

## Basic Science Advances

# Down Syndrome: Advances in Molecular Biology and the Neurosciences

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**ABSTRACT.** The entire DNA sequence for human chromosome 21 is now complete, and it is predicted to contain only about 225 genes, which is approximately three-fold fewer than the number initially predicted just 10 years ago. Despite this remarkable achievement, very little is known about the mechanism(s) whereby increased gene copy number (gene dosage) results in the characteristic phenotype of Down syndrome. Although many of the phenotypic traits show large individual variation, neuromotor dysfunction and cognitive and language impairment are observed in virtually all individuals. Currently, there are no efficacious biomedical treatments for these central nervous system-associated impairments. To develop novel therapeutic strategies, the effects of gene dosage imbalance need to be understood within the framework of those critical biological events that regulate brain organization and function. *J Dev Behav Pediatr* 22:40–59, 2001. Index terms: Down syndrome, chromosome 21, gene expression, brain development, neurobiology, cognitive impairment, Alzheimer's disease.

One hundred thirty-five years ago, John Langdon Down, an Englishman, published the first clinical description of the condition that now bears his name. While working as superintendent of the Earlswood Asylum for Idiots, he described patients with similar Asiatic or "Mongolian" features in an article titled "Observations on an Ethnic Classification of Idiots."<sup>1</sup> Down's original report attributed the condition to maternal tuberculosis. In 1932, Waardenburg suggested that the syndrome was a consequence of a chromosomal abnormality. In 1959, Lejeune et al<sup>2</sup> confirmed the presence of trisomy 21 in nine infants. The first neuropathologic description of the brain in Down syndrome (DS) was provided by Fraser and Mitchell in 1876.<sup>3</sup> Not only did they remark upon the comparatively simple gyration pattern of the cerebral hemispheres, but they called special attention to the narrowness of the superior temporal and inferior frontal gyri and noted a possible relationship to speech production.<sup>3</sup> Eleven years before Lejeune et al's findings, Jervis<sup>4</sup> described classic neuropathologic stigmata of Alzheimer's disease in three adults with DS aged 35, 42, and 47 years. During the past 50 years, great strides have been made toward understanding and treating the myriad medical conditions associated with DS.<sup>5</sup>

Throughout this century distinct genetic, neurobiologic, metabolic, developmental, and medical models of DS have

evolved, each having its own set of principles, pedagogy, and practices that has produced a "separate definition of reality" among basic scientists, health care practitioners, and parents, as well as an array of treatment options both real and imagined. This review is focused specifically on the central nervous system (CNS) manifestations of trisomy 21, its relevance for early developmental function, and certain aspects of aging.

DS may be understood best as a syndrome complex of genetic and epigenetic origin with protean neurobiologic consequences and several characteristic neurodevelopmental manifestations.<sup>6–8</sup> At present, there are no efficacious, biomedically based treatments for the CNS impairment seen in children with this condition. To advance our understanding of this biologically complex condition, and toward the development of novel therapeutic approaches, clinician-scientists must be able to integrate information from many disparate disciplines, including molecular genetics, developmental biology, and the neurosciences.

### CYTOGENETICS

Down syndrome (DS) is a chromosomal disorder that occurs in approximately 1 in 800 to 1000 live births. DS most often results from complete trisomy of chromosome 21 due to nondisjunction during gamete formation.<sup>2</sup> In approximately 95% of cases of trisomy 21, the nondisjunction is of maternal origin.<sup>9</sup> Such cases of nondisjunction seem to occur "randomly" during meiosis, as the extra

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chromosome is not “inherited,” per se. Rarely, nondisjunction will occur after fertilization is complete, resulting in two different cell lines. This condition is referred to as mosaicism because one trisomic and one euploid cell line exist within the same embryo-fetus. A small number of cases result from either complete or partial translocation of chromosome 21 to another chromosome, usually in the D (13–15) or G (21–22) group. Some forms of translocation DS are associated with a familial pattern of inheritance.<sup>10</sup> Overall, 90% to 95% of cases of DS result from full trisomy 21; 2% to 4% result from translocation; and 2% to 4% are the result of mosaicism.<sup>11,12</sup>

## MOLECULAR GENETICS

Chromosome 21, the smallest human autosome, contains 33.8 million base pairs of DNA and is predicted to contain just 225 genes, many or all of which contribute to the pathogenesis and phenotype of DS.<sup>13</sup> It is acrocentric, with two arms and its centromere close to one end. The short arm (21p) consists of the nucleolar organizer region that contains multiple copies of genes coding for ribosomal RNA and a more proximal region composed of highly repetitive DNA sequences. The genes on 21p do not seem to be essential for normal development, because duplications or deletions in this region usually have few observable phenotypic manifestations. All of the other genes on chromosome 21 map to the long arm (21q). At least 94 known genes are listed as mapping to 21q.<sup>14</sup> Rapid advances in understanding the genes implicated in DS are due, in part, to technologic innovations that have allowed researchers to isolate small, discrete portions of the chromosome or individual genes to determine the DNA sequence and construct detailed chromosome maps. Several different types of maps of chromosome 21 are being developed (see reviews by Antonorakis<sup>16</sup> and Onodera and Patterson<sup>15</sup>).

In an attempt to assign the individual phenotypic features of DS to specific subregions of chromosome 21, investigators have begun constructing phenotypic maps. These are constructed using DNA from individuals with rare partial duplications (i.e., segmental trisomy) of 21q and correlating this cytogenetic information with clinical phenotype. The conceptual bases for phenotypic mapping of aneuploid syndromes have been put forth by Epstein.<sup>17</sup> The most important a priori assumption is that the resulting phenotype of any aneuploid condition results from an abnormal number of gene copies present on the unbalanced chromosome, and that specific components of the phenotype are attributable to an imbalance of specific genes. Thus, in trisomy 21, the phenotype is a direct consequence of a gene dosage imbalance of the genes on 21q, which are present in three copies. The existence of a putative minimal or “critical region” on 21q, which is responsible for most of the physical features of the DS phenotype, has also been suggested.<sup>17,18</sup> Transcription maps of 21q are being constructed in an attempt to identify those DNA sequences that are expressed within a given tissue compartment. A transcript map of a small 1.2-Mb region within the putative critical region (around D21S55) has recently been estab-

lished.<sup>19</sup> This endeavor will serve as a model, as efforts continue to generate gene expression data for the remainder of 21q.

## CNS Gene Expression

It is estimated that 50% to 65% of all genes in the human genome contribute to the development and/or function of the CNS.<sup>20</sup> Accordingly, of the 225 genes predicted to map to the long arm of q21, between 110 and 150 may be expressed in the brain and spinal cord. Theoretically, one extra copy of each of these genes should lead to a 50% increase in messenger RNA (mRNA) and its gene product (protein). The developmental consequences of increased gene dosage depend, in part, on the biological function of the gene product itself (e.g., enzyme, structural protein, transcription factor, intracellular signaling molecule, cell surface marker, receptor subunit, etc.).

At this time, at least 10 genes listed in the OMIM catalog are known to exert an influence on CNS structure or function (Table 1, see names in boldface type). The function of these genes and/or protein products have been partially characterized, and many are being tested to determine their role in the neuropathogenesis of DS.

**Amyloid Precursor Protein.** The gene for amyloid precursor protein (*APP*) maps to q21.3–22.05. Mutations in *APP* are associated with some cases of familial Alzheimer’s disease (AD), as well as one form (Dutch-type) of cerebral amyloid angiopathy.<sup>21</sup> The *APP* gene codes for a large, transmembrane protein expressed in both neurons and astrocytes. Although the function of APP protein is not precisely known, various fragments are associated with the promotion of cell survival, stimulation of neurite outgrowth and synaptogenesis, modulation of synaptic plasticity, regulation of cell adhesion, and neuroprotection against excitotoxic and oxidative insults.<sup>22</sup> *APP* gene expression is regulated during CNS development and has been found to be overexpressed in the brain of at least one fetus with DS.<sup>23</sup> Full-length APP mRNA undergoes alternative splicing to produce at least three different isoforms (695, 751, and 770AA) that can be cleaved at different sites by  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases during normal cellular metabolism (Fig. 1). APP processing seems to be regulated by a heterogeneous assortment of stimuli, including acetylcholine, glutamate receptor activation, synaptic activity, cytokines, and neurotrophic factors. Those stimuli that regulate the production of a secreted form of APP (sAPP) via the  $\alpha$ -secretase pathway seem to be especially important in modulating effects on plasticity, neurite outgrowth, and neuroprotection.<sup>22</sup>

**Superoxide Dismutase.** The gene for superoxide dismutase (*SOD-1*) maps to q22.1. Mutation of *SOD-1* is associated with a familial form of amyotrophic lateral sclerosis (ALS).<sup>24,25</sup> ALS is a progressive degenerative disorder of large motor neurons in the brain and spinal cord. The cascade of events leading to neuronal degeneration is believed to be initiated by harmful byproducts of oxidative reactions within affected cells.<sup>26,27</sup> SOD-1 protein is a cytoplasmic enzyme that catalyzes the dismutation of superoxide radicals ( $O_2^{\cdot-}$ ), a product of normal oxidative

