The 50th anniversary of the discovery of trisomy 21: The past, present, and future of research and treatment of Down syndrome

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Abstract: Trisomy 21 or Down syndrome is a chromosomal disorder resulting from the presence of all or part of an extra Chromosome 21. It is a common birth defect, the most frequent and most recognizable form of mental retardation, appearing in about 1 of every 700 newborns. Although the syndrome had been described thousands of years before, it was named after John Langdon Down who reported its clinical description in 1866. The suspected association of Down syndrome with a chromosomal abnormality was confirmed by Lejeune et al. in 1959. Fifty years after the discovery of the origin of Down syndrome, the term “mongolism” is still inappropriately used; persons with Down syndrome are still institutionalized. Health problems associated with that syndrome often receive no or little medical care, and many patients still die prematurely in infancy or early adulthood. Nevertheless, working against this negative reality, community-based associations have lobbied for medical care and research to support persons with Down syndrome. Different Trisomy 21 research groups have already identified candidate genes that are potentially involved in the formation of specific Down syndrome features. These advances in turn may help to develop targeted medical treatments for persons with Trisomy 21. A review on those achievements is discussed. *Genet Med* 2009;11(9):611–616.

Key Words: Down syndrome, trisomy 21, mouse, treatment, genes

Fifty years ago, Lejeune et al.¹ discovered that Down syndrome (DS) results from the presence of an additional Chromosome 21. A common defect present in about 1 in 700 liveborn children, it is the most frequent cause of mental retardation and a recognized genetic etiology of Alzheimer disease (AD). Recent progress in studies of mouse models of Trisomy 21 suggests a link between characteristic phenotypic changes in DS and increased dosage of specific genes, which may allow an understanding of the molecular basis of these abnormalities. If so, it may soon be possible to understand and to effectively treat the most distressing symptoms and signs of this disorder.

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Disclosure: The authors declare no conflict of interest.

Submitted for publication May 21, 2009.

Accepted for publication June 14, 2009.

Published online ahead of print July 24, 2009.

DOI: 10.1097/GIM.0b013e3181b2e34c

HISTORICAL REVIEW

Clinical description

By examining artifacts from the Tumaco-La Tolita culture, which existed on the border between current Colombia and Ecuador approximately 2500 years ago, Bernal and Briceno² suspected that certain figurines depicted individuals with Trisomy 21, making these potteries the earliest evidence for the existence of the syndrome. Martinez-Frias³ identified the syndrome in a terra-cotta head from the Tolteca culture of Mexico in 500 patients with AD in which the facial features of Trisomy 21 are clearly displayed. Examining more recent artifacts, different authors reported apparent depictions of the syndrome in 15th and 16th century paintings.^{4,5} On the basis of this evidence, it is likely that people with Trisomy 21 have been a part of human culture for thousands of years.

Esquirol, a psychiatrist from the school of Pinel, was interested in the phenotypic differences between mental retardation and psychosis: he was the first to write a phenotypic description of Trisomy 21 in 1838 (Table 1). At the same time, Seguin established the first training program for mentally retarded children and published in 1846 a treaty on “the education of idiots” in which he gives an extended description of Trisomy 21 named “idiotie furfuracée.” Twenty years later, an English physician, John Langdon Down published an essay describing the phenotype of children with common features distinct from other children with mental retardation. He was the first to make the distinction between children who were “cretins” (later found to have hypothyroidism) and what he referred to as “Mongoloids.” Down based the name on his feeling that these children looked like people from Mongolia, who were erroneously thought to have an arrested development.⁶ By the turn of the century, mongolism had unfortunately become a widely used descriptive term for DS.

