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Protein expression in Down syndrome brain

Review Article

E. Engidawork and **G. Lubec**

Department of Pediatrics, University of Vienna, Austria

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Summary. Down syndrome (DS) is the most common chromosomal abnormality associated with early mental retardation and neurological abnormalities followed by precocious age dependent Alzheimer-type neurodegeneration later in life. Knowledge of the pathological mechanisms involved in DS is far from complete, but overexpression of genes residing in chromosome 21 was considered to be the central point for the DS phenotype. In this regard, ßamyloid precursor protein (APP), CuZn superoxide dismutase (SOD1) and S100β have been implicated in causing apoptosis, a mechanism thought to be responsible for neuronal loss in DS, in one way or another. The gene dosage hypothesis has been challenged, however, and dysregulation of expression of genes located on other chromosomes has been described, which may well be secondary to chromosomal imbalance or a direct consequence of the disease process. The present review focuses on the protein expression profile in DS and we postulate that abnormalities in the coordinated expression, as well as interaction of proteins may be responsible for the neuropathology of DS. A series of candidate proteins are discussed that may be directly causing or reflecting the DS phenotype, in particular the brain abnormalities in DS.

Keywords: Down syndrome – Protein expression – Chromosome 21 – Fetal brain – Neurodevelopment – Apoptosis – Oxidative stress

1 Introduction

Down syndrome (DS), the most common genetic cause of mental retardation, results from triplication of the whole or distal part of human chromosome 21. The DS brain is characterized by reduced brain weight, decreased number and depth of sulci in the cerebral cortex, neuronal heterotopias, and reduced numbers of specific neuronal populations, such as granule cells, in the cerebral cortex. Neuronal abnormalities, primarily of dendrites, are observed in por-

tions of the neuraxis, such as hippocampus, cerebellum, and cerebral cortices (Coyle et al., 1986). Abnormalities of brain development that result in learning disability and intellectual impairment are compounded by Alzheimer disease (AD)-like neuropathological changes by the fourth decade of life. The onset of AD is accompanied by a number of neurodegenerative changes characterized by progressive accumulation of senile plaques and neurofibrillary tangles. These changes are complemented by neuronal loss, proliferation of dystrophic neurites, granulovacoular degeneration, Hirano bodies, astrocytic and microglial response, and vascular changes (Cairns, 1999; de la Monte, 1999; Mann, 1988; Wisiniewski et al., 1985).

The pathogenesis of DS is still obscure, but overexpression of genes located on chromosome 21, as a result of extra gene load, has been considered to be the central point. Chromosome 21 is the smallest of the human autosomes constituting approximately 1–1.5% of the haploid genome. The major part of chromosome 21 is the long arm (21q), which is essential for normal development and function, and harbours almost all genes of known function, except ribosomal RNA (Epstein, 2001). The sequencing effort of this arm of chromosome 21 provided evidence for the existence of 225 genes embedded within the 33.8 Mb of chromosome 21 genomic DNA and labeled chromosome 21 as a gene poor chromosome (Hattori et al., 2000). What's more intriguing is that about 41% of genes that were identified on chromosome 21 have no functional attributes. Earlier phenotype-genotype correlation studies suggested that a subregion of 21q may contain the critical genes and therefore should be the focus of gene discovery efforts. These studies then led to the proposal that there is a region of about 4 Mb between DNA markers D21S17 and ETS-2 that, if triplicated, is responsible for the clinical features of DS. This region was termed "Down syndrome critical region (DSCR)" (Antonarakis, 1998) and is mapped to 21q22.3. It now appears that essentially no segment of 21q can be excluded from containing genes relevant to the DS phenotype (Korenberg et al., 1994; Shapiro, 1999). Whilst a large body of evidence exists for aberrant expression of proteins in adult DS brain, information in fetal brain is scanty. In the present review, we discuss the findings in our laboratory and elsewhere, and consider the possible developmental and age related events that could contribute to neuropathology of DS.

2 Neurotrophic factors and/or cytokines

Cytokines are secreted molecules, characteristically expressed constitutively in low amounts, which are promptly upregulated in response to various stimuli, or by other cytokines. The group includes neurotrophic cytokines, such as S100β and interleukin-1, and inflammatory cytokines, such as interleukin-6. Although β-amyloid precursor protein (APP) is neither a neurotrophic factor nor a cytokine in a strict sense, it does enhance survival of cultured neurons and regulates the effect of nerve growth factor on neurite outgrowth and cell adhesion (Mrak et al., 1995). Thus, it is included in this group.