Table 1. Partial List of Genes Mapped to Chromosome 21

Symbol	Location	Name
AABT	chr 21	$\beta$ -amino acids renal transport
ABC8	q22.3	Drosophila homolog of (ABC-binding cassette transporter 8)
ADARB1	q22.3	Adenosine deaminase, RNA-specific, B1
AIRE	q22.3	Autoimmune regulator
AML1	q22	Acute myeloid leukemia oncogene
APECED	q22.3	Autoimmune polyglandular disease, type I
<b>APP</b>	<b>q21.3–22.05</b>	<b>Amyloid-<math>\beta</math> precursor protein</b>
ASNSL2	chr 21	Asparagine synthetase-like 2
ATP5O	q22.1–22.2	ATP synthase, H <sup>+</sup> transporting mitochondrial F1 complex, O subunit
BAS	chr 21	$\beta$ adrenergic response
C21orf2	q22.3	Chromosome 21 open reading frame 2
CAF1A	q22.2	Chromatin assembly factor I, p60 subunit
CBR	q22.12	Carbonyl reductase
CBS	q22.3	Cystathionine- $\beta$ -synthetase
CBFA2	q22.3	Core-binding factor, runt domain, $\alpha$ subunit
CNN2	q11.1	Calonin 2
COLA6A1	q22.3	Collagen, type VI $\alpha$ -1
COLA6A2	q22.3	Collagen, type VI $\alpha$ -2
COLA18A1	q22.3	Collagen, type XVIII $\alpha$ -1
CRFB4	q22.1	Cytokine receptor, family II, member 4
CRYA1	q22.3	Crystallin, $\alpha$ polypeptide 1
<b>CSTB</b>	<b>q22.3</b>	<b>Cystatin B (stefin B)</b>
CTBP2	q21.3	C-terminal binding protein 2
DCR	q22.3	Down syndrome chromosome region
DFNB8	q22.3	Deafness, autosomal recessive 8
<b>DSCAM</b>	<b>q22.2–22.3</b>	<b>Down syndrome cell adhesion molecule</b>
<b>EPM1</b>	<b>q22.3</b>	<b>Epilepsy, progressive myoclonic 1</b>
ERG	q22.3	Avian erythroblastosis virus E26 avian oncogene
ES1	q22.3	ES1 (zebrafish) protein, homolog
ETS2	q22.3	Avian erythroblastosis virus E26 oncogene homolog 2
EZH2	q22.2	Enhancer of zeste (Drosophila) homolog-2
FPDMM	q22.1–22.2	Platelet disorder, familial, with myeloid malignancy
GABPA	q21–q22.1	GA-binding protein transcription factor, $\alpha$ subunit
<b>GART</b>	<b>q22.1</b>	<b>Phosphoribosylglycineamide formyltransferase</b>
<b>GARS</b>	<b>q22.1</b>	<b>Phosphoribosylglycineamide synthetase</b>
<b>AIRS</b>	<b>q22.1</b>	<b>Phosphoribosylglaminoimidazole synthetase</b>
<b>GRIK1</b>	<b>q22</b>	<b>Glutamate receptor, subunit 5</b>
GT355	q22.3	GT335 gene
GPXP2	chr 21	Glutathione peroxidase pseudogene 2
HLCS	q22.1	Halocarboxylase synthetase
HMG14	q22.3	Nonhistone chromosomal protein HMG-14
HPE1	q22.3	Holoprosencephaly-1, alobar
HRMT1L1	q22.3	HMT1 (hnRNP methyltransferase, <i>S. cerevisiae</i> )-like
HSPA3	chr 21	Heat shock 70-kd protein 3
HTOR	chr 21	5-hydroxytryptamine oxygenase regulator
IFNAR	q22.1	Interferon, $\alpha$ and $\beta$ receptor
IFNGT1	q22.1–22.2	Interferon, $\gamma$ receptor 2
ITGB18	q22.3	Integrin $\beta$ -2, antigen CD18, lymphocyte function-associated antigen-1
ITSN	q22.1–22.2	Intersectin
KCNJ6	q22.1–22.2	Potassium inwardly-rectifying channel, subfamily J, 6
KCNJ7	q22.1	Potassium inwardly-rectifying channel, subfamily J, 7
KCNJ15	q22.2	Potassium inwardly-rectifying channel, subfamily J, 15
KCNE1	q22.1–22.2	Potassium voltage-gated channel (Isk-related subfamily) member 1
KCNE2	q22.1	Potassium voltage-gated channel (Isk-related subfamily) member 2
KNO	q22.3	Knobloch syndrome
LSS	q22.3	Lanosterol synthase
MACSL1	chr 21	Myristoylated alanine-rich protein kinase 1 substrate
MAP80	chr 21	Minichromosome maintenance 3-associated protein
<b>MNBH</b>	<b>q22.1</b>	<b>Minibrain (Drosophila) homolog</b>
MST	q11.2	Myeloproliferative syndrome, transient
MX1	q22.3	Myxovirus (influenza) resistance 1
MX2	chr 21	Myxovirus (influenza) resistance 2

Table 1. (Continued)

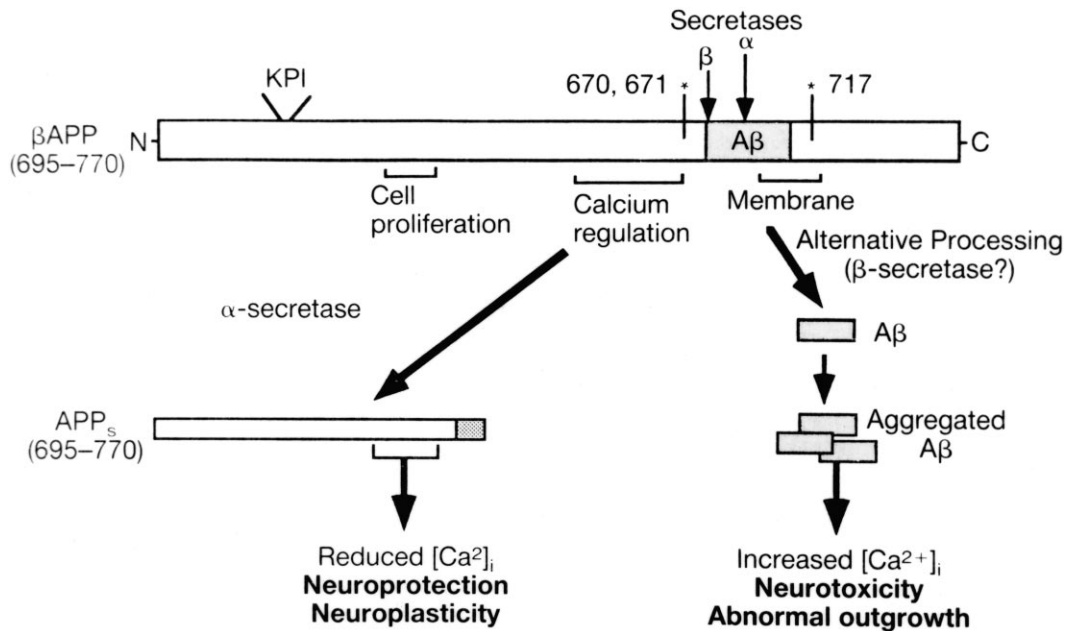
Symbol	Location	Name
NCAM2	q21	Neural cell adhesion molecule 2
NDUFV3	q22.3	NADH-ubiquinone oxidoreductase flavoprotein 3
NRIP1	q11	Nuclear receptor interacting protein
PCNT	q22.3	Pericentrin
<b>PCP4</b>	<b>q22.3</b>	<b>Purkinje cell protein 4</b>
PDE9A	q22.3	Phosphodiesterase 9A
PKFL	q22.3	Phosphofructokinase, liver-type
PKNOX1	q22.3	PBX/knotted 1 homeobox 1
PRSS7	q21	Protease, serine 7
PWP2H	q22.3	PWP2 (periodic tryptophan protein, yeast) homolog
RNR4	p12	Ribosomal RNA-4
S14	chrs 21	Surface antigen (chromosome 21)
<b>S100B</b>	<b>q22.2–22.3</b>	<b>S100 calcium-binding protein, <math>\beta</math> subunit</b>
SH3GBR	q22.3	SH3 domain binding glutamine acid-rich protein
SIM2	q22.2	Single-minded (Drosophila) homolog 2
SLC5A3	q22	Solute carrier family 5 (inositol transporter), member 3
SLC19A1	q22.3	Solute carrier family 19 (folate transporter), member 1
SMT3H1	q22.3	SMT3, yeast, homolog 1
<b>SOD1</b>	<b>q22.1</b>	<b>Cu/Zn superoxide dismutase, soluble</b>
SON	q22.1–22.2	SON DNA-binding protein
STCH	q11.1	Stress 70, protein chaperone, microsome associated
TFF1	q22.3	Trefoil factor 1 (breast cancer, estrogen inducible)
TFF2	q22.3	Trefoil factor 2 (spasmolytic prtein-1)
TFF3	q22.3	Trefoil factor 3 (intestinal)
TIAM1	q22.1	T-cell lymphoma invasion and metastasis 1
TMEM1	q22.3	Transmembrane protein 1
TMPRSS2	q22.3	Transmembrane protease, serine 2
TRPC7	q22.3	Transient receptor potential channel 7
TTC3	q22.2	Tetratricopeptide repeat domain 3
U2AF1	q22.3	U2 (RNU2) small nuclear RNA auxilliary factor 1
UBE2G2	q22.3	Ubiquitin-conjugating enzyme E2G2
USHIE	q21	Usher syndrome 2A
VDAC2	chrs 21	Voltage-dependent anion channel 2
WRB	q22.3	Tryptophan-rich basic protein

metabolism, to produce hydrogen peroxide ( $H_2O_2$ ) and molecular oxygen ( $O_2$ ).<sup>28</sup> SOD-1 activity is present in most tissues, including the brain, in which it is expressed in both neurons and glia.<sup>29</sup> Generally, SOD-1 is regarded as a protective enzyme because it scavenges free superoxide molecules in the cell.<sup>30–32</sup> However, the  $H_2O_2$  generated by SOD-1 action may itself, under certain conditions, become toxic. In the presence of  $Fe^{2+}$ ,  $H_2O_2$  will breakdown and form the highly toxic hydroxyl radical ( $OH\cdot$ ), which can result in profound cellular damage. Excess  $OH\cdot$  causes peroxidation of lipid membranes, as well as direct damage to proteins and DNA molecules. Gene dosage effects for *SOD-1* have been documented in DS. Elevations in SOD-1 activity and increased lipoperoxidation are observed in the brain of those with DS as early as 15 to 25 weeks gestation.<sup>33</sup>