Chromosomal basis

Until the middle of the 20th century, the cause of Trisomy 21 remained unknown. However, its presence in all races, the association of its incidence with increasing maternal age, and its exceptional occurrence in siblings had already been noted. The possibility that Trisomy 21 might be due to a chromosomal abnormality was suggested in 1932 by Waardenburg (a Dutch ophthalmologist)⁷ and Davenport⁸ (an American geneticist). In 1950, a study of chromosomes on a testicular sample taken from patients with Trisomy 21 was undertaken by Penrose. The conclusion was that neither triploidy nor aneuploidy was the cause.⁹

With the discovery of karyotyping techniques, and the assignment, in 1956, of the exact chromosome number in humans Lejeune et al.^{1,10} were able to show, in 1959, that Trisomy 21 resulted from an additional chromosome. The extra chromo-

Table 1 Principle dates in Trisomy 21 syndrome

500 BC: First representation of Trisomy 21
1838: First phenotypic description of trisomy 21 by Esquirol
1846: Treaty on “the education of idiots” and extended description of Trisomy 21 by Séguin
1866: John Langdon Down describes the phenotype of children with Trisomy 21
1932: A possible chromosomal origin suspected by Waardenburg and Davenport
1959: Lejeune et al. and Jacobs et al. find an extra Chromosome 21
1961: Geneticists complained about the term “mongolism”; replaced by “Down Syndrome or Anomaly,” or “Trisomy 21 Anomaly”
1989: Individualization of the DCR
1990s: First trisomic mouse lines
1997: Cold Spring Harbor meeting defining DCR-1 as a region with the largest number of associated DS features
2000: Sequencing of Chromosome 21 by Hattori et al.

DCR, Down syndrome critical region.

some was subsequently labeled Number 21, and the clinical condition was thus called Trisomy 21. After the report by Lejeune et al., rapid confirmation was provided by Jacobs et al.¹¹ and Fraccaro et al.¹² A year later, Harnden et al.¹³ reported the first case of double aneuploidy (a boy with Trisomy 21 and Klinefelter syndrome); this was followed rapidly by the discovery that some familial cases of Trisomy 21 were caused by translocations and the fact that the phenotype of Trisomy 21 could also result from patients with partial Trisomy 21.

At the start of 1961, Asian geneticists complained about the term “mongolism”; a letter cosigned by a group of geneticists urged that this expression, which implies a racial basis for the condition, be abandoned.¹⁴ Some of the undersigned suggested that “mongolism” be replaced by “Down Syndrome or Anomaly,” whereas others indicated the desire to honor the discovery of Lejeune et al., by using the term “Trisomy 21 Anomaly,” which could include cases of both complete trisomy and translocations.

CURRENT STATUS

The most compelling hypothesis for the pathogenesis of Trisomy 21 is the gene-dosage hypothesis. It states that all changes are due to the presence within the genome of an extra copy of Chromosome 21 and the genes contained therein; the immediate consequence of the overexpression of certain genes is the induction of specific mechanisms responsible for identified clinical anomalies. Stated differently, the expectation is that specific gene dosage changes will be linked to specific phenotypic abnormalities.

Gene dosage and medical care

In the absence of detailed information regarding gene expression, early attempts to treat the pathogenesis noted the possible deleterious effects of: (1) increased gene dose for Cu/Zn superoxide dismutase, cystathionine β synthase, S-100 β protein, and phosphofructokinase; (2) disturbed thyroid function with elevated thyroid-stimulating hormone with low rT3 as well as

changes in bipterin metabolism; and (3) the peculiar sensitivity of trisomic 21 lymphocytes to methotrexate.¹⁵ To counter the presumed effects of abnormal gene dose, some authors suggested vitamin supplements. In the 1960s, Dr Henry Turkel claimed that a mixture of 48 ingredients could improve the intelligence of children with DS, which he administered to his patients for more than 40 years. No double-blind study was ever performed. No evidence emerged that this was beneficial. Few years later, Harrell et al.¹⁶ reported that supplementary vitamins and minerals and thyroid hormone improved intelligence quotient scores and normalized physical appearance in children with mental deficiency. Reportedly, three children with DS showed the best results. The study was not performed scientifically, and seven studies performed in the next decade using this mixture showed no benefit.¹⁷ Antioxidants and folic acid were also suggested as treatments.^{18,19} Although on the surface this suggestion is reasonable, no proof has been provided for a beneficial effect on psychomotor development.