2.1 Amyloid precursor protein (APP)

A number of human pathologies are characterized by the abnormal deposition of proteins in a fibrillar form, causing disruption of cellular functions. In brains of Alzheimer's disease patients, extracellular amyloid deposits are composed of fibrils of the β-amyloid (Aβ) peptide (Selkoe, 1996). Aβ is derived from the proteolytic processing of APP, an ubiquitously expressed integral membrane protein with multiple isoforms generated by differential splicing of the gene that maps to the region 21q21–q22 of chromosome 21 (Huret et al., 1987; Kang et al., 1987; Tanzi et al., 1988). APP is processed by at least two different pathways, involving α-, β-, and γ-secretase. α-secretase cleaves APP within the Aβ sequence resulting in the release of the soluble N-terminal ectodomain of APP (sAPP) into the extracellular space, thereby precluding generation of the full length of Aβ. An alternative pathway operates in parallel with α-secretase processing, whereby β-secretase cleaves APP at the N-terminus of Aβ to produce a shortened form of sAPP leaving a C-terminal fragment. Aβ then can be liberated from this fragment by the action of γ secretase and released into the extracellular space (Haass and Selkoe, 1993; Mills and Reiner, 1999; Roßner et al., 1998). APP is involved in neuronal development, synaptogenesis, synaptic plasticity and transducing signals from the extracellular matrix to the interior of the cell (Neve et al., 2000). It is developmentally expressed and the different isoforms exhibit specific expressional changes during periods of neuronal differentiation and synaptogenesis in vivo (Roßner et al., 1998). APP undergoes fast axonal transport and is localized to synaptic sites in the brain (Arendt, 2001; de la Monte, 1999).

In DS, APP expression is considered to increase due to the extra gene load and this condition is associated with the appearance of cortical amyloid plaques by the middle age that are very similar to those seen in Alzheimer's disease (Mann, 1988, Wisniewski et al., 1985). The gene dosage-related difference in APP message was first shown by increased mRNA intensity in hybridization experiments in human fetal DS brain (Tanzi et al., 1988). This was later confirmed by mRNA quantification in human fetal (Epstein, 1995, 2001) and adult (Oyama et al., 1994) DS brain. However, increased mRNA expression was complimented by increased protein expression only in adult DS patients. Immunohistochemical (Griffin et al., 1998) and radioimmuno assay (Rumble et al., 1989) studies revealed overexpression of APP protein in adult DS brain, but immunoblot (Engidawork et al., 2001a) and immunohistochemical (Griffin et al., 1998) analyses failed to replicate this observation in fetal brain. Moreover, using different antibodies generated against distinct epitopes of the APP molecule, control and DS fetal, neonatal, and infant brains were shown to have similar levels and distribution of APP immunorectivitiy corresponding to the N- and C-terminal fragments and to Aβ1–28 (Arai et al., 1997). These observations suggest that the gene dosage effect may not be manifested, at protein level, in fetal DS. The lack of correlation between mRNA and protein expression in fetal DS brain could be attributed to altered mRNA/protein stability. It is possible that there may be developmental and/or other factors that cause rapid turnover of mRNA and these factors may be

over-ridden with disease progression. The fact that DS is associated with synaptic loss at a later stage, a situation which can lead to upregulation of APP (Arendt, 2001) can argue for this notion. If APP is not overexpressed in fetal DS brain then how does it contribute to the neuropathology? The detection of Aβ42 in DS subjects aged from 21 gestational weeks' to 61 years and its absence in age matched controls (Teller et al., 1996) appears to answer this question. DS patients almost invariably develop amyloid deposits by middle age, although they are free of them at earlier ages (Mann, 1988; Wisniewski et al., 1985). Given this, the neuropathology of plaque free DS individuals can thus be explained by the presence of soluble Aβ. Indeed, soluble Aβ has been viewed as the primary candidate for the neurotoxic element in AD (Klein et al., 2001). Aβ is a normal metabolite produced through highly regulated proteolysis and the presence of soluble Aβ in brain of DS but not in controls indicates that the mechanism that clears soluble Aβ in the brain is not fully functional in DS. Overexpression of APP can be considered as a possible cause for the malfunctioning of the clearance mechanism. However, the unchanged expression of APP in fetal DS brain points to the presence of other causative factors. These factors may include abnormalities related directly to the clearance mechanism per se. Taken together, soluble Aβ or amyloid deposit obtained as a result of gene dosage effect and/or impaired clearance mechanism could contribute to the early cortical dysgenesis and the late neurodegeneration in DS.

2.2 S100β

S100β is a small acidic calcium binding protein encoded by a gene mapped to the Down's locus of chromosome 21 (Allore et al., 1988). It is synthesized and released by astrocytes in the brain and functions as a neurotrophic factor for selected neurons as well as a gliotrophic factor, inducing mitosis and morphogenesis of astrocytes. Release of S100β can be stimulated by serotonin (5-HT) through stimulation of 5-HT1A receptors on astrocytes and can function as a serotonergic neurite extension factor (Azmitia et al., 1992). The protein increases intraneuronal free calcium levels and stimulates growth of neuronal processes (Barger and Van Eldik, 1992; Kligman and Marshack, 1985). It therefore plays an important role in development and maintenance of the central nervous system.

The number of astrocytes expressing S100β protein in DS is reported to be significantly higher than in controls at all ages (Griffin et al., 1989, 1998). In AD, reactive astrocytes that encircle the overgrown neurites surrounding the amyloid cores of plaque are shown to contain elevated levels of S100β (Marshack et al., 1992). Because of the neurodegenerative component, DS is characterized by the presence of overgrown neurites composed of APP and abnormally phosphorylated tau, proliferation and morphogenesis of astrocytes, and mineral deposits. Astrocytic changes seen in newborn DS decades before the appearance of neuritic plaques and neurofibrillary tangles suggested the potential role of S100β in dystrophic neurite formation, and

evolution of plaques and neurofibrillary tangle changes (Sheng et al., 1997). In addition, high concentration of S100β is suggested to mediate cell death in neuronal precursor cells and postmitotic cells when Bcl-2 is downregulated (Wang et al., 1999). Taken together, increased expression of S100β in DS may contribute to the dendritic abnormalities, mental retardation, apoptosis and could play a role in a cascade of events that eventually culminate in neurodegeneration. However, the observation that subjects who were trisomic for all, but for distal 21q22.3, including S100β manifested all the stigmata of DS, including mental retardation (Pangalos et al., 1992) cast doubt on the accountability of S100β for DS neuropathology.

2.3 Interleukins (IL-1 & -6)

IL-1 is an acute phase cytokine that has numerous systemic effects. The principal source of both α and β isoforms are macrophages in the periphery and microglia in the central nervous system. Both isoforms are encoded by genes residing on chromosome 2 (Boutwood et al., 1989) and are synthesized as 33 kDa proteins. Proteolytic cleavage of both isoforms yields a 17 kDa biologically active fragment, but in case of the β-isoform although the parent protein is also active, it is only the 17 kDa protein that is secreted (Mrak et al., 1995). In Down syndrome, there is a striking early overexpression of IL-1 (Griffin et al., 1989), which progressively increases with age (Baggott et al., 1993). IL-1 promotes the synthesis (Goldgaber et al., 1989) and processing (Buxbaum et al., 1998) of APP. It also induces overexpression of S100β (Sheng et al., 1996). It is because of these effects that IL-1 is suggested to play a key orchestrating role in plaque evolution (Akiyama et al., 2000).

IL-6 is a pleiotropic cytokine that mediates immune responses and inflammatory reactions affecting central nervous system cell growth and differentiation. These actions occur through interactions with specific soluble or membrane bound receptors that form the biologically active IL-6 receptor complex (Akiyama et al., 2000). IL-6 overexpression is commonly regarded as detrimental that exacerbates the pathology associated with several central nervous system disorders. Published reports indicate that elevated levels of IL-6 were found in AD brain and DS patients who developed AD (Berkenbosch et al., 1992). Moreover, serum IL-6 level was found to correlate with age and disease severity in both DS and AD (Kalman et al., 1997). In an effort to investigate the possible viral etiology of DS, we also examined the levels of IL-6 in DS brain. The data demonstrated an increased prevalence of IL-6 in DS brain pointing to the possibility that neuroinflammtion in DS may be caused by viral mediated upregulation of IL-6 (Cheon et al., 2001a).

3 Antioxidant proteins

Although reactive oxygen species (ROS) are considered as harmful to cells, low levels may serve as signaling molecules. Besides, normal cellular physiology involves the continuous production of ROS mainly derived from mitochondrial respiration. Thus, cells have evolved protective mechanisms to

maintain levels of these dangerous species within the required limit (Castagne et al., 1999), including enzymatic defense, low molecular weight scavengers, and reductants. Under normal circumstances, the balance between oxidant and antioxidant mechanisms is maintained. Any perturbation of this balance towards the oxidant or antioxidant pathway as a result of overproduction or deficient production results in oxidative or reductive stress. The brain is particularly vulnerable to oxidative stress because it contains disproportionately large amounts of oxidizable lipids and metals, and is relatively deficient in antioxidant systems (Castagne et al., 1999). Since oxidative stress as a result of alterations in the activity or expression of cellular antioxidant systems has been implicated in DS neuropathology, many studies have been performed to address this issue.

3.1 CuZnSuperoxide dismutase (SOD1)

SOD1 is a key enzyme in the metabolism of oxygen free radicals encoded by a gene localized to 21q22.1. Virtually all individuals with DS have an extra copy of this locus, even though the DS phenotype, including mental retardation can occur without triplication of SOD1 (Epstein, 2001). A number of studies have demonstrated that SOD1 levels are elevated in a variety of cell types and organs in DS. SOD1 activity was reported to be increased in DS fetal brain (Brooksbank et al., 1984) and fibroblasts (Anneren and Epstein, 1987) as well as in trisomy 16 fetal mice (Anneren and Epstein, 1987). Although SOD1 mRNA was found to be increased in fetal DS brain (de Haan et al., 1997), no apparent change was observed in at the protein level (Gulesserian et al., 2001a). By contrast, increased SOD1, but not SOD2, protein expression was noted in adult DS brain (Gulesserian et al., 2001c). SOD1 catalyzes the dismutation of superoxide anion into hydrogen peroxide, which is then converted to water by catalase or glutathione peroxidase. SOD1 is generally regarded as a protective enzyme. However, in DS where there is not a corresponding increase in catalase or glutathione peroxidase (see below), increased expression of SOD1 is suggested to lead to accumulation of hydrogen peroxide, which is toxic by itself or gives rise to the more noxious hydroxyl radical. Lack of such an adaptive response renders cells vulnerable to oxidative stress, which accounts for the early onset of apoptotic cell loss and activation of pro-apoptotic genes in the brains of individual with DS (de la Monte, 1999). This notion was upheld by the finding of apoptotic death of fetal DS neurons in vitro caused by increased generation of ROS, which was prevented by free radical scavengers (Busciglio and Yankner, 1995). Evidence from animal studies also supports the pathogenic role of SOD-1 in neurodevelopmental abnormalities and neurodegeneration. SOD transgenic mice were found to exhibit withdrawal and destruction of terminal axons and associated development of multiple small terminals (de la Monte, 1999), cognitive deficits and impaired long-term potentiation (Gahtan et al., 1998), increased lipid peroxidation and oxidative damage (Ceballos-Picot et al., 1992). Nonetheless, no evidence for the pathogenic role of SOD, lipid

peroxidation or ROS was found in adult DS brain (Hayn et al., 1996), despite overexpression of S0D-1.

3.2 Hydrogen peroxide handling enzymes

Hydrogen peroxide produced as a result of cellular metabolism is removed by three major types of enzymes, catalases, glutathione peroxidases, and peroxiredoxins (Kang et al., 1998a, b). Catalases and glutathione peroxidases are independently involved in the conversion of hydrogen peroxide generated by the action of SOD to water. Northern blot studies in different fetal organs, including brain did not show any change in catalase as well as glutathione peroxidase expression in DS (de Haan et al., 1997). This observation is also confirmed at protein level in fetal DS brain (Brooksbank and Balazs, 1984; Gulesserian et al., 2001a) as well as trisomic 16 mice fetal brain (Anneren and Epstein, 1987) providing evidence for the lack of adaptive response in DS brain.

Many proteins from a wide variety of species that show sequence similarity to yeast thioredoxin peroxidase have been identified and these homologues are referred to as peroxiredoxins (Chae et al., 1994). So far, six subfamilies of mammalian peroxiredoxin (Prx) proteins (Prx I–VI) have been identified based on their amino acid sequences and immunological properties (Chae et al., 1999; Kang et al., 1998a; Okado-Matsumoto et al., 2000; Zhou et al., 2000). Whilst Prx-VI uses glutathione as electron donor (Kang et al., 1998a), Prx I-V use thioredoxin and thus are also known as thioredoxin peroxidases (Kang et al., 1998b). In addition to their antioxidant property, several lines of evidence indicate that Prx proteins are also involved in the regulation of various hydrogen peroxide mediated signaling processes associated with growth, differentiation, and apoptosis (Jin et al., 1997; Kang et al., 1998b; Prosperi et al., 1994; Shau et al., 1998; Zhang et al., 1997). To assess the possible role of Prx proteins in DS, we investigated their expression in fetal and adult DS brain. Whereas Prx-I, -II, and -VI were decreased in fetal brain, Prx-IV was unchanged (Gulesserian et al., 2001a). A different pattern of expression emerged in adult DS brain. Prx-I and Prx-II were increased, while Prx-III was decreased (Kim et al., 2001a). These findings underscore the occurrence of oxidative stress in both fetal as well as adult DS brain. The decrease in Prx proteins in fetal brain may be related to impaired hydrogen peroxide mediated signaling that could result in developmental abnormalities. Failure to remove hydrogen peroxide instantaneously leads to its accumulation, which eventually culminates in oxidative stress. The different direction of alteration of Prx proteins in adult DS brain may indicate that with age and disease progression compensatory mechanisms become operative to save the remaining neurons from an eerie of oxidative stress and its consequences.

4 Guidance and adhesion molecules

Pathfinding, guidance of axons and cell migration play an important role in brain morphogenesis. Most neurons travel long distances through the com-

plex extracellular terrain of the developing embryo to reach their final position. Navigation of axons through the growth cone, a sensory-motor structure at the tip of the growing axon, to their appropriate target is a major mechanism that underlies formation of neuronal networks during brain development. This process is facilitated by guidance molecules encoded on various genes that provide sequential cues, including cell adhesion, chemoattraction, contact inhibition, and chemorepulsion. Recognition of these cues by the growth cone eventually directs the axon to its ultimate target. Nerve cell heterotopias have been found in fetal and infantile brains with DS that are attributed to a disturbance in embryonic cell migration (Coyle et al., 1986; Epstein, 1995, 2001).

4.1 Dihidropyrimidinase related proteins (DRPs)

The genes within the DRP family were originally identified in humans by their homology to dihydropyrimidinase (Hamajima et al., 1996). Four members of this gene family, DRP 1–4 are expressed mainly in the fetal and neonatal brains of mammals and chickens, and have been implicated in the axonal outgrowth and pathfinding by transmitting and modulating extracellular signals (Byk et al., 1996; Goshima et al., 1995; Minturn et al., 1995). Proteome analysis using two-dimensional electrophoresis coupled to matrix assisted laser desorption ionisation mass spectroscopy (2-DE/MALDI-MS) allow comparison of subsets of expressed proteins among a large number of samples. We used this form of analysis combined with software assisted quantification to study the protein levels of DRPs to get insight into the cause of abnormal neuronal migration and axonal path finding in DS. In adult brain, DRP-2 was decreased both at mRNA as well as protein level (Lubec et al., 1999). The same trend was also noted regarding DRP-2 in fetal DS brain. However, DRP-3, and DRP-4 were increased, and DRP-1 was unchanged (Weitzdoerfer et al., 2001a). DRPs were represented by multiple spots, the number of spots being more numerous in fetal than adult brain which probably indicates their role in brain development. Given the presumed roles of DRPs, their dysregulated expression both at early and later in life may contribute to abnormal wiring and plasticity of the DS brain.

4.2 Down syndrome cell adhesion molecule (DSCAM)

A variety of substrate and cell-attached factors influence neural development by regulating adhesion properties of cells. Interaction occurs between neural cells or between neural cells and the extracellular matrix of the microenvironment. The molecules mediating these interactions have been implicated in regulating the specificity and timing of cell-cell adhesion and the consequences on cell morphology and physiology. Many of the glycoproteins involved in the adhesion of neural cells belong to one of the three major structural families: the immunoglobulin superfamily, the cadherins, and the integrins. Whilst the first two mediate adhesion between the surfaces of neural

cells, integrins mediate adhesion of neural cells to glycoprotein in the extracellular matrix. DSCAM is a protein that shows high homology to members of the immunoglobulin superfamily and is encoded on the Down syndrome critical region of chromosome 21 at band 21q22.2–22.3 (Yamakawa et al., 1998). Consistent with the gene dosage effect, expression of DSCAM protein was shown to be higher in DS than controls (Saito et al., 2000). A growing body of evidence indicates that neural imunoglobulin superfamily members play a critical role in neural development and function, including cell migration and sorting, axon guidance and fasciculation, formation of neural connection, and synaptic plasticity (Edelman and Crossin, 1991; Schuster et al., 1996). The spatial and temporal dependent expression of DSCAM in relation to neurite outgrowth (Yamakawa et al., 1998) and its role as an adhesion molecule regulating myelination (Saito et al., 2000) reflects the potential role of DSCAM in the neurological abnormalities seen in DS.

4.3 Collagen type VI α1 chain precursor

Collagens are a family of highly characteristic fibrous heterotrimeric or homotrimeric protein found in all multicellular organisms. They are secreted by connective tissue cells as well as by a variety of other cell types and so far at least 15 collagen suptypes have been identified. Collagen type VI is a heterotrimer of three genetically distinct $\alpha 1$, $\alpha 2$, and $\alpha 3$ chains (Jander et al., 1983). Whilst the $\alpha 1$ and $\alpha 2$ chains are encoded by genes located on chromosome 21 within the region thought to be critical for congenital heart defects, the gene encoding the $\alpha 3$ chain is located on chromosome 2 (Weil et al., 1988). Decreased expression of $\alpha 1$ chain precursor of collagen type VI was found in fetal DS syndrome (Engidawork et al., 2001a), contrary to what is predicted from the gene dosage effect. These findings add further evidence to the view that the DS phenotype may not be explained by the gene dosage effect. Collagen type VI is thought to participate in cell adhesion and migration through interaction with member of the integrin receptor family or the NG2 proteoglycans (Levine and Nishiyama, 1996). Integrin receptors provide for developing neural cells a system capable of linking adhesion/migration information with other development signals controlling proliferation and differentiation. Since collagen functions as integrin ligand, the decrease in one of the chains could lead to formation of a homotrimeric molecule that would have a lower structural stability (Chu et al., 1988) thereby altering the adhesion between neural surface and extracellular matrix.

5 Neurotransmission associated proteins

Besides the anomalous development of the central nervous system described macroscopically and microscopically, neurotransmission systems, including cholinergic, seretonergic, noradrenergic, and glutamatergic are also affected in DS brain (Godridge et al., 1987; Reynolds and Warner, 1988; Seidl et al., 1999c; Yates et al., 1986).

5.1 Neurotransmitter receptors

Receptors are molecules that transduce signals when occupied by messenger molecules which specifically relay signals into a cell or between compartments within the cell (Barnard, 1997). Synaptic transmission involves the regulated release of transmitter molecules to the synaptic cleft, where they interact with postsynaptic receptors that subsequently transduce the information. The neurotransmitters also interact with presynaptic receptors whose activation leads to attenuation or accentuation of further release of the transmitter. In AD the cognitive impairment has been linked to loss of nicotinic acetylcholine receptors (NAChRs) (Whithouse, 1998) and reduced activity of cholineacetyl transferase (ChAT) (Bierer et al., 1995). Since DS patients invariably develop AD-like pathology by middle age, studies were undertaken to investigate these cholinergic markers in adult DS. The results revealed reduced activity of ChAT (Schneider et al., 1997) and aberrant expression of NAChRs (Engidawork et al., 2001d) providing explanation for the cognitive impairment seen in DS. By contrast, neