EGCG), a polyphenol of green tea with antioxidant properties, has generated much interest in recent years. It is a natural inhibitor of the enzyme encoded by the gene *DYRK1A*.

Experiments with murine models appear to support the efficiency of EGCG in rescuing various aspects of neurogenesis. For example, in the course of the study mentioned before, Thomazeau et al. (2014) administered drinking water containing 25 % green tea decaffeinated extract (.08 mg/ml) and 25 % glucose to adult male mice Tg189N3 (mBACtgDyrk1a) aged 4-6 months for 4 to 6 weeks. These mice with a third copy of *DYRK1A* expressed more DYRK1A brain proteins compared to wild-type littermates. Green tea extracts contained 45% EGCG. Daily doses ranged between 120 and 200 mg per kilo of weight. Thomazeau et al. observed a normalization of spine density in deep layer pyramidal cells of the prefrontal cortex associated with a rescue of long-term potentiation (i.e., strengthening of synapses) in memory structures. This study suggests that the origin of the morphological and functional *DYRK1A*-related deficits in the prefrontal

[3] No precise information is given in Nakano-Kobayashi et al.'s paper on the nature and chemical composition of this molecule or molecular compound.

cortex is not merely developmental. Continued overexpression of *DYRK1A* in adult times may also be detrimental to brain prefrontal functioning. Contrasting *DYRK1A* excessive activity also in adolescents and adults years may possibly be justified in DS.

Hibaoui et al. (2014) induced iPSCs from primary fetal skin fibroblasts obtained post-mortem from discordant monozygotic twins, one with T21 (twin-DS) and the other a normal (twin-N) in an attempt to control for the genomic background. Both the iPSCs and the NPSCs (i.e., iPSCs derived into neural cells) exhibited defects associated with changes in the architecture and density of neurons, glial cells and with the expression of genes involved in neurogenesis. In particular, a two-fold increase in DYRK1A enzymes was found in NPSCs.

Hibaoui and associates were able to rescue neurogenesis impairment in these cells through *DYRK1A* inhibition using EGCG or a short-hairpin RNA silencing (shRNA), the last one in order to exclude a simple anti-oxidant effect of EGCG.

EGCG improved the number of NPSCs derived from twin-DS-iPSCs by promoting cell proliferation and preventing cell apoptosis.

When these NPSCs were further induced into mature neurons, *DYRK1A* inhibition through EGCC or shRNA treatment improved the expression of neuronal markers B3-TUBULIN and MAP2, an indication of improved neurogenesis.

The reduced expression of several genes, up to 30%, in NPSCs derived from twin-DS-IPSCs, was promoted by restoring *DYRK1A* expression to near normal levels by shRNA, confirming that the *DYRK1A* gene when overexpressed is a major contributor to DS impaired neurogenesis.

Moreover, the genetic profiling of twin-DS-iPSCs compared to twin-N-iPSCs revealed that TR21 not only affects the expression of trisomic genes but also that of disomic genes. As many as 96 genes related to brain

functions were found to be downregulated. This finding suggests that TR21 actually determines an alteration of the whole transcriptome (i.e., the whole set of RNAs resulting from gene expression), as already suggested by Lyle et al. (2009).

Corresponding data have been published by Guedj et al. (2009). They submitted transgenic Ts65Dn and Ts1Cje mice carrying an additional copy of gene *DYRK1A* to a diet rich in green tea polyphenols from gestation to adult age. The study included four groups of mice: wild-type and trangenic, fed either with water or green tea. Green tea infusions were given daily. The chronic polyphenol-based diet rescued major features of the transgenic phenotype, as demonstrated by significant statistical differences between the groups in brain weight, brain magnetic resonance imagery, and volume of the thalamus-hypothalamus region (at the $p < .01$ and $p < .05$ levels of error).

The above studies and others (see Stagni et al., 2016, for a summary of additional works) suggest the interest of the EGCG strategy for improving neurogenesis in mice genetically modified or transgenic for the gene *DYRK1A*. It must be noted, however, that the studies differ from each other in several respects that may interact with the effect of EGCG: for example, length of treatment, age of the animals, dosage, administration of other green teat extracts in ill-defined combinations with EGCG (see Stagni et al., 2016, for an overall discussion). This prevents solid conclusions to be drawn.

Very few studies have been conducted for testing the effect of EGCG at the human level. In a first experiment, De la Torre et al. (2014) witnessed positive effects on visual recognition in a group of young adults with DS following three months of daily treatment with EGCG (green tea extracts, low in caffeine, 9 mg per kilo of body weight, administered orally). No adverse event linked to the medication was observed. However, three

months after the end of treatment, the participants'performances returned to pre-intervention level.