Nowadays, cardiac surgery, vaccinations, antibiotics, thyroid hormones, leukemia therapies, and anticonvulsive drugs (e.g., vigabatrin) have considerably improved the quality of life and life expectancy of individuals with DS. Indeed, life expectancy that was barely 30 years in the 1960s is now reaching more than 50 years of age. Simultaneously, early rehabilitation allowed better socialization. Nevertheless, physicians managing patients on a daily basis still have to face practical questions such as pain detection, sleep apnea, depression, and prevention of premature ageing. The clinical trials meant to improve cognitive functions have been so far deceiving.^{20–26} Most of them have relied on vitamins or trace elements supplementation. Despite the physicians’ involvement, the way these trials have been conducted is somehow questionable because of clinical variability and a limited number of patients and “bottom effect” of the psychometric tests.

Phenotype–genotype correlation

Focusing on the role of specific genes in the pathogenesis of the disorder, the molecular genetic analysis of individuals with partial Trisomy 21 was coupled with studies of their phenotype and comparison with individuals with full Trisomy 21. This comparative analysis allowed investigators to define the presence or absence of phenotypes in individuals whose aneuploidy was due to different sets of genes. Several research groups identified a region on Chromosome 21 that was argued to contain the major genes responsible for the pathogenesis of the disorder. This region from 21q21 to 21q22.3 was called the Down syndrome critical region (DCR).^{27,28} Other or overlapping definitions were given.^{29–31} Finally, a common nomenclature was endorsed at a Cold Spring Harbor meeting in 1997 defining DCR-1 as a region with the largest number of associated features, including facial and hand phenotypes and mental retardation. Regions specific for a unique feature were named “DCR-name of the feature.” Later, Ronan et al.³² described a family with a 4.3-Mb duplication within 21q22.13–q22.2, encompassing *DYRK1A* gene (OMIM 600855) but not *DSCR1* (OMIM 602917) or *DSCAM* (DS Cell Adhesion Molecule) (OMIM 602523). Karyotype analysis and metaphase fluorescent in situ hybridization were normal, but interphase fluorescent in situ hybridization revealed a microduplication within 21q22 in all three individuals, confirmed by array comparative genomic hybridization and covering part of the DCR-1. The phenotype in this family is of Trisomy 21 with mild learning disability but no major organ malformation. Recently, Lyle and coworkers³³ reported the identification and mapping of 30 pathogenic chromosomal aberrations of human Chromosome 21 (HSA21), con-

sisting of 19 partial trisomies and 11 partial monosomies for different segments of HSA21. The breakpoints mapped to approximately 85 kb with the majority (26 of 30) in partial aneuploidies mapping within a 10-Mb region. These data argued against a single DCR, explaining the whole phenotype and identified susceptibility regions for 25 phenotypes for DS and 27 regions for Monosomy 21.³³

The search for those genes whose dose is most significant for specific phenotypes was markedly enhanced by the fact that Chromosome 21 is the smallest human autosome and the second human chromosome to be sequenced.³⁴ So far, at least 300 genes have been located on Chromosome 21. The gene-dosage hypothesis has been modified to state that Trisomy 21 is a direct consequence of either an additional copy of protein-coding genes that are dosage sensitive or an additional copy of non-protein-coding sequences that are regulatory or otherwise functional.³⁵ The effect of some dosage-sensitive genes on the phenotypes might be allele specific and could depend on the combination of alleles with qualitative (alleles with amino acid variation) or quantitative (alleles with variation in gene expression level) traits. Dosage-sensitive genes could act directly to induce pathogenesis or indirectly by interacting with genes or gene products of either aneuploid or nonaneuploid genes or gene products. It is a reasonable speculation that the genetic background of individuals plays an important role in the variability of phenotypic severity that is seen in DS.