**S100 $\beta$  Protein.** The gene for *S100 $\beta$*  ( $\beta$  subunit) maps to q22.2–22.3. The S100 protein is a 10.5-kD, dimeric, zinc- and calcium-binding protein implicated in signal transduction pathways, which regulate the cell-cycle and neuronal differentiation.<sup>34</sup> *S100 $\beta$*  inhibits the protein kinase C-dependent phosphorylation of several proteins, including the tumor suppressor p53.<sup>35</sup> Levels of S100 are especially high in CNS tissue. In the brain, the  $\beta$ -subunit is found

primarily in astroglial cytoplasm, although large amounts are also secreted extracellularly. *S100 $\beta$*  also acts as a mitogen, which stimulates glial proliferation, and it possesses neurotrophic properties on serotonergic neurons.<sup>36,37</sup> *S100* is detectable in human brain by approximately 10 weeks gestation, and levels increase in specific regions concomitant with advancing maturation.<sup>38</sup> *S100 $\beta$*  mRNA and protein expression increase dramatically during postnatal maturation of the cerebellum, and gene dosage effects have been documented in the brains of those with DS.<sup>39</sup>

**Glutamate Receptor Subunit 5.** The gene coding for glutamate receptor subunit 5 (*GluR5*) maps to q22. Glutamate is one of the most abundant and important excitatory neurotransmitters in the brain. An optimal amount of glutamate activity is necessary to mediate dendritic outgrowth and synaptogenesis during development.<sup>40,41</sup> Underactivation of glutamate receptors may result in delayed maturation or disruption of neural differentiation, whereas overactivity can produce neuronal damage.<sup>42,43</sup> The diversity of effects generated by the activation of glutamate receptors may be attributable to multiple receptor subtypes.<sup>44</sup> The *GluR5* subunit forms a critical component of the ionotropic kainate (KA)-preferring glutamate receptor.<sup>45</sup> Although the functional role of KA-preferring receptors in



**FIGURE 1.** Structure of amyloid precursor protein (APP). The functional domains, sites of mutations, and alternative processing pathways that influence neuronal survival are indicated. APP is a transmembrane glycoprotein that exists in forms lacking a Kunitz protease inhibitor (KPI) domain near the aminoterminal (extracellular) ( $APP_{695}$  and  $APP_{714}$ ) or containing it ( $APP_{751}$  and  $APP_{770}$ ). Enzymatic processing of APP by secretases ( $\alpha$  and  $\beta$ ) liberates secreted forms ( $APP_s$ ) from the cell.  $APP_s$  contains several functional domains including the KPI region, a region that stimulates cell proliferation, and a region involved in  $Ca^{2+}$  regulation and neuroprotection. Alternative processing of APP can liberate  $\beta$ -amyloid peptide ( $A\beta$ ), which can form potentially neurotoxic aggregates that can destabilize  $Ca^{2+}$  homeostasis and increase neuronal vulnerability to excitotoxicity. (\*Sites of mutations in inherited forms of Alzheimer's disease.) (Reprinted from *Trends in Neurosciences*, vol. 16, Mattson MP, Barger SW, Cheng B, Lieberburg I, Smith-Swintosky VL, and Rydel RE:  $\beta$ -amyloid precursor protein metabolites and loss of neuronal  $Ca^{2+}$  homeostasis in Alzheimer's disease, pp 409–414, 1993, with permission from Elsevier Science.)

vivo is incompletely understood, overactivation may lead to seizure activity and neurotoxicity.<sup>46</sup> Heteromeric complexes composed of KA-sensitive receptor subunits (GluR5, GluR6, GluR7, KA1, and KA2) form calcium-conducting channels under certain conditions. Calcium permeability is determined by RNA editing of the transmembrane segment of the GluR5 and GluR6 subunits, which comprise most KA-preferring receptors.<sup>47</sup> In rodents, *GluR5* mRNA editing results in a sharp reduction in calcium conductance through KA channels. A temporal analysis of *GluR5* mRNA editing shows it to be up-regulated in the cortex during fetal and early postnatal life.<sup>48</sup> *GluR5* expression is most robust in cortical layers II, III, and IV, and expression peaks during the period of greatest developmental plasticity in the somatosensory cortex.<sup>49</sup> In adult primates, a monoclonal antibody recognizing GluR5/6/7 KA receptor subunits shows labeling of neurons throughout the neocortex.<sup>50</sup> Postsynaptic densities located on dendritic shafts and spines showed the most intense staining, particularly those associated with pyramidal cells in cortical layers II, III, and V. There are no studies documenting a gene dosage effect for *GluR5* in the brain of persons with DS.

**Single-Minded Gene.** One of two human homologs of the *Drosophila* single-minded (*SIM*) gene, *SIM2*, maps to q22.2. *SIM2* codes for a basic helix-loop-helix nuclear protein with putative transcriptional repressor activity.<sup>51</sup> In *Drosophila*, *SIM2* is expressed in the precursor cells of the CNS midline, where it is required for synchronized cell division and establishment of proper cell lineage.<sup>52</sup> It is

believed to function as a master-regulator of CNS midline development. In fetal rats, *SIM2* expression is high in the neuroepithelium of the cerebral vesicle,<sup>53</sup> whereas in fetal mice, expression is highest in the hypothalamus, ventral diencephalon, and brachial arches.<sup>54</sup> In the human brain, *SIM2* expression has been detected in the germinal matrix of the developing cerebral cortex.<sup>55</sup> *SIM2* has been excluded as a candidate gene for a genetic form of holoprosencephaly caused by *HPE1*, which maps nearby within q22.3.<sup>55</sup>

**Minibrain Gene.** The human homolog of the *Drosophila* minibrain (*MNB*) gene maps to q22.1. *MNB* codes for a novel type of serine-threonine protein kinase. In *Drosophila*, *MNB* is expressed during neuroblast proliferation and is believed to be important in regulating cell-cycle kinetics during cell division.<sup>56</sup> This dual-specificity tyrosine phosphorylation-regulated kinase is also expressed in human fetal brain as well as in nonneuronal tissues.<sup>57</sup> In the adult mouse brain, mRNA expression is localized to the olfactory and cerebral cortices, pyriform cortex, pyramidal cells of the hippocampus, cerebellum, and hypothalamus. Transgenic mouse models suggest that overexpression of *MNB* results in impaired learning and memory function.<sup>58</sup> Its putative role in the regulation of neuronal proliferation and cell division in humans awaits further study.

**Cystatin B.** The gene coding for Cystatin B (*CSTB*) maps to q22.3. Mutation of *CSTB* is the cause of an inherited

form of progressive myoclonic epilepsy (EPM1).<sup>59</sup> *CSTB* codes for the cysteine protease inhibitor Cystatin B, which inactivates proteases such as cathepsins L, H, and B, which tend to “leak out” of lysosomes, potentially exposing the nerve cell to autoproteolysis.<sup>60,61</sup> This seems to represent a novel mechanism of epileptogenesis, and its role in the neuropathogenesis of myoclonic epilepsy and DS remains to be established.

*GARS-AIRS-GART.* The human gene coding for the trifunctional protein glycinamide ribonucleotide synthetase (*GARS*), aminoimidazole ribonucleotide synthetase (*AIRS*), and glycinamide ribonucleotide formyltransferase (*GART*) maps to q22.1. The GARS-AIRS-GART protein complex catalyzes the second, third, and fifth steps, respectively, in de novo purine synthesis.<sup>62</sup> Purines are essential for nucleic acid synthesis and energy metabolism.<sup>63</sup> Interestingly, levels of uric acid, xanthine, and hypoxanthine, the end-products of purine catabolism, are increased in those with DS.<sup>64,65</sup> The GARS-AIRS-GART complex is normally expressed during prenatal development of the human cerebellum, but in the brain of those with DS, overexpression continues for several months postnatally.<sup>66</sup>

*Purkinje Cell Protein.* The gene for Purkinje cell protein (*PCP4*) maps to q22.2–22.3. *PCP4* codes for the peptide PEP-19, which is found exclusively in the brain.<sup>67</sup> *PCP4* gene expression is developmentally regulated,<sup>68</sup> and PEP-19 is most abundant in cerebellum, where it localizes to the cell body, axon, and dendrites of Purkinje cells.<sup>69</sup> The function of PEP-19 is currently unknown.

*DS Cell-Adhesion Molecule.* The DS cell-adhesion molecule (*DSCAM*) gene maps to q22.2–22.3. *DSCAM* is expressed in all regions of the brain and in cells of neural crest origin.<sup>70</sup> The *DSCAM* protein is believed to be a member of the immunoglobulin superfamily involved in axonal outgrowth during development of the nervous system.<sup>71</sup> Its role in the neuropathogenesis of DS is unknown.

## NEUROBIOLOGY

In DS, as in other trisomic conditions, the developmental expression of normal genes present in triplicate results in altered patterns of development, histoanatomic, and/or physiologic function.<sup>72</sup> Our understanding of how an extra copy of chromosome 21 leads to the development of microcephaly, cognitive and language impairment, and neuromotor dysfunction (hypotonia, diminished reflexes, and motor delays) remains poorly understood. Ultimately, the effects of gene dosage imbalance must be understood within the framework of those critical developmental events which regulate brain organization and function.<sup>73</sup> Accordingly, the impact of gene overexpression on the cellular, molecular, and biochemical processes which regulate neuronal proliferation, migration, differentiation, and organization will continue to be an area of active research in the future.