In a second study, De la Torre et al. (2016) tested the effect of a daily EGCG treatment (with a dosage identical to the one in the preceding study), lasting for one year, coupled with a behavioral program of cognitive training. The sample of subjects counted 84 adults with DS (about as many women as men) aged between 16 and 34 years. They were divided into two groups: one group treated with EGCG and undergoing cognitive training; the other one receiving a placebo and the same cognitive training as the first group. Longer-term administration of the medication in the experimental group of subjects appeared to be well tolerated

A battery of neuropsychological tests was administered at the end of the study. Participants treated with EGCG and exposed to cognitive training showed a statistically significant superiority in two cognitive tests (memory and visual recognition) and in several adaptive tasks (measuring daily routines abilities). A retest 16 months later showed partial persistence of the effects measured at the end of the intervention.

The above data suggest that EGCG may improve brain defects in TR21 mice, genetically modified or transgenic. It is questionable, however, whether EGCG actually eliminates these defects. At the human level, 12 months of treatment with green tea extracts containing EGCG plus cognitive training seems to have a positive effect on cognitive performance, albeit of a modest magnitude, and this effect is partially retained after discontinuation of the treatment. This latter trend of research also suggests that some neurobehavioral intervention is necessary at the human level to trigger or stabilize an EGCG-induced effect.

Else, Xicota et al. (2019) tested the effect of EGCG on the lipid profile of individuals with DS. It is known that these persons tend to have higher rates of obesity. A double-blind clinical trial compared the effect of EGCG administered during 12 months to that of a placebo on the body lipidic

composition of 77 young adults with DS. Individuals receiving a placebo showed the expected increase in body weight and body mass index (ratio of body weight to square body height) over time A similar increase was not observed in participants having received EGCG treatment. However, the group difference was statistically significant only for male subjects.

An anonymous survey has been conducted by Long, Drawbaugh, Davis, Goodlett, and Roper (2019) on parents'attitudes regarding the administration of green tea extracts containing EGCG. Parents who give green tea extracts to their DS children are mostly younger, highly educated, and they consult scientific sources. DS individuals who receive green tea extracts are characterized as less severely disabled to begin with. Most caregivers who do not give green teas extracts report that they are concerned about potential negative side effects and/or that they have doubts regarding treatment effectiveness.

EARLY DIAGNOSIS

The DS brain starts at a disadvantage and the disadvantage is cumulative. It is essential that the attempts at improving neurogenesis and neural connectivity be conducted as early as possible. Major phenotypic features of DS can already be traced back to the fetal period. Several of the studies summarized above suggest the need for an efficient intervention to start early in life, which implies an early diagnosis of the condition.

Prenatal screening for DS is possible from the eleventh week of pregnancy through the analysis of fetal DNA fragments or mRNAs in maternal blood samples. Ninety-nine percent reliability can be reached in combining ultrasound, blood analysis, cardiac rhythm, and nuchal translucency (Sparks, Truble, Wang, Song, & Oliphant, 2012; Nicolaides, Syngelaki, Poon, & Wright, 2014; Sun, Lu, & Ma, 2019].

A less expensive technique has been experimented by Shan et al. (2019). They analyzed the peptidome (complete set of peptides expressed in cells) of urine samples of pregnant women carrying fetuses with DS and with normal karyotypes. A classification model was constructed based on candidate peptides that could differentiate fetuses with DS from controls reaching a sensitivity of 95,7 % and a specificity of 70 %. This suggests that maternal urinary peptidome could offer a prospect for a noninvasive biomarker screening of fetal DS.

However, amniocentesis and chorionic villus sampling are still the only fully reliable diagnosis techniques. However, they are intrusive and there is a risk of a miscarriage between 0.5 and 1 % (Reena et al., 2013).

Progresses are being made towards the development of noninvasive prenatal diagnostic methods. Asim, Kumar, Muthuswamy, Jain, and Agarwal (2015) have published an analysis of the advantages and disadvantages of a series of molecular methods for prenatal diagnosis of DS (e.g., cytogenetics analysis, fluorescence in situ hybridization).. A noninvasive diagnostic technique has been experimented by Zbucka-Kretowska et al. (2019). They studied the expression level of miRNAs in the plasma of 198 pregnant women with fetal DS at 15-18 weeks of gestation and 12 women with uncomplicated pregnancies who delivered healthy newborns at term.

Out of 800 miRNAs analyzed by Zbucka-Kretowska et al., six were upregulated and seven downregulated in plasma samples of women with fetal DS. The genes regulated by these miRNAs are involved in central nervous system development, congenital abnormalities, and heart defects. This study opens the way for designing a panel of miRNAs as a non-invasive technique for prenatal DS diagnosis.

Genetic corrections in the early stages of prenatal development could become possible in the future. The ultimate target in this respect would be embryonic gene correction inducing normalized genetic expression in all or

most body cells. The technology exists pending major research, refinements, and security check. It can further cell mosaic development given that there is no way to modify the entire embryo's line cell in the present state of gene editing knowledge and technology[4]. Mosaicism itself should not be a deterrent given that mosaic cases exist in DS and do not preclude healthy development.

It is known that neurogenesis continues in the ventricular and subventricular zones of cerebral cortex during the third trimester of pregnancy (Malik, Vinukonda, Vose, Bhimavaparu, & Hu, 2013). Later corrective intervention in fetuses with DS therefore could still be of therapeutic interest.

MOLECULAR PHARMACOLOGY

The neurobiological consequences of DS result in reduction of synaptic density and plasticity (Wisniewsky, 1990). Much attention has been devoted recently to the neurotransmitters, these biochemical

[4] He Jiankui, a Chinese researcher, performed what he claimed to be the first gene editing experiment with human embryos using the CRISP-Cas9 tool. The experiment was mentioned in the November issue of the *MIT Technological Review* (the story was subsequently eliminated from the journal's archives). Using a pre-implantation genetic diagnosis process, this researcher altered a gene called *CCR5* (bêta-chemokine receptor) located on C3, which when functional allows the aids-causing HIV virus to infect a class of cells in the immune system. He Jiankui's experiment, illegal in Europe and the United States of America, and also prohibited in China, has been widely condemned by the scientific community and in the international press, on several grounds including a technical one (as it clearly cannot achieve its declared goal of guaranteeing lifetime full immunity from HIV infection in the twin babies). He Jiankui now faces criminal investigation from the Chinese authorities who claim to have been unaware of the experiment. He has been fired from his (Shenzhen) university.

substances that permit synaptic functioning. Gotti, Caricati, and Panzica (2011), have published a review of studies on the alterations of brain circuits that can be identified in murine models of DS. It shows that different neurotransmission systems are deregulated (downgraded or upgraded) in several cerebral regions including the hippocampus (a part of the limbic system involved in memory processes), the locus coeruleus (a subcortical structure located in the cerebral trunk), and the Broca area in the frontal cortex (an important language structure).

Major neurotransmitter subsystems involved in brain functions are the cholinergic system (an excitatory neurotransmitter), the noradrenergic system (with the mostly excitatory neurotransmitter noradrenaline), the glutamate system (brain's major excitatory neurotransmitter), the GABAergic system (major brain's inhibitory neurotransmitter), and the serotoninergic subsystem (also inhibitory).

Particular drugs are being developed for contrasting the neurotransmission deficits caused by TR21. Promising results have been obtained with the Ts65Dn murine model of DS. Several substances have been shown to rescue at least partially learning and memory deficits. In some cases, attempts have been made to extent the intervention to humans with DS.

1. Regarding **the cholinergic subsystem**, inhibitory agents of acetylcholinesterase (which catalyzes acetylcholine post-synaptically reducing it to its basic constituents and allowing neurons to return to a state of rest following activation) have been tested: in particular, donepezil, rivastigmine, and physostigmine. In adults with DS and already in DS children, atrophy of cholinergic neurons is observed in several brain areas.

Acute injection of physostigmine has been proved to inhibit acetylcholinesterase and increase concentration of acetylcholine in the synaptic area in Ts65Dn mice at 4 months of age (but no longer at 10 and 16 months), and to improve learning and memory (Chang & Gold, 2008).

At the human level, Heller et al. (2003) have reported an open-label case series of six non-demented adults DS subjects (aged 20-41 years) receiving 5 then 10 mg donepezil over 24 weeks. Despite transient side-effects expectable from cholinergic overstimulation, all subjects tolerated the 10 mg dosage. A small but significant improvement ($p < .05$) in expressive language was observed at 24 weeks in four sub-tests from the Clinical Evaluation of Language Function – Revised (CELF-R). However, the small sample of subjects and the lack of a control group in this experiment render the outcome difficult to interpret.

Heller et al. (2004) have also reported the results of a 16-week pilot clinical trial on the effects of donepezil on language in 5 children with DS aged 8 to 13 years. The drug was dosed orally at 2.5 mg once daily for 8 weeks and 5 mg for the remaining 8 weeks. Two language measures were used: the Test of Problem Solving (TOPS) and the Clinical Evaluation of Language Fundamentals (CELF-3). The effect of the medication was measured by change from the baseline performance at weeks 8 and 16. No subject experienced serious adverse effects from the administration of the medication. T-tests for repeated measures yielded no significant indication of change in language performance between mean base line and the TOPS scores at 8 and 16 weeks. However, a significant improvement (at the $p < .003$ level of error) was registered in the mean CELF-3 performance from baseline to week 16. The results of this study are also difficult to interpret from an efficiency standpoint owing to the lack of a control group, the absence of a statistical correction for multiple comparisons, and the small sample of subjects.

Kishnani et al. (2009) tested the efficiency of donepezil in a sample of 123 young DS adults (aged 18-35 years) with no evidence of AD in a 12-week, randomized, double-blind, placebo-controlled study. The experimental subjects were treated with doses of 5 mg per kilo of weight for 6 weeks and then 10 mg/kilo in the following 6 weeks. Cognitive measures

included the Severe Impairment Battery (SIB), the Rivermead Behavioral Memory Test for Children, and the Clinical Evaluation of Language Fundamentals. Additionally the Vineland Adaptive Behavior Scales (a test assessing daily living skills, basic communication, interpersonal relations, and motor skills) were given. Administration of donepezil appeared safe except for transient and moderate impairment in a few subjects. However, two subjects had to be withdrawn from the double-blind phase for an hypertension rated severe by the investigators. Outcomes suggest efficacy of the drug in some but not all subjects, which the authors estimate consistent with the phenotypical variability of DS. However, an improvement observed also in the placebo group, particularly on the SIB, during the double-blind phase of the study, prevents a clear-cut conclusion regarding the cognitive specificity of the molecule.

Kishnani et al. (2010) assessed the efficiency and safety of donepezil with 129 DS children and adolescents (aged 10-17 years) in a 10-week, randomized, double-blind, placebo-controlled, multicenter study. Participants received a dose of 2.5 mg/kilo donepezil in the first part of the experiment, increased every 14 days until reaching 10 mg/kilo. Measures included the Vineland-II Adaptive Behavior Scales Parent/Caregiver Rating Form (VABS II/PCRF). The medication appeared to be well tolerated. During the double-blind phase, the VABS II/PCRF scores improved significantly ($p < .0001$) in both groups but with no significant difference between groups. The authors concluded that their trial failed to demonstrate a benefit for donepezil vs. placebo in their subjects.

Rivastigmine is a molecule with globally the same biochemical properties as donepezil. Its efficiency was tested by Heller and associates (2006, 2010) in two open-label studies with 10 DS children and adolescents (8 boys and 2 girls) aged 10 to 17 years. Doses at the beginning of the intervention were 1.5 mg/kilo increased to 3 mg/kilo and 4.5 mg/kilo in the following weeks. Five subjeccts reported no adverse event with the

medication, but five others signalled transient vomiting, diarrhea, fatigue, or insomnia related to cholinergic enhancement. After 16 weeks, results showed statistically significant gains in expressive language on the Test of Verbal Reasoning and Expression (TOVER; $p < .02$), on two memory mesures emphasizing language (narrative memory and immediate memory for names at the Developmental Neuropsychological Assessment Test (NEPSY; $p < .02$) , and in attention at the Leiter-R Attention Sustained Tests A and B ($p < 01$ and $p < .02$, respectively). Five participants (4 boys and 1 girl) continued the treatment for another 38 months. After an average of 38 months, all subjects returned to determine the safety and efficacy of longer-term rivastigmine use.

Longer-term use of rivastigmine appeared to have no adverse effect on overall health. A comparison of median performance change between the two subgroups of subjects (those who had continued the treatment for 38 months against those who had not) yielded no statistically significant group difference. However, two subjects demonstrated an important improvement in adaptive function (measure on the VABS) over the longer-term period with continued rivastigmine administration.

This research raises the interesting challenge of the assessment of cognitive change in clinical trials (here with DS subjects but the problem is more general) relying on group versus individual data.

The results of the above studies suggest that the effects of donepezil and rivastigmine administration are not consistent across individuals with DS. Many participants show little to no gains but a small subset of individuals seem to respond positively to cholinergic intervention; This suggests that genetic or epigenetic factors could affect the response to cholinergic therapy in DS (Heller et al., 2010).

2. Regarding **the noradrenergic subsystem**, one observes a reduction of the concentration of thyroid hormones in the locus coeruleus of Ts65Dn mice from 6 months of age in comparison with normal mice. This can be

hypothesized to reduce the supply of noradrenaline in the hippocampus. Ts65Dn mice were treated with formoterol, which stimulates the adrenergic receptors. The treatment appeared to have a limited positive effect on neurogenesis (Gardiner, 2015).

3. Molecules have been tested that target the receptors NMDA (N-methyl-D-aspartate) of **the neurotransmitter glutamate** in order to reduce an excess of activation of this neurotransmitter. Costa, Scott-McKean, and Stasko (2008) observed that acute injections of the NMDA receptor antagonist memantine improves learning in Ts65Dn mice. Administered to young adults with DS, in preliminary studies, memantine has shown encouraging clinical signs (Capone unpublished in Capone, 2011).

4. Also in the pipeline of cognitive pharmacotherapy, is **the GABA neurotransmitter subsystem** (gamma aminobutyric acid). GABA receptor antagonists picrotoxin and pentylenetetrazole have been tested by Fernandez et al. (2007). Two weeks of daily injection for each drug appeared to rescue maze learning and object recognition in Ts65Dn mice. Pentylenetetrazole is controversial for clinical application, however, because it is known to be convulsant in humans.

5. As to **the serotoninergic subsystem**. A series of studies with Ts65Dn mice has shown that chronic treatment with fluoxetine, an antidepressant antagonist of serotonin synaptic recapture, from postnatal days, rescues abnormalities in neurogenesis and stimulates the production of neurons and their incorporation into functional networks. Drug concentration must be carefully controlled, however, for higher levels of fluoxetine in daily consumption may provoke seizures (Gardiner, 2015).

Guidi et al. (2014) administered fluoxetine to pregnant mice Ts65Dn in continuity from embryonic stage until birth. After delivery, the mice whose mothers had been given fluoxetine exhibited normal brain neuronal proliferation and dendritic growth up to a control at 45 days. Fluoxetine is known as a molecule interacting with the class of enzymes histone

deacetylase, which catalyzes the loss of the acetyl group on the histones (a kind of coil around which ADN is winded more or less tightly), thereby reducing genic expression.

Another series of biochemical agents have **nootropic** (nonspecific brain boosting) **and antioxydant effects.** They contrast the continued production in cells of the biological residus of oxygen reduction, more or less 2% of the regular oxygene intake, which is harmful to the biochemistry of the body and is listed as participating to cellular aging in the long term.

Piracetam increases brain oxygenation and improves GABA neurotransmission. Lobaugh et al.(2001) assessed the cognitive and adaptive effects of piracetam in a double-blind study with 18 DS children (aged 7-13 years). The experimental group received 80-100 mg/kg per day for 15 weeks. No statistically significant benefit was observed in the treated suject in comparison with the control group in 30 tests measuring learning, attention, and memory, and several parent and teacher adaptive scales. Treatment induced side effects of irritability and poor sleep in 7 subjects during treatment.

An antibiotic commonly used to treat acne, minocycline, seems to have neuroprotective effects and inhibit apoptosis. Three months of treatment in 10-month old Ts65Dn mice significantly improved performance in several cognitive tasks ($p < .05$ and less) (Plane, Shen, Pleasure, & Deng, 2010). Chen et al. (2014) observed that the astrocytes (glial cells supplying nutrients to the neurons and detoxifying the extracellular milieu in neutralizing excess glutamate) induced in vitro from stem cells derived from fibroblasts of persons with DS, do not favor in vivo neurogenesis when transplanted into iPSCs mice brains. Minocycline corrects this deficiency by modulating the expression of gene *S100B*, located on chromosome 21 at locus 21q22.3. This gene regulates various cellular processes including the glial function.

Broze, Svonenko, Devenney, Smith, and Reeves (2019) tested the ability of hydroxyurea, a derivative of urea, to activate neural pathways in Ts65Dn mice. Treatment was initiated when the mice were three-month old and lasted until 6 months of age. A significant improvement of memory retention of spatial information was measured in the treated animals.

In conclusion, experiments with Ts65Dn mice suggest that different drugs can rescue learning and memory in cognitive tasks and in some cases appear to improve neurogenesis. However, there is a need for a standardization of the experimental protocols. Some works supply acute single doses of drugs, others chronic administration extending from a few weeks to several months. The ages of theparticipating mice varies from early prenatal to several months following birth. Sex variation is rarely carefully controlled.

A conundrum in with murine models concerns the non-Hsa ortholog genes in genetically modified mice. For example, these gene are estimated to be 50 in Ts65Dn mice. It is unlikely that overexpressed they would not contribute to the phenotype of these animals. Also the molecular basis of drug responses must be better defined before envisaging the conduct of pre-clinical and clinical trials (Gardiner, 2015).

The neurobiological gap between mice and human beings cannot be underestimated. Generalizations must be considered with maximum caution and conclusions envisaged only hypothetically. Cognitive pharmacotherapy in DS is still in infancy and the results obtained with the drugs tested so far are preliminary and inconsistent.. Drug safety and the occurrence of adverse events while subjects are under medication and in following weeks need to be better assessed.

A major challenge regarding the efficacy of the pharmacological products is to evaluate correctly the maturational level of the target children for a pharmacological intervention. Capone (2011) indicates: “Given that pharmacological agents require binding to external cell surface receptors

that are linked to a network of signalling mechanisms in order to produce a deliberate cellular response, it becomes critical to determine which children with DS achieve the requisite level of maturation necessary to experience a pharmacological response when challenged" (p. 98).

ALZHEIMER DISEASE

Alzheimer disease (AD) is more frequent and precocious in DS than in the general population. It affects approximately 20% of DS persons beyond 40 years of age, 40% beyond 50 years, and close to 80% beyond 60 years. In some cases, the degenerative process is relatively slow going and may cover a dozen years. In other cases, the pathological involution is rapid. In the general population, sporadic cases of AD are found most often in persons beyond 55. Familial (hereditary) cases usually have an earlier onset.

The evolution of the AD pathology is globally the same in persons with DS and in the general population except for chronicity. However, there are a few potential differences deserving additional investigation. Symptoms of depressions appear more pronounced in persons with DS in the early phases of the disease. Epileptic crises seem also to be more frequent in DS people with AD again particularly in the early phases of the pathology (Prasher, 2005).

DS persons with a partial translocation not involving the upper part of Hsa21 do not present a particular susceptibility to AD while showing the regular DS phenotype. Those with a partial translocation involving the upper part of this chromosome often present a clear AD pathology. This has suggested that one point of departure for the AD etiopathology in DS may be found in an amyloid cascade induced by overexpression of the gene *APP*

located on the upper part of the long arm of Hsa21. Although gene *APP* is not triplicated in non-DS persons, it has been suspected that the AD pathogeny in these persons is also related to an overexpression of this gene (e.g., Sinet, Nicole, Ceballos, & Delabar, 1987).

Important quantities of nontoxic amyloid-alpha proteins are produced naturally in the brain of all people. In DS, a phenomenon not yet identified switches this production to toxic peptides amyloid-bêta 40 and 42, which cannot be dissolved in brain fluids. These peptides initiate the formation of amyloid plates. They are tiny structures of 40 microns diameter composed of degenerated axons and dendrites, glial cells, and astrocytes centered around amyloid deposits.

The plates aggregate around the junctions between neurons disturbing neurotransmission. They end up circumventing neuronal bodies. This seems to accelerate a tauopathy, which may start independently of the amyloid pathophysiology. It is characterized by the aggregation within the brain pyramidal neurons of a protein called TAU made of helicoidal filaments of 10 nm diameter, responsible for the neurofibrillation of the cells'cytoplasm. TAU is a natural protein involved in the construction of the microtubules (communication channels within cells). In the AD pathology, TAU proteins become hyperphosphorylated (i.e., they incorporate an excess amount of phosphorus), which destroys the neuronal tissue and annihilates it functional capacity.

The amyloid plates gradually invade all cortical layers, mid-brain structures, brain trunk, and cerebellum. Neurofibrillation follows a route going from entorhinal and hippocampal cortices in the medial temporal lobe to adjacent temporal cortex, the frontal cortex, and then gradually the entire cerebral cortex. This mapping fits the evolution of the pathology major symptoms: from memory limitations to language difficulties, behavioral and overall disorders. If the general framework of the pathological evolution has begun to be known, the intermediate steps are still insufficiently specified.

The primum movens of the amyloid cascade remains unknown. A massive destruction of the Meynert nucleus, located in the brain basis and supplying the point of departure of the cholinergic neurons, has been suggested to be at the orign of the amyloid cascade (Dubois, 2019).

Prionic mechanisms are also suspected in the etiology of AD. In prion pathologies, a protein located on a brain cell surface folds abnormally and becomes pathological determining other proteins to unfold abnormally, which leads to cerebral lesions. It is believed that in AD, bêta-amyloid and TAU proteins display prion-like autoreplication properties.

Not all DS persons develop AD, however. Compensatory mechanisms operate of which nothing is known for sure in the condition.

Other genes on other chromosomes than the 21st have been shown to influence the determinism and the course of AD in non-DS aging persons. A gene encoding apolipoprotein E (associated with cholesterol metabolism and intervening in a number of neurophysiological processes), located on C19, exists in three allelic variants. *APOE3* is the most frequent one in the population, followed by *APOE4* and *APOE2*. Higher dosages in *APOE3* and *APOE4* are correlated with increased and earlier risk for AD. They are associated with higher concentrations of protein amyloid-bêta in the brain. Variant *APOE2* seems to have a protective action (Firth et al., 2018). A variant form of *APOE3* gene, labelled *APOE3ch* (for Christchurch in New Zealand where it was first identified), seems to have also a protective role (overall against the TAU pathology) particularly when present in two copies in the genome (Arboleda-Velasquez et al., 2019).

Allelic variants of the gene *SORL1* (sortilin receptor, located on C1) have been identified as additional risk factors for a later AD onset (Rogaeva et al., 2007). When *SORL1* is overexpressed, one observes an increase in brain amyloid deposits. Alterations of genes *PSEN1* (presenilin) on chromosome 14 and *PSEN2* on chromosome 1 also appear to be involved at various moments in the amyloid cascade; most often in the early stages in

the hereditary forms of the *PSEN1* mutation, called *PSEN* E280A (Wallon et al., 2012). Vascular cholesterol may also be involved in interaction with genotype *APOE* (Hochino, Kamino, & Matsumoto, 2002).

The first molecule proposed (and approved by the US.Federal Drug Administration, FDA) for trying to contrast the early manifestations of AD was tacrine (tetrahydroaminoacridine), an inhibitor of acetylcholine-esterase, thus increasing intracerebral concentration of the neurotransmitter acetylcholine by reducing its synaptic degradation. In spite of a limited clinical efficiency, the heavy hepatic toxicity of tacrine led the pharmaceutical firms to retire the product from the market.

Other inhibitors of acetylcholine-esterase have been developed with no marked hepatic toxicity, for example, donepezil, and rivastigmine, already mentioned in a preceding section, and galantamine (Malegiannaki, Katsarou, Liolios, & Zisi, 2019, for a review). They have been approved by the FDA.

As indicated, a moderate cholinergic deficit exists in DS. It is augmented in AD. In Ts65Dn mice, overexpression of the molecule amyloid-bêta has been shown to be associated with degeneration of cholinergic and noradrenergic neurons particularly in the brain hippocampal region (Sanchez et al., 2012). On this basis, one has proposed to reduce the natural elimination of neurotransmitter acetylcholine with donepezil, rivastigmine, or galantamine, in chromic administration. Acetylcholine-esterase inhibitors are generally well tolerated. If side effects occur, they commonly include nausea, vomiting, loss of appetite, and diarrhea, but are mostly transitory. So far, however, attempts to rescue cognitive functioning in DS persons with AD using acetylcholine-eserase have met with limited success only and uniquely in patients at the early stages of the AD pathology (Pritchard & Kola, 2007).

As indicated, memantine (also approved by the FDA) is a molecule that regulates the activity of the neurotransmitter glutamate. It acts on the

NMDA receptors for regulating the quantity of ions calcium entering neurons in the propagation of the neural influx. Excess glutamate increases the activity of NMDA receptors causing an excess of calcium entering the cell. This alters neural transmission. Similar side effects to those observed with cholinergic medication have also been observed with the chronic administration of memantine. Clinical trials with memantine have met with limited success only in improving cognitive functioning in DS persons with AD (Boada et al., 2012).

Immunotherapy addressing the first leg of the amyloid cascade has been tried with transgenic mice (Schenk et al., 1999). The principle was to inoculate the APP protein in the vascular system, which determined the production of antibodies acting against the injected antigene but also against the amyloid plates in the brain of the treated mice. Positive results were obtained involving a drastic reduction of the amyloid plates associated with important cognitive benefits. Clinical trials with human subjects at the early stages of AD were undertook and did indeed determine a reduction of the amyloid charge in the brain of the persons treated but no improvement of the clinical symptoms was observed.

Lithium has been tested by Matsunaga et al. (2015) in an attempt to limit cognitive deterioration in DS persons at the first stages of AD. They reported positive results in comparison with a control group. However, as said, AD incipiens in DS is characterized by episodes of depression. Lithium is known to be a mood stabilizer. It is not clear whether this molecule acts mostly as an antidepressant or whether it may constitute a genuine antagonist to early cognitive deterioration in AD.

Antioxidant molecules, for example, nicotinamide, levocarnitine, and lipoid acid, have been tried for reducing the oxidative stress, thought to play a role in the etiology of AD, but seemingly without encouraging results (Rasore-quartino, 2012). A long series of other molecules (e.g., phenserine, xaliproden, bapineuzumab, huperzine, intravenous hemoglobin,

methylthioninium chloride, raloxifene) are in various phases of clinical trials (Sabbagh, 2009). They may lead to new pharmacotherapy options for the treatment of AD.

Among the primary suspects in the etiopathology of AD, one finds the gene *DYRK1A*. Overexpression of this gene is thought to be one of the major culprits of the hyperphosphorylation (i.e., excess addition of a particular phosphate group to a protein) of the TAU protein leading to neurofibrillation of the neuronal bodies.

Normalizing *DYRK1A* dosage by breeding Ts65Dn mice with a triplication of this gene with mice trisomic for the same DNA segment but without the gene *DYRK1A*, yielded Ts65Dn mice with normal dosage of this gene and a normal concentration of protein APP in cerebral cortex, hippocampus, and cerebellum (Garcia-Cerro, Rueda, Vidal, Lantigua, & Martinez-Cue, 2017).

Kawakubo, Mori, Shirotani, Iwata, and Asai (2017) injected harmine, an inhibitor of protein DYRK1A, in fibroblasts obtained from DS persons with an AD pathology. They measured an important increase in the concentration of the enzyme neprilysin and correlatively a decrease in the concentration of DYRK1A proteins in the fibroblasts.

Accumulation of amyloid plates in the brain intensifies some 5 to 20 years before a significant cognitive decline is observed. Global cortical atrophy, increased concentration of TAU proteins, and neurofibrillation of cells cytoplasm can be objectified 1 to 5 years before a diagnosis of AD is warranted.

Current work concentrate on the validation of a list of biological markers of early AD, including concentration of proteins APP and TAU in blood plasma (Lee, Chien, Hwu, 2017; Hartley et al., 2017; Alhajraf, Ness, Hye, & Strydom, 2019), blood expression of *MTRNR2L12*, a gene located on C3 (at 3q11.2), almost identical to the mitochondrial gene *MT-RNR2*, which encodes the micropeptide HUMANIN considered to be a protective

factor in familial AD (Bik-Multanovsky, Pietrzyk, & Midro (2015), and blood plasma neurofilament light chains (Shinomoto et al., 2019; Rafii et al., 2019).

Blood tests could possibly predict onset of AD up to 10 years in advance in combining measures of amyloid-bêta proteins, proteins IRS-1 (involved in insulin signaling in the brain and commonly defective in people with AD), the presence of genetic variants *APOE3* and *APOE4* in blood plasma, and differences in levels of miRNAs. The question is still pending, however, because the presence of amyloid-bêta proteins in blood although correlating positively with the presence of amyloid plates in the brain, does not ineluctabily means AD incipiens. A proportion of aging people have them without developing the pathology.

Neurological examination by transcranial magnetic stimulation (a noninvasive technique able to measure the activity of brain circuits) offers a promise for revealing synaptic dysfunctions linked to AD and predicting cognitive decline in the early phases of the disease (Motta et al., 2017).

Assuming the validity of the amyloid cascade hypothesis, a genuine curative strategy for AD would be to prevent accumulation of amyloid plates, proliferation of TAU proteins, and neurofibrillation of neuron's cytoplasm. A number of molecules and drugs are being tested that may prove efficient in these respects, such as the inhibitors of the APP protein, vaccines and antibodies, inhibitors of the TAU protein, as well as a number of other biochemical agents thought to be able to boost brain defenses against APP and TAU toxicity.

Nawa et al. (2019) derived dermal fibroblasts from patients with DS. These cells showed a severe limitation of proliferation and signs of premature senescence accompanied by perturbation of protein homeostasis leading to accumulation of protein aggregates. They treated these cells with sodium 4 - phenylbutyrate (4-PBA), a drug used to treat urea cycle disorders, and observed a decrease in the protein aggregates of the

fibroblasts. This study suggests that a treatment with 4-PBA could be of help for inhibiting protein aggregates in early AD.

Studies involving protein VPS35 (vacuolar sorting 35) that has the capability of altering the amyloid preprotein/amyloid-bêta metabolism, are also relevant. Li, Chiu, and Praticò (2019) observed that triple transgenic mice (3xTg) overexpressing VPS35 exhibit better spatial learning and short-term memory compared to control animals, an improvement associated with a significant reduction of amyloid-bêta levels and TAU phosphorylation. In vitro studies revealed reduced synaptic pathology and neuroinflammation.

Vagnozzi et al. (2019) showed that an enzyme labelled TPT-172 can induce higher levels of protein VPS35. In vitro studies showed that overexpression of VPS35 leads to a reduction of critical TAU levels in neurons. In contrast, silencing gene VPS35 is associated with an accumulation of TAU proteins. In vivo experiments with a transgenic mouse model of TAU pathology, down-regulation of VPS35 caused an increase of TAU proteins and a reduction of synaptic integrity. It appears that active cathepsin D encoded by gene *CTSD* on chromosome 4, is the agent mediating the VPS35 effect on TAU accumulation, due to its role in degrading toxic proteins in the brain.

These latter studies open new perspectives for biochemically contrasting the second leg of the amyloid-cascade, i.e., the one concerned with TAU toxicity and neurofibrillation.

CONCLUSION

Important developments are taking place in the field of DS with a perspective of improvement in the life and cognitive functioning of the persons affected with the condition. Genetic therapy is still largely

experimental and mostly restricted to in vitro manipulations. It will require an important number of additional experimental works before being assessed pre-clinically and tried clinically.

Cognitive pharmacotherapy is a very active field not the least regarding cognitive disabilities and DS. Decisive outcomes have been relatively rare so far. However, a number of molecules are in the research pipeline. Some of them could prove efficient as adjunct treatments for boosting brain development and neurotransmission in DS persons in particular. However, short- and longer-term negative secondary effects need to be better controlled and the drugs possible toxicity further assessed.

Biological research on Alzheimer disease has identified a significant part of the causal chain leading to brain degeneration. AD in DS and non-DS persons seems to proceed in the same way, even if only persons with standard TR21 present a full triplication of Hsa21. Pharmacological treatments have not demonstrated particular efficiency so far except perhaps mostly symptomatically at the early stages of the pathology.

Further research on AD in non-DS aging persons will also benefit persons with DS and vice versa. AD represents a very active field of biological and pharmacological research and for good reasons. In the United States alone, at present time, there are 4.7 million persons diagnosed with AD. It is estimated that 20% of the persons aged beyond 80 years are affected with AD and 40% beyond 90 years. A further increase is expected in coming decades associated with gains in average life expectancy. In this respect, it is interesting to mention that recent statistics in the United Kingdom suggest a higher prevalence of AD in women (65% of the cases), possibly linked to longer life expectancy; 72 years 8 months in women vs. 68 years 4 months for men (according to the United Nations World Population Project, revision 2015; The Guardian, July 17, 2019).

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