Deutsch et al.³⁶ investigated the patterns of gene expression variation in a 25-Mb region of HSA 21 and identified loci involved in their regulation. Using expression arrays of lymphoblastoid cell lines, Ait Yahya-Graison et al.³⁷ found that 29% of the expressed Chromosome 21 transcripts are overexpressed in cells from people with Trisomy 21. Among these, 22% are increased proportional to the gene-dosage effect (i.e., 1.5-fold) and 7% are amplified (i.e., above the expected 1.5 ratio). The other 71% of expressed sequences are either compensated (i.e., with a ratio not statistically different from 1) (56%), with a large proportion of predicted genes and antisense transcripts) or highly variable among individuals (15%). Thus, most of the Chromosome 21 transcripts are compensated for by the gene-dosage effect. In contrast to compensated genes, overexpressed genes are likely to be involved in the Trisomy 21 phenotypes.³⁷ These data, together with expression data for interesting genes and functional data, are enhancing the ability to identify candidate genes for specific Trisomy 21 features.

In this context, it is interesting to consider which genes might be most important. Overexpression in genes such as *CAFLA* (OMIM 601245), *CBS* (OMIM 236200), and *GART* (OMIM 138440) might be harmful to DNA synthesis and repair. *COL6A1* (OMIM 120220) overexpression may cause heart defects, and *CRYA1* (OMIM 123580) overexpression might contribute to the development of cataracts. *DYRK1A* (OMIM 600855) overexpression could possibly result in mental retardation, whereas the overexpression of *ETS2* (OMIM 164740) may be the cause of leukemia and skeletal abnormalities. In addition, researchers have demonstrated that overexpression of the latter gene results in apoptosis and that transgenic mice overexpressing *ets2* developed a smaller thymus and lymphocyte abnormalities, similar to features observed in Trisomy 21.³⁸ *IFNAR* (OMIM 107450), the gene for expression of interferon, may interfere with the immune system as well as other organ systems when overexpressed. Premature aging and decreased function of the immune system may be caused by the overexpression of *SOD1* (OMIM 147450). Recently, Gardiner³⁹ reviewed data on four Chromosome 21 proteins: NRIP1, GABPA, DYRK1A, and SUMO3 and suggested how perturba-

tion of their dose may be relevant to the etiology of Trisomy 21 phenotypes. Other possible candidates for inducing one or more aspects of pathogenesis include *APP* (OMIM 104760), *GLUR5* (OMIM 138245), *S100B* (OMIM 176990), *TIAM1* (OMIM 600687), *Synaptojanin* (OMIM 604297), *PFKL* (OMIM 171860), and *KCNJ6* (OMIM 600877). It is important to note, however, that despite much progress, no human gene has yet been conclusively linked to causing a specific Trisomy 21 phenotype.

Mouse models to define genotype-phenotype correlation and pathogenesis

Studies of mouse models of DS are a potentially valuable source of information in understanding the etiology of the disorder. Indeed, under the assumption that certain phenotypes can be recreated in the mouse by introducing into their genome a copy of the gene(s) that causes the human phenotype, it should be possible to test the gene-dosage hypothesis and, in so doing, to define dosage relationships that are significant. The advantages inherent to studies in mice include the following: (1) access to a large number of subjects; (2) enhanced access to tissues of interest, including the brain; (3) the ability to use advanced technologies to characterize phenotypes both qualitatively and quantitatively; (4) access to mice engineered to contain one or more defined gene segments or individual genes; (5) control of genetic background; (6) shortened time and cost of screening; (7) use of in vitro and in vivo models to explore pathogenesis; and (8) far easier conduct of studies to explore therapeutic interventions. Taken together, the advantages are considerable. Although not replacing human studies, experiments using mouse models are expected to greatly increase the pace of discovery and accelerate the time when effective treatments will be available for people with DS.

In the mouse genome, orthologs of *HSA21* genes are located with the same synteny and orientation mostly on Chromosome 16 (MMU16) but also on Chromosome 17 (MMU17) and Chromosome 10 (MMU10). A few trisomic mouse lines have been developed so far. Three are trisomic for subregions located on MMU16, including respectively 132 genes from *APP* to *Zfp295* in Ts65Dn, 85 from *Sfrs15* to *Zfp295* in Ts1Cje, and finally for 33 genes on a smaller segment delimited by *Cbr* and *Mx2* (Ts1RhR). The Ts65Dn and Ts1Cje mice present features of patients with Trisomy 21.^{40–43} Trisomic mouse models provide good representation of important aspects of cognitive dysfunction in DS. For instance, a robust learning and memory deficit have been demonstrated in Ts65Dn mice (fear conditioning, Morris water maze, and object recognition). Impairments in these three paradigms have also been observed in models with a smaller number of genes or focusing on a single gene such as the *Dyrk1a* models.

Recently, an example has been provided that speaks to the utility of mouse models to dissect the genetic basis of phenotypes of interest. All patients with DS older than 40 years show the neuropathological hallmarks of AD.⁴⁴ The brains of patients with AD and aged individuals with DS⁴⁵ show large numbers of plaques and tangles, significant brain atrophy, and basal forebrain cholinergic neurons (BFCNs) degeneration.^{46,47} The potential significance of this observation is 2-fold: (1) the two disorders may share an underlying pathogenesis; and (2) it may be possible to know at birth if a person will develop full blown AD-related neuropathology in 40 years. Thus, a promising strategy for understanding pathogenesis, and its emergence over time, focuses on linking increased expression of one or more genes on Chromosome 21 in people with DS to AD-like degen-

eration of specific populations of neurons. BFCN dysfunction and loss contribute to difficulties in attention and memory.⁴⁸ Human studies indicate increased gene dosage for APP because a case study of an elderly woman with DS who died without age-related decline in cognition and without AD-related pathology was shown to harbor a partial trisomy that did not include the gene for APP.⁴⁹ In recent studies, increased APP gene dosage was linked in several families to typical clinical and neuropathological features of AD and cerebral amyloid angiopathy.⁵⁰

BFCNs degenerate in DS and AD.^{51–53} Ts65Dn mice recapitulate a variety of DS morphological changes including synaptic structural abnormalities in territories that receive BFCN projections.^{54,55} Although 6-month-old Ts65Dn mice do not show changes in the size or number of BFCNs in the medial septal nucleus, there is a significant reduction in both parameters by 12 months of age. Intracerebroventricular injection of nerve growth factor (NGF) reversed these degenerative changes in Ts65Dn mice.⁵⁶ To discern the genetic basis for decreased NGF transport and BFCN degeneration, studies in mice harboring a shorter segment were examined, leading to the conclusion that a gene(s) of interest must be present in a rather small segment of perhaps 25 genes. Intriguingly, within this segment was the mouse gene for APP (i.e., *App*). By crossing Ts65Dn mice to mice in which one copy of *App* was deleted, Ts65Dn mice were produced that contained either the normal three copies of *App* (*App*^{+/+/+}) as well as mice that contained only two copies of the gene (*Ts65Dn; App*^{+/-}). NGF transport was markedly increased in *Ts65Dn; App*^{+/-} mice; even more exciting was that BFCN degeneration was not detectable.⁵⁷ These results are evidence that an extra copy of the gene for *App* contributes markedly to decreased NGF transport and to degeneration of BFCNs. The possibility is exciting that by finding treatments to reduce the level of APP gene expression, it may be possible to lessen the severity or even prevent AD in people with DS.

FUTURE

So, what does the future hold? Although the presence of an extra copy of 21 creates a marked change in the genome, the studies cited earlier indicate the possibility of linking specific gene(s) to specific phenotype(s), thus shedding light on the pathogenesis of the disorder and ways to overcome its severe features.

Some of the properties of potential candidate genes have already been used to decrease the level of the corresponding active proteins, as in the case of *Dyrk1a*: polyphenols from green tea have been used to rescue phenotypes observed in a YACtgDyrk1a model: long-term memory assessed by a novel object recognition paradigm is corrected after the treatment.⁵⁸ The findings for APP are also illustrative. If APP gene dosage can be confirmed to induce neurodegenerative phenotypes in mice, it will be important to confirm whether this is the case in people with Trisomy 21. At present, the data to support this are scanty but intriguing. Studies of individuals with partial Trisomy 21 whose genetic lesion does not include APP will be uniquely informative because they will allow us to examine whether the phenotypes that are absent in mice bearing only two copies of APP are also absent in people with Trisomy 21 whose genome harbors only two copies of APP. Experiments to explore in humans APP gene expression in brain and peripheral tissues, status of vulnerable neurons, and variety of Alzheimer-related pathologies should be sufficient to show whether mouse studies are predictive of events in humans. One can readily

envision the battery of tests that would be conducted and the possibility that ongoing studies in mice will suggest additional tests for demonstrating the extent of concordance.

If this can be demonstrated, studies in mice that focus on the mechanism by which a candidate gene dose acts to compromise neuronal function are likely to be informative as will experiments to explore therapeutic options. Among these would be attempts to decrease the level of expression of candidate genes, interfere with the underlying pathogenetic mechanism(s) engaged by increased gene dosage and perhaps other strategies proven effective in mouse models.

Thus, in studies in mice the following seems to be important in mice: (1) vigorous continued study of DS-relevant phenotypes in mouse models of the disorder; (2) as part of this effort, the creation of additional segmental trisomies and specific gene deletions to allow for more rapid genetic dissection of phenotypes; (3) once genes can be shown to be implicated, a vigorous pursuit of underlying mechanisms; (4) the search for therapeutic targets to allow for regulation of expression of important genes and approaches to blunt or prevent the pathogenetic mechanisms created by increased levels of their products. Studies in humans will also be crucial, including the following: (1) the identification of people with partial Trisomy 21; (2) the enrollment of as many of these individuals as possible in phenotype correlation studies; (3) the creation of a battery of tests that allow one to carefully and quantitatively measure phenotypes of greatest interest—e.g., those that define cognitive strengths and weaknesses; (4) accelerated efforts to collect phenotypic data to support or refute phenotype–genotype linkages; (5) whenever possible to test for the presence or absence in humans of mechanisms defined in mouse studies; and (6) robust attempts to develop effective treatments. The latter will almost certainly require: (1) the participation of industry and governmental partners for drug development; and (2) the cooperation of the larger community of people with Trisomy 21 for the clinical trials needed to test new agents.

International collaboration for clinical trials

Regarding improvements of cognitive functions in individuals with DS, clinical trials monitored with high and internationally recognized clinical standards are an urgent need. To achieve this task, physicians have to face several problems. First, noticeable difficulty for conducting such trials is to have access to large cohorts of patients with Trisomy 21. The centers that take care of patients with Trisomy 21 do not always have a sufficient recruitment basis. Second, the psychometric tests used to assess cognitive or behavioral functions are not always adapted to patients with Trisomy 21 because these tests often show a “bottom effect.” To assess cognitive improvement related to new treatments, the development of internationally recognized psychometric tests with no language or cultural influence is a priority. Third, knowing the complexity and intricacies of the biochemical pathways, drugs or nutrients should be carefully chosen or combined because one molecule may be useful for the patient but not when used alone. For these reasons, an international collaboration on clinical trials is needed, which will have to avoid the drawbacks of multicenter trials. The formula for success is far easier to define than to achieve, but recent progress brought the hope that effective treatments can be found and successfully applied to enhance the lives of people with Trisomy 21 and their families. The financial resources needed to make this progress possible cannot be compared to the benefits that will be derived.

CONCLUSION

Fifty years after the discovery of the origin of Trisomy 21, people with this disorder continue to suffer the consequences of an extra copy of Chromosome 21. Compromised well-being and the presence of cardiac disorders and other health problems, including cognitive dysfunction, place these people outside of the mainstream, even in highly advanced cultures. However, recent advances are showing that it may be possible soon to decipher the underlying genetic and molecular bases for their disability and for creating effective treatments. Continued and increasing investments in research on the genetic and molecular basis of Trisomy 21 promise to transform the lives of these individuals and the communities in which they live.

ACKNOWLEDGMENTS

We thank Dr Henri Bléhaut, Celine du Boispeán, and Gwenael Poret for their support.

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