### Developmental Neuropathology

The neurobiologic sequelae of trisomy 21 include a variety of anatomic, histologic, ultrastructural, and bio-

chemical alterations. No single finding, however, is pathognomic for DS, because many of these alterations are seen in the brains of individuals with other mental retardation/major congenital anomaly syndromes. Furthermore, not every individual with DS necessarily manifests each of these changes to the same degree. The brain of persons with DS is said to have a characteristic morphologic appearance, which permits it to be easily identified at autopsy. Decreased size and weight with foreshortening of the anterior-posterior diameter, reduced frontal lobe volume, flattening of the occiput, and a narrow superior temporal gyrus are characteristic. Primary cortical gyri may appear wide, whereas secondary gyri are often poorly developed or absent with shallow sulci.<sup>74</sup> The cerebellum and brain stem are frequently noted to be markedly reduced in size compared with forebrain structures.<sup>75</sup> During the first half of gestation, brain morphology does not seem to be markedly different in fetuses with DS.<sup>76</sup> Overall, brain growth may appear normal up to 5 or 6 months postnatal before decelerating later in the first year of life.<sup>77</sup> During this period, the growth of dendritic arbors, which normally results in expansion of the neuropil and increased head circumference, begins to go awry.

Detailed examination at autopsy indicates that the critical periods of brain development primarily affected by trisomy 21 include neuronal proliferation, differentiation, and organization. Myelination is somewhat more variable. Generalized hypocellularity of the brain seems to be due to both primary reduction in neuron proliferation and increased neuronal apoptosis early in fetal development.<sup>78–80</sup> Reduction in neuron number and density have been demonstrated for most brain regions examined.<sup>81</sup> In the cerebral cortex, there is neuronal reduction in all cortical layers, with a striking paucity of small interneurons from layers II and IV and layer III pyramidal neurons.<sup>81,82</sup> Interneurons use the neurotransmitter  $\gamma$ -aminobutyric acid and provide the primary inhibitory influence onto the pyramidal cells of the cerebral cortex. Additionally, interneurons are believed to perform a critical role in the higher-order information processing capabilities of the cerebral cortex vis-à-vis local intracortical circuits.<sup>83</sup> Pyramidal neurons, which provide the major cortical efferents to subcortical and corticocortical pathways, are excitatory and use the amino acid glutamate as a primary neurotransmitter. Depletion of cortical interneurons, as well as other factors which disrupt the delicate balance between excitation and inhibition within cortical circuits, may also explain the co-occurrence of seizures in individuals with DS.<sup>84</sup> The magnitude of reduction in small interneurons and pyramidal neurons undoubtedly differs among individuals with DS<sup>7</sup> and may partially explain the variability of cognitive and neurodevelopmental impairments observed.

Ultrastructural studies of cortical pyramidal neurons reveal abnormalities within dendritic arbors and a reduced number of postsynaptic spines.<sup>85–87</sup> Surviving spines may be abnormally long, thin, or irregular in contour and appearance.<sup>88</sup> Dendritic spine density within layer V seems to develop at a rate close to that of controls for the first few months of life before showing a dramatic decline, whereas

synaptic density within layer III seems to be reduced at birth (Fig. 2A).<sup>89</sup> Reductions in synaptic density and surface area are also present postnatally.<sup>81</sup> These reductions, along with accompanying alterations in spine morphology, almost certainly result in dysfunctional synaptic transmission in the cerebral cortex, which further contributes to the cognitive and neurodevelopmental impairments observed in children with DS. There is also evidence to suggest that unlike controls, the pattern of dendritic connections actually becomes less complex during the first 5 years of life (Fig. 2B).<sup>87</sup> Such findings indicate a dysregulation of those mechanisms mediating regressive events in the developing brain of young children with DS.<sup>90</sup>

Delays in myelination are sometimes observed in DS.<sup>91</sup> During the first year of life, decreased white matter formation is observed throughout the cerebral hemispheres, basal ganglia, cerebellum, and brain stem. This pattern is interesting because it identifies structures known to regulate muscle tone and neuromotor function. Because muscle tone generally improves with age, it is tempting to hypothesize that these improvements are in part the result of "catch-up" myelination and subsequent neuromaturation. Beyond the first 2 to 3 years of life, myelination delay affects primarily those fiber tracts with a late beginning and slow myelination cycle, especially intercortical and U-fibers of the frontal and temporal cortices.

### Neuroimaging

Magnetic resonance imaging (MRI) has allowed researchers to document volumetric relationships among various structures within the brains of children with well-defined cognitive and behavioral syndromes. This approach offers the opportunity to understand which brain structures are implicated in specific neurobehavioral conditions and may also yield insight into the question of individual variation among persons with the same neurogenetic syndrome.

Several MRI studies have demonstrated volume reduction for whole brain, cerebral cortex, white matter, and cerebellum in DS.<sup>92-94</sup> Specifically, the frontal cortex, uncus, amygdala, hippocampus, and parahippocampal gyrus all show dramatic reductions in size, compared with subcortical structures, including regions of the thalamus, putamen, and globus pallidus, which seem nearly normal in size. Some of these imaging studies have tended to focus on patterns of morphologic difference between Down and William syndromes (WS). Two MRI studies have highlighted frontal lobe involvement in DS. Wang et al<sup>95</sup> described decreased width of the rostral-most portion of the corpus callosum and increased bend angle when comparing typical children and children with WS. However, no mention of morphologic variation or volume reduction of specific cortical gyri with correlation among cognitive and behavioral function has ever been reported in children with DS.

### Oxidative Stress and CNS Development

Evidence suggesting that increased oxidative stress is related to the early cognitive manifestations of DS has been

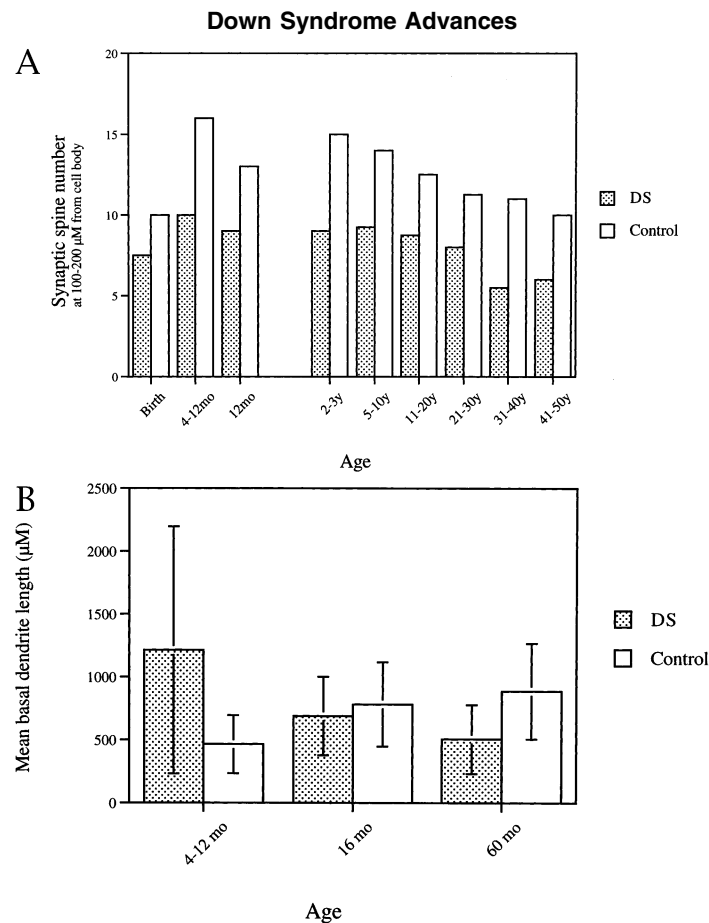
argued for more than two decades. Sinet et al<sup>96</sup> reported a highly positive correlation between erythrocyte glutathione peroxidase (GSHPx) activity and IQ in children with DS as indicative of the impact of oxidative status on cerebral function. Brooksbank and Balazs<sup>33</sup> demonstrated increased SOD1 activity (60%) as well as increased lipid peroxidation (36%) in the cerebral cortex of fetuses with DS occurring as early as the 25th week of gestation.<sup>33</sup> Recent evidence has also emerged showing that fetal DS neurons exhibit a three- to four-fold increase in reactive oxygen species (ROS) and elevated levels of lipid peroxidation *in vitro* before the onset of degeneration and cell death, which differs significantly from control neurons.<sup>97</sup> Pretreatment of cultures with a variety of antioxidant compounds enhanced neuronal viability and in some cases inhibited the generation of ROS and subsequent lipid peroxidation. This finding is consistent with the hypothesis that oxidative stress, present during fetal and early postnatal development, could exert a critical influence on the differentiation and survival of neurons in the brains of individuals with DS. However, this hypothesis has not been tested *in vivo* and may prove a difficult question to answer given the practical constraints of conducting such research. Measures of oxidative damage in living subjects with DS during the early years of postnatal brain development demonstrate that biomarkers of both lipid and DNA oxidation are increased in young children with DS compared with their siblings.<sup>98</sup>

The effects of chronically increased membrane lipid peroxidation on synaptic and cellular function may be particularly important for understanding how neurocognitive impairment evolves over time in DS. Lipid membrane peroxidation negatively affects ion homeostasis by modifying membrane transporters and ion channels, especially Na<sup>+</sup>/K<sup>+</sup>-ATPase and the glucose- and glutamate-transporters (Fig. 3). Disturbances in Ca<sup>+2</sup> homeostasis may also result from alterations in endoplasmic reticulum and mitochondria, two organelles critical for intracellular Ca<sup>+2</sup> sequestration and signaling (see the review by Mattson<sup>99</sup>). Additional studies that support the negative effects of oxidative stress on neuronal function in DS derive from work using the Ts16 mouse model.<sup>100-102</sup>

Certain species of reactive oxygen also serve as important signaling molecules under normal physiologic conditions.<sup>103</sup> At least two gene transcription factors, nuclear factor (NF)-*κ*B and activator protein (AP)-1, are regulated by intracellular redox state.<sup>104</sup> Thus, in DS tissue, the resultant pattern and timing of gene expression in cells of neuronal and glial lineage may not be determined by gene dosage considerations alone and could result in complex gene-gene interactions, with consequences for brain development and function that are difficult to predict.<sup>105</sup>

### Neurodevelopmental Function

*Cognition.* To discuss the issues of cognitive decline, developmental plateauing, or the cognitive benefits of early intervention programs is to touch the "third rail" of a highly charged topic. Delays in early language-based and performance-based cognitive milestones are most often

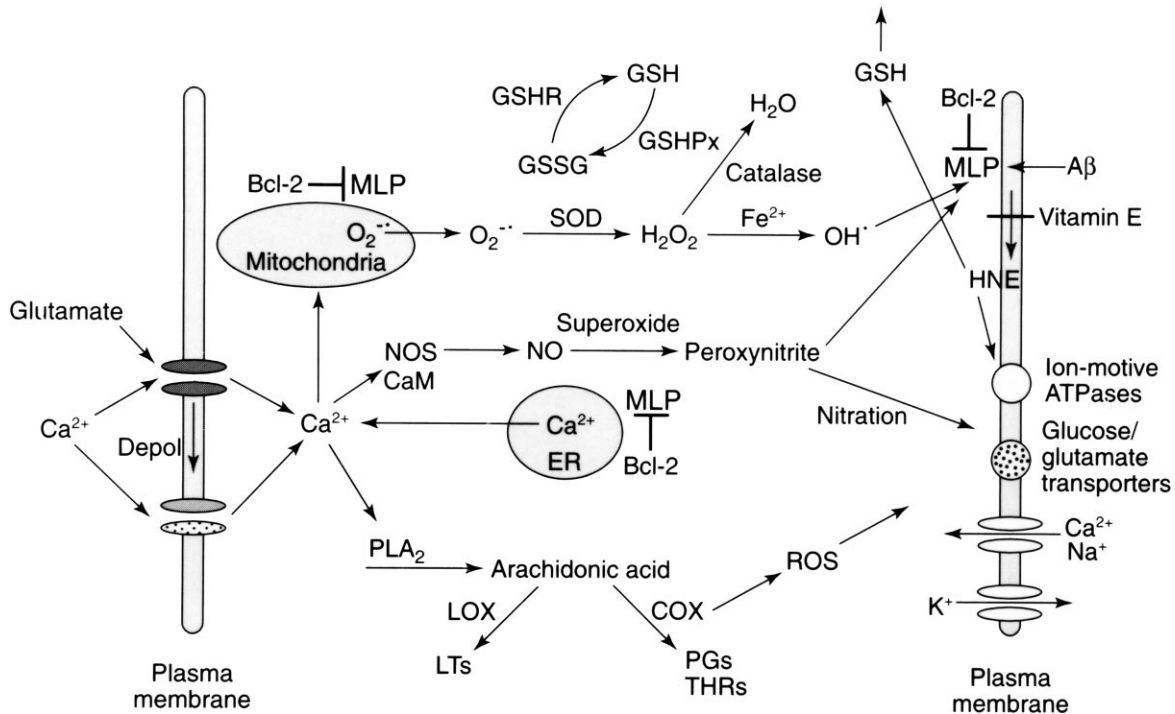


**FIGURE 2.** (A) Development and aging of dendritic spines on basal dendrites of layer III pyramidal neuron, from visual cortex of DS and controls subjects. Note the relationship between (mean) spine density and age. The peak spine density in DS is only approximately 60% to 70% of that of controls early in life. By the third decade, reductions in density approach 50%. (Data from Takashima et al.<sup>89</sup>) (B) The mean length ( $\mu\text{M}$ ) of basal dendrites of layer III pyramidal neurons from visual cortex of DS and controls subjects during the first 5 years of life. Differences are significant at 4 to 12 months ( $p < .01$ ) and 5 years ( $p < .001$ ). Values for maximum branching, number of dendritic intersections, and number of dendritic branches are also significantly different in the brain of subjects with DS at these ages (not shown). (Data from Becker et al.<sup>87</sup>)

evident toward the end of the first year of life. As expectations for language and cognitive growth increase with age, these delays usually become apparent to both parents and professionals alike. Numerous studies have demonstrated certain trends that seem to be characteristic of cognitive development in children with DS. Several investigators report a nonlinear rate of cognitive growth during the first decade of life.<sup>8,106,107</sup> These investigators use declining developmental quotients (DQ) or intellectual quotients (IQ) to argue for a slowing in cognitive growth over time. Some of these same studies report a plateau in the rate of cognitive development during the first decade. At least one study found no evidence of decline in either DQ or IQ during the first 3 years of life.<sup>108</sup> Proposed sources of individual cognitive variation may include, but are not limited to, genetic and neurobiologic factors, associated medical conditions, and social and educational opportunities. Variables noted to be negatively associated with cognitive function include the presence of severe congenital heart disease or hypotonia,<sup>109</sup> seizures,<sup>110</sup> and severe sensory impairments.<sup>111</sup> The factor which correlates positively with enhanced cognition is mosaicism for

trisomy 21.<sup>112</sup> Additional factors which are reported to positively influence cognitive outcome, but which have been insufficiently demonstrated in well-designed, controlled studies, include the effect of early intervention programs<sup>113,114</sup> and the parents' level of education or socioeconomic status.<sup>115,116</sup>

Several recent reviews of the effectiveness of early intervention in DS have concluded that despite benefits in social adaptive function, any effects on cognition (as measured by IQ) are short-lived.<sup>117,118</sup> Given the current availability of early-intervention programs, it would be difficult (although not impossible) to design clinical trials to test hypotheses regarding the best methods and/or frequency of specific types of intervention. For the foreseeable future, developmentally based intervention programs will continue to provide social enrichment and educational opportunities to young children and their parents. However, the fact remains that despite widespread participation in such programs, significant numbers of children with DS will function in the moderately retarded (40%–60%) to severely retarded (30%) range of mental retardation during the first decades of life.<sup>119,120</sup>



**FIGURE 3.** Pathways involved in the induction of membrane lipid peroxidation (MLP) and the mechanisms whereby MLP leads to alterations in ion homeostasis and energy metabolism. The preeminent source of oxyradicals is mitochondria, wherein  $O_2^-$  is generated by the electron-transport process (top). Superoxide dismutases (SODs) convert  $O_2^-$  to  $H_2O_2$ , which, in the presence of  $Fe^{2+}$ , generates  $OH^\cdot$ ;  $O_2^-$  can also interact with nitric oxide (NO) to form peroxynitrite (middle). Both  $OH^\cdot$  and peroxynitrite induce MLP, which can occur in the plasma membrane, mitochondrial membranes, and endoplasmic reticulum (ER) membranes. Additionally, exogenous agents such as amyloid  $\beta$ -peptide ( $A\beta$ ) can induce MLP. MLP liberates 4-hydroxynonenal (HNE), which binds to membrane transporters and ion channels and thereby alters their activities (right side). Impairment of the  $Na^+/K^+$ -ATPase, glucose transporter, and glutamate transporters results in membrane depolarization and excessive activation of glutamate receptors, resulting in excitotoxicity (left side). MLP also perturbs ion homeostasis in ER and mitochondria and thereby comprises their important  $Ca^{2+}$  sequestration function. The antiapoptotic gene Bcl-2 might act in part by suppressing MLP in plasma, mitochondrial, and ER membranes. It should be noted that not only does MLP lead to an elevation of  $(Ca^{2+})_i$ , but conversely, elevation of  $(Ca^{2+})_i$  promotes MLP by inducing NO and  $O_2^-$  production and by activation of phospholipases, resulting in production of arachidonic acid (bottom). This is then acted upon by cyclooxygenases (COX) and lipoxygenases (LOX) with resultant generation of reactive oxygen species (ROS). (CaM,  $Ca^{2+}$ /calmodulin kinase; Depol, depolarization; GSH, glutathione; GSHPx, glutathione peroxidase; GSHR, glutathione reductase; GSSG, glutathione disulphide; LTs, leukotrienes; NOS, nitric oxide synthase; PGs, prostaglandins; PLA, phospholipase  $A_2$ ; THR, thromboxanes.) (Reprinted from *Trends in Neurosciences*, vol. 20, Mattson M: Modification of ion homeostasis by lipid peroxidation: Roles in neuronal degeneration and adaptive plasticity, pp 53–57, 1998, with permission from Elsevier Science.)

It is likely that additional cognitive benefits can be gained by safely and effectively enhancing the brain's neurochemical capacity to learn and retain new information. Advancing neurocognitive function beyond the limits imposed on the brain as a consequence of its ontogenetic heritage will require new therapeutic strategies. Given what is known about excitatory neurotransmission and its role in learning, memory, and neuronal plasticity,<sup>121,122</sup> pharmacologic interventions that act directly on the biochemical and functional substrate of developing excitatory and cholinergic synapses will be required to further advance neurocognitive potential for young children with this condition.<sup>123</sup>

**Language.** Most, but not all, children with DS will learn to speak. Several studies have documented large individual differences in the age of onset and complexity of spoken language in children with DS.<sup>124</sup> As a group, children with DS demonstrate greater deficits in verbal-linguistic skills relative to visual-spatial skills.<sup>125,126</sup> Asynchrony of language development has been well documented in this

population.<sup>127</sup> Language production skills often lag behind language comprehension skills. Some of the factors that have been postulated to impact specifically verbal-linguistic skills in children with DS include hearing loss, altered auditory perception, family and environmental variables, and specific neurobiologic impairments which impact language-based learning.<sup>128</sup> One study found a significant correlation between enhanced language production at 3 years of age and higher cognitive level, less severe hypotonia, and gender, and females generally performed better than males.<sup>129</sup> Remarkably, there is a dearth of data demonstrating the effects of early speech-language intervention for children with DS.<sup>117</sup>

There is substantial literature dealing with certain aspects of linguistic competency in DS. For example, most children will demonstrate especially poor auditory sequential memory skills.<sup>130</sup> Relative to auditory short-term (working) memory, they remain significantly more adept at visual-spatial-based short-term memory tasks. Varnhagen et al<sup>131</sup>

suggest that those with DS fail auditory memory tasks because they have a deficiency in retrieval and short-term storage of lexical information. Surprisingly little research has focused on the neurobiologic substrate of auditory-based receptive language impairment in this condition. A notable exception has been the use of electrophysiologic methods, which are instructive in discerning a unique profile of auditory processing. Contrary to findings of right-ear advantage for dichotic-listening tasks in the general population, a left-ear advantage for auditory stimuli has been reported in those with DS.<sup>132,133</sup> Interestingly, those subjects with DS who had the most severe language impairments demonstrated the most atypical ear advantage.<sup>134</sup> Additional support for auditory-processing difficulties comes from a recent study, which reported an abnormal pattern of left-right ear brain stem auditory-evoked responses in DS children and adults compared with mentally retarded control subjects.<sup>135</sup>

A quasineurologic model of atypical cerebral specialization has been proposed to explain the discrepancy between language comprehension and speech expression skills.<sup>136–138</sup> This model proposes that a functional disassociation exists between right hemispheric systems subserving auditory perception and those left hemispheric systems associated with movement production, including speech sounds. It predicts that a breakdown in communication results from a “partial loss” of linguistic information secondary to the “separation” of speech perception and movement production systems.

Given the central role of the temporal and frontal lobes in speech-sound processing and production and their known involvement in the neuropathogenesis of DS,<sup>95,139</sup> it is anticipated that a more complete understanding of neuro-linguistic dysfunction in this condition will soon emerge. Functional neuroimaging combined with electrophysiologic methods should prove highly informative in this regard.

### Neuropathology and Aging

Several important age-related changes in the brain have been described in association with DS. Calcification of the basal ganglia (BGC) has been described in several reports.<sup>140,141</sup> Typically, the globus pallidus and putamen are affected. Massive calcium deposition within brain parenchyma is frequently observed in those who die during the first decade of life. Those surviving beyond this period and into the fourth decade often show perivascular calcium deposition in or around blood vessel walls themselves. Using computerized tomography (CT), Wisnewski et al<sup>140</sup> demonstrated that 27% of individuals with DS of various ages showed BGC. None of these individuals had any clinical evidence of movement disorder, dysfunction of serum calcium homeostasis, parathyroid hormone secretion, or vitamin D regulation. In contrast, 100% of the brains of those with DS showed histopathologic evidence of BGC at postmortem examination. Interestingly, only 11% of these autopsied brains showed evidence of BGC when evaluated by CT scan before autopsy. Thus, BGC seems to be a nearly universal finding in all individuals with DS, the pathogenesis and clinical significance of which remain obscure.

Neuropathologic stigmata of Alzheimer’s disease (AD) is also a consistent finding in DS. All older persons (>35–40 yr) with DS develop senile plaques (SP), neurofibrillary tangles (NFT), and granulovacuolar bodies (GVB), which is virtually identical with the AD pathology seen in the general population.<sup>142–144</sup> SPs consist of extracellular deposits of  $\beta$ -amyloid peptide (AB) and the remnants of degenerating neuronal cell bodies. NFTs are composed of the hyperphosphorylated cytoskeletal protein *tau*, which forms an intracellular precipitate with a characteristic paired-helical configuration. GVBs consist of clear areas of vacuolization containing clusters of dense, granular material within the neuronal perikaryon. AD-type neuropathologic changes are most pronounced throughout the cerebral cortex and limbic structures.<sup>145</sup> Autopsy studies have convincingly demonstrated that SPs and NFTs are present in all individuals with DS by the fourth decade of life, with some individuals showing a much earlier onset.<sup>146–149</sup> The exact mechanisms by which neurons ultimately die in the brain of aging subjects with DS remain unknown, although it may involve an apoptotic mechanism.<sup>150</sup>

In addition to histopathologic similarities, a similar pattern of neurochemical deficits is observed in aged individuals with DS. Presynaptic markers for cholinergic, noradrenergic, and serotonergic markers are all reduced in the brains of aged individuals with DS.<sup>151,152</sup> These neurochemical changes seem to be caused by degeneration and cell loss of the cortical projection neurons arising from the nucleus basalis of Meynert (cholinergic), locus ceruleus (noradrenergic), and dorsal raphe nuclei (serotonergic). Progressive degeneration and loss of neurons from these subcortical nuclei are associated with the appearance of SPs and NFTs in the cerebral cortex and hippocampus.<sup>153</sup> Degenerative changes and cell loss in association with Lewy body pathology in the substantia nigra and ventral tegmental area (dopaminergic) may also be present.<sup>154</sup> Pharmacologic interventions, designed to enhance cholinergic neurotransmission in the brain, seem to hold some promise for enhancing communication and adaptive behavior in young adults with DS.<sup>155</sup>

### Neuroimaging

MRI has permitted researchers to document changes in the relationship of various brain structures associated with aging in the general population and in DS. It would be particularly helpful to know which brain regions are most vulnerable to atrophy as AD pathology progresses, as well as whether such changes are associated with specific cognitive or neuropsychiatric symptoms of the disease. In a study of healthy, nondemented adults with DS, Kesslak et al<sup>156</sup> reported enlargement of the parahippocampal gyrus with reduction in the hippocampus and neocortex compared with normal controls. In a similar study, Raz et al<sup>157</sup> found evidence of shrinkage in both cerebral and cerebellar hemispheres, ventral pons, mamillary bodies, and hippocampus compared with normal controls. The dorsolateral prefrontal cortex, anterior cingulate gyrus, and inferior temporal and parietal cortices were also affected. Similar to

the findings of Kesslak et al, they also noted a larger parahippocampal gyrus that was negatively correlated with measures of general intelligence. More recently, Pearlson et al<sup>158</sup> compared elderly subjects with DS with and without dementia and found more generalized atrophy (for age), mesial temporal shrinkage, and third ventricular enlargement in subjects with clinical dementia. In a companion study, Aylward et al<sup>159</sup> determined that hippocampal volume, although disproportionately small for brain size, remains fairly constant throughout the fifth decade in those without dementia. Subjects older than 50 years with dementia showed further volume reductions in hippocampus and marked volume reduction in amygdala that exceeded reductions in total brain volume. In the near future, quantitative MRI measures may prove useful as outcome measures in clinical trials designed to prevent or ameliorate AD progression in individuals with DS.

### Oxidative Stress and AD Neuropathology

Buoyed by evidence suggesting a role for oxidative injury in the progression of AD, the contribution of oxidative stress in the manifestation of age-related changes in the brain of subjects with DS is beginning to attract more research interest.<sup>160,161</sup> Several lines of evidence are now converging to paint a dynamic picture of the putative mechanisms involved: (1) increased lipid peroxidation in the brain of subjects with AD, particularly in those regions in which the neuropathologic lesions are most severe<sup>162-165</sup> and increased 4-hydroxynonenal, an aldehyde product of advanced lipid peroxidation,<sup>166</sup> with resultant disturbances in ion homeostasis<sup>99</sup>; (2) increased protein and DNA oxidation in the brain of subjects with AD<sup>167-171</sup>; (3) studies showing that A $\beta$  peptide is capable of generating free radical damage and neuronal death<sup>172-176</sup>; and (4) diminishing mitochondrial function and increased susceptibility to excitatory amino-acid induced cell death in the brain of subjects with AD.<sup>177-181</sup>

Direct evidence for the role of oxidative injury and its relationship to the AD pathology in DS has proved elusive. In one autopsy study, neither malondialdehyde, a marker of lipid peroxidation, nor glutathione peroxidase, which catalyzes the breakdown of hydrogen peroxide, was significantly altered in the brain samples of adults with either AD or DS.<sup>182</sup> In a companion study, levels of 8-OHdG, an indicator of oxidative DNA damage, was also not increased in nuclear DNA isolated from brain samples of adults with either AD or DS.<sup>183</sup> Compared with nuclear DNA, mitochondrial DNA (mtDNA) is 10 times more vulnerable to oxidative damage in vivo. One recent study documented defective repair of oxidative mtDNA damage in DS fibroblasts compared with controls.<sup>184</sup> Several proteins necessary for nucleotide excision repair have been studied in brain samples from adult individuals with DS or AD and compared with control subjects.<sup>185</sup> The expression of excision repair-cross-complementing proteins 80 and 89 were consistently higher in frontal and temporal cortex samples from DS subjects and in all brain regions studied from AD subjects. Such findings are consistent with increased oxidative DNA damage in vivo and suggest that chronic oxidative injury constitutes a risk factor for subsequent neuronal death in aged individuals with DS.<sup>97,186</sup>

### Chromosome 21, Inflammatory Mechanisms, and Alzheimer's Neuropathology

*APP and A $\beta$ .* The amyloid-cascade hypothesis currently offers one coherent picture of disease progression in DS.<sup>187</sup> It is hypothesized that the overexpression of *APP* leading to increased A $\beta$  formation (A $\beta$ 40/A $\beta$ 42) is the central event leading to AD-type pathology in DS. Recently, an amended model of selective neuronal vulnerability and A $\beta$  deposition based on synaptic remodeling and neurite repair mechanisms has also been proposed.<sup>188</sup> Under normal physiologic conditions, a soluble form of A $\beta$  is produced from APP after proteolytic cleavage by  $\alpha$ -secretase (Fig. 1). Because the putative A $\beta$  fragment is destroyed, this pathway, if functionally intact, cannot contribute to plaque formation. Instead, a soluble sAPP is produced and is either excreted or internalized and recycled. A second pathway entails cleavage on either side of the putative A $\beta$  peptide by  $\beta$ - and  $\gamma$ -secretases, resulting in the release of either A $\beta$ 40 or A $\beta$ 42 fragments.<sup>189,190</sup>

Several studies have focused on the early aspects of A $\beta$  deposition in subcortical and cortical structures in DS.<sup>146,147</sup> One study noted increased A $\beta$ -staining in medial-temporal structures by age 8 years, which increased linearly up to age 40 years; considerable variability was noted among individuals.<sup>148</sup> Both A $\beta$ 40 and A $\beta$ 42 forms are increased two- to three-fold in plasma from DS subjects compared with normal controls.<sup>191</sup> This increase is greater than that predicted by gene dosage considerations alone and suggests that other factors are important for determining A $\beta$  production. Recently, the soluble form of A $\beta$ 42 has been detected in the brain during the first decade of life in DS, clearly preceding plaque formation by many years.<sup>192</sup> Therapies targeting the production and/or clearance of A $\beta$ 40 and A $\beta$ 42 peptides before their aggregation and deposition in the brain are likely to be beneficial in halting disease progression. Such therapies are currently the focus of intensive research.<sup>193,194</sup>

*Apolipoprotein E.* The gene encoding the cholesterol-carrying apolipoprotein E (*APOE*) maps to chromosome 19 and has three allelic forms and is inherited as an autosomal codominant trait.<sup>195</sup> In the general population *APOE E3* is the most frequent allele (0.78), followed by *APOE E4* (0.14) and *APOE E2* (0.07). Accordingly, three different homozygous or heterozygous genotypes are possible, resulting in six distinct phenotypes. Certain phenotypes have been linked to increased risk for developing both late-onset, familial, and sporadic forms of AD.<sup>196,197</sup> Gene dose for *APOE E4* is correlated with both increased risk and earlier onset of AD, whereas the *APOE E2* gene dose seems to confer some protective effect.<sup>198</sup> To determine whether this relationship holds true in DS, several studies have examined *APOE* allele frequency in adults with and without clinical symptoms of dementia.<sup>199-205</sup> Differences in sample size, ascertainment methods, and determination of dementia onset make it difficult to compare studies. The study by Prasher et al<sup>205</sup> in 1997 included a meta-analysis of data from six previous studies from which several important conclusions have emerged: (1) the overall frequency of the three *APOE* alleles is similar in control populations with and

without DS; (2) there is a trend for higher *APOE E4* allele frequency in DS subjects with clinical dementia compared with those without dementia (15.1% vs 10.4%); and (3) There is a trend for lower *APOE E2* allele frequency in DS subjects with clinical dementia compared with DS subjects without clinical dementia (5.8% vs 10.0%). In those subjects for whom the age of dementia onset was available, this same study reported a trend for lower mean age of onset in the presence of *APOE E4* allele compared with *APOE E2* (45.7 vs 52.4 yr). Since the publication of that article in 1997, three more studies have reported a greater frequency of *APOE E4* allele in DS with dementia compared with those without (18.8% vs 6.9%; 17.7% vs 10.9%; 18.0% vs 13%).<sup>206-208</sup> The study by Tyrrell et al<sup>207</sup> also reports a significantly lower frequency of *APOE E2* allele in DS with dementia compared with age-matched controls without dementia (0% vs 8.3%), thus supporting previous findings of a protective effect of *APOE E2* in the expression of clinical disease. Another more recent meta-analysis also confirms the protective effect of *APOE E2*.<sup>209</sup>

The mechanism of how ApoE protein affects the risk for AD is far from clear. In brain tissue, ApoE is synthesized by astrocytes and macrophages, where it participates in the redistribution of cholesterol and lipid breakdown products following neuronal injury. Specific cellular interactions are mediated via binding to the low-density lipoprotein (LDL) receptor<sup>195</sup> and the LDL receptor-related protein/ $\alpha_2$ -macroglobulin receptor (LRP).<sup>210</sup> It has been suggested that ApoE production is necessary for the clearance of  $A\beta$  protein through binding to the LDL and LRP receptors.<sup>211</sup> It has also been shown that ApoE3 and ApoE4 isoforms demonstrate different binding affinities for  $A\beta$  protein and may thus vary in their ability to remove it from the neuropil.<sup>212</sup> Support for this hypothesis comes from autopsy studies of subjects with late-onset AD with increased numbers of plaque and vascular  $A\beta$  deposits in cerebral cortex of subjects with one or two *APOE E4* alleles compared with those with one or both *APOE E3* alleles.<sup>213</sup> Similarly in DS, inheritance of the *APOE E4* allele seems to confer an additional, and independent, risk factor for developing higher levels of amyloid accumulation.<sup>214</sup>

**SOD1.** Alterations in antioxidant defense systems have also been hypothesized to play a role in the AD-type pathology observed. Interestingly, adults with DS and AD-type pathology have significantly lower SOD-1 activity in erythrocytes compared with age-matched DS controls without AD-type pathology.<sup>215</sup> In the mature brain, particularly intense SOD-1 immunostaining is found in large pyramidal neurons of the neocortex and hippocampus.<sup>216,217</sup> In the brains of aged persons with DS, SOD-1 immunoreactivity is only transiently expressed in a subset of mature SPs; such staining was also found in the brain of individuals with AD, but was rarely seen in normal aged controls.<sup>218</sup> SOD-1 immunoreactivity is also absent from early diffuse plaques; thus, elevated SOD-1 may not be necessary in the initial pathologic process that leads to SP formation in DS. The neurobiologic manifestations of elevated SOD-1 activity will continue to be an important and active area of research.<sup>219</sup>

**S100.** Elevated levels of S100 protein and increased size and number of S100-immunoreactive astrocytes have also

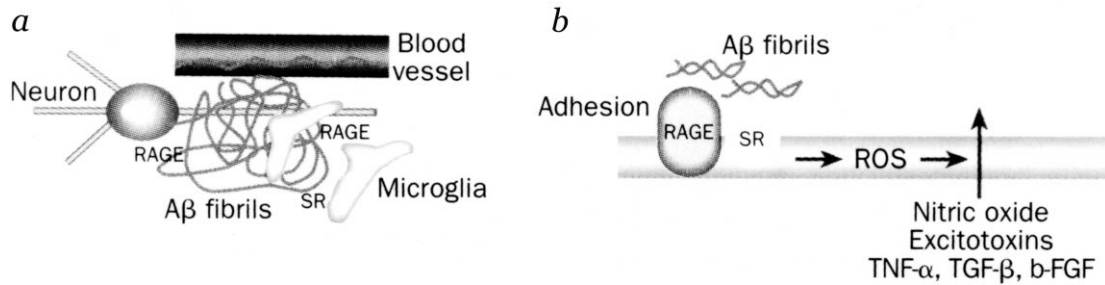
been detected in DS brain from aged persons, and a role for S100 $\beta$  has been proposed in the pathogenesis of AD-type neuropathology.<sup>220-223</sup> Based on findings of astrogliosis and increased levels of the cytokine interleukin 1 (IL-1), it has been proposed that elevated S100 $\beta$  secreted from astrocytes may stimulate the proliferation and activation of nearby microglia.<sup>224</sup>

**Microglia.** The role of inflammatory mechanisms as contributing to the progression of AD has not been emphasized until recently. Microglia are cells derived from the mononuclear-phagocyte lineage. They comprise up to 20% of the glial cell population and function as the predominate immune-effector cell population in the brain. In response to neuronal injury, they are swiftly activated to differentiate into phagocytic brain macrophages, whereupon they may release a number of toxic secretory products including proteases, cytokines, reactive oxygen species, and reactive nitrogen species.<sup>225,226</sup> In AD, the number of microglia is markedly increased.<sup>227</sup> Microglia associated with amyloid deposits display a phenotype consistent with the activated state, including immunoreactivity to class II histocompatibility antigens and inflammatory cytokines (interleukin-1, interleukin-6, and tumor necrosis factor- $\alpha$ ).<sup>228-231</sup> Recently, it was reported that serum levels of interleukin-6 are increased in persons with AD and in aged persons with DS and correlate with the severity of dementia.<sup>232</sup>

Studies of the intimate relationship between microglia and amyloid deposition have been examined in the brains of both AD and DS subjects and suggest that neuronally derived APP is the source of  $A\beta$  peptide, whereas microglia act as processing cells.<sup>233</sup> In DS the deposition of  $A\beta$  seems to precede the presence of microglia in plaques. Thus, microglia may play a role in processing or modifying the  $A\beta_{42(43)}$  species into  $A\beta_{40}$ .<sup>234,235</sup> Specific interactions between amyloid fibrils and cell-surface receptor sites, such as the receptor for advanced glycation end products and associated intracellular signaling pathways in microglia, are being defined<sup>236,237</sup> (Fig. 4).

### Neuropsychiatric

The prevalence of psychiatric disorders increases with age among persons with DS. Overall, approximately 25% of adults have a psychiatric diagnosis, which is somewhat lower than the 30% to 60% prevalence rates often cited for other groups of individuals with mental retardation.<sup>238</sup> The incidence of depression, obsessive-compulsive disorder, and dementia is significantly increased.<sup>239,240</sup> Depression, which may be responsive to medication, is frequently misdiagnosed as AD in young and middle-aged adults. An incorrect diagnosis of dementia may lead health care workers and family members to abandon a search for effective treatments prematurely. Treatable medical conditions that may be associated with behavioral change need to be ruled out before arriving at a diagnosis of either depression or AD. Conditions include vision or hearing loss, cardiovascular decompensation secondary to uncorrected congenital heart disease or aortic regurgitation, hypothyroidism, hypoxemia secondary to obstructive sleep apnea, and medication effects.<sup>241,242</sup>



**FIGURE 4.** Neuritic plaques in the brain of subjects with AD are composed of fibrils of  $\beta$ -amyloid peptide ( $A\beta$ ) that are in close apposition to degenerating neurons and activated microglia;  $A\beta$  is also deposited in blood vessels.  $A\beta$  binds to the receptor for advanced glycation end products (RAGE) on the surface of neurons, microglia, and vascular endothelial cells, and to the scavenger receptor (SR) on microglia (left side).  $A\beta$  interaction with, and activation of microglia. Binding of  $A\beta$  fibrils to RAGE or the SR on the surface of microglia promotes cell adhesion and induces reactive oxygen species (ROS), with activation of the microglia resulting in microglial generation of nitric oxide, excitotoxins, cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and transforming growth factor- $\beta$  (TGF- $\beta$ ), and the neurotrophic factor basic fibroblast growth factor (b-FGF) (right side). (Reprinted with permission from *Nature*, vol. 382, pp. 674–675, 1996, Macmillan Magazines, Ltd.)

Several studies have confirmed a bimodal peak in the prevalence of new-onset seizures in adults with DS.<sup>243–245</sup> The first peak occurs between 20 and 30 years of age, with a second peak occurring around age 45. Typically, partial complex and partial simple seizures are seen in the early-onset group and are not associated with cognitive decline or diffuse electroencephalographic (EEG) abnormalities. Generalized tonic-clonic seizures or myoclonus are typically seen in the later-onset adult group and are most often associated with both cognitive decline and diffuse EEG abnormalities—findings which herald the onset of an AD-type dementia.

Despite the apparent universal finding of AD-type neuropathologic changes by the fourth decade of life, the implications for the expression of a clinical dementia syndrome are less clear. In one retrospective review of 16 studies, both postmortem brain tissue and clinical findings were used to support the diagnosis of AD in 33 people with DS between the ages of 35 and 60 years.<sup>246</sup> All individuals had evidence of SPs and neurofibrillary tangles at autopsy, and one or more clinical manifestations were found in 75% of subjects: seizures (58%), change in personality (46%), focal neurologic signs (46%), apathy (36%), loss of conversational skills (36%), incontinence (36%), EEG abnormalities (33%), loss of self-help skills (30%), tremors or myoclonus (24%), visual or auditory deficits (24%), gait or mobility problems (21%), stubborn or uncooperative behavior (21%), depression (18%), memory loss (18%), increased muscle tone (12%), disorientation (12%), and delusions or hallucinations (3%). Prospective studies reveal a very different clinical picture of AD-related behavioral changes. Memory loss, temporal disorientation, and reduced verbal skills have been reported as the earliest signs of AD in higher-functioning individuals.<sup>247</sup> Those individuals in the severe-to-profound range of mental retardation more often manifest apathy, inattention, and decreased social interaction. Motor impairments, new onset of seizures, and loss of self-help skills have also been reported. The natural history of AD in DS indicates that the mean age for onset of clinical symptoms is 51.3 years in the moderately retarded and 52.6 years in the severely retarded.<sup>243</sup> The prevalence of symptoms increases from 11% between 40 and 49 years of age to 77% between 60 and 69 years and approaches 100% in all subjects older than 70

years.<sup>248</sup> Once recognized, the clinical symptoms of dementia progress rapidly in all subjects. Advanced cases are also more likely to show extrapyramidal signs or parkinsonian features.<sup>249</sup>

### FUTURE RESEARCH

In the coming decades, continued scientific advancement is likely to occur on a number of fronts.

#### Sequencing and Mapping of Chromosome 21

The DNA sequencing of chromosome 21 is now virtually complete.<sup>13</sup> The greatest challenge ahead is to characterize the biological role of newly identified genes, characterize their temporal and spatial pattern of expression during CNS development, and then determine the mechanism(s) by which overexpression affects normal development and function in trisomy 21. This promises to be an exceedingly daunting task, even once all of the genes have been identified. Because of the complexity of genetic and epigenetic regulatory mechanisms operative during development, it will be some time before we are able to grasp the very intricate mechanisms by which major developmental events are disrupted in trisomy 21. Sophisticated computer modeling of gene expression in regionally and neurochemically specific cell populations in the developing brain will be a necessary tool to both aid our understanding of and to catalog these events.

#### Animal Models and Gene Function

It is well understood by physicians and scientists, but less so by the general public, that isolating any gene from chromosome 21 only marks the first step in elucidating its function and role in producing the phenotype of DS. The technology for producing rodent models with gene dosage imbalance has also advanced markedly during the past decade, and there now exist several animal models that can be used to understand the genetic basis for the DS phenotype.<sup>250–253</sup> Transgenic and transomic mice (which are partially trisomic for mouse chromosome 16—a partial homolog to human chromosome 21) have proven especially promising in studies describing the developmental con-

sequences of gene dosage imbalance as it relates to brain development.<sup>254-257</sup> These models will continue to be of great interest in determining how alterations in brain ultrastructure and neurochemistry results in specific cognitive and behavioral deficits.<sup>258-260</sup>

### Functional Neuroimaging and Multidisciplinary Neuroscience Data

Increasingly sophisticated computerization is making it possible to represent detailed graphic images of the human brain in two and three dimensions for the purpose of interactive mapping.<sup>261</sup> Soon it will be possible to access images of the neuroembryologic development of the human brain with detailed information regarding cell type, regional connectivity, neurotransmitter phenotype, and gene expression profile.<sup>262</sup> The advantages of a computerized system dedicated to understanding the structural and neurochemical organization of the DS brain are compelling.

### Neurocognitive Enhancement

A revolution in our understanding and conceptualization of adult-onset neuropsychiatric disorders is taking place at this time. Once thought untreatable, neurodegenerative conditions such as AD, Huntington's disease, and Parkinson's disease are now the targets of intensive research and drug development on the part of the National Institutes of Health, biotechnology companies and the pharmaceutical industries.<sup>263</sup> The vision and spirit of excitement that characterizes this movement has not been recognized within the arena of childhood-onset neurocognitive disorders. There may exist a window of opportunity during which carefully targeted pharmacologic intervention could produce a more favorable biological outcome for children with some forms of mental retardation, including DS.<sup>123,264</sup>

### Neuroprotection Against AD

The high risk of developing AD-type dementia in individuals with DS makes it clear that a neuroprotection strategy offers the best hope for palliation or prevention.<sup>265</sup> Current clinical research strategies are focused on the use of antioxidants, anti-inflammatory agents, neurotrophic factors, or hormone replacement.<sup>266-268</sup> Recent epidemiologic studies and clinical trials have been encouraging regarding possible benefits derived from using indomethacin (a nonsteroidal anti-inflammatory drug),<sup>269,270</sup> estrogen,<sup>271,272</sup> and the antioxidants selegiline (monoamine oxidase inhibitor) and  $\alpha$ -tocopherol (vitamin E).<sup>273</sup> Novel compounds designed to improve the clearance and/or to prevent

deposition of the neurotoxic A $\beta$  peptide are also on the horizon.<sup>274</sup> Large randomized clinical trials of neuroprotective agents have yet to be conducted in persons with DS.

### SUMMARY

The neurobiologic consequences of trisomy 21 remain incompletely understood. As a syndrome complex of genetic origin with multiple and variable neurobiologic and neuropsychologic manifestations, it will be some time before a complete understanding of this condition emerges based on molecular genetic and developmental biological principles. As this review intends to make clear, the clinically dominant model of DS as a developmental disorder is, by itself, incomplete and unsatisfactory for advancing knowledge of this condition based on emerging biological concepts. Discussions of DS as a disorder characterized primarily by developmental delay are rightfully enjoyed by parents, educators, and early-intervention personnel; after all, it is the language of their craft and it permits highly complicated biological events to be easily conceptualized in terms of the whole child. It would seem, however, that it is time to *deemphasize* reliance on developmental models as having indelible explanatory power or prognostic significance, especially considering what we know about the risks for adult-onset neurocognitive and psychiatric disorder. Moreover, current child-based developmental concepts, taught either in isolation or to the exclusion of genetic and neurobiologic paradigms, are woefully inadequate for training today's clinician scientists to conduct meaningful biomedical research. Only as new information from molecular genetics, developmental biology, and the neurosciences are incorporated into clinically testable hypotheses will our understanding of DS advance accordingly. To produce the most meaningful clinical research requires that trainees in the fields of neurodevelopmental pediatrics, behavioral pediatrics, medical genetics, child neurology and psychiatry receive the necessary mentorship and research experience that will prepare them to address these critical questions. However, such opportunities are not readily available within the corridors of most departments of pediatrics, child neurology, or university-affiliated developmental disability programs, the primary mission of which is to train leaders in neurodevelopmental medicine. Until this situation is addressed, newborns with DS born in the year 2001 can expect to live longer lives compared with their peers from previous generations, but they will be unable to avoid the lengthening shadow of neurocognitive impairment that awaits them.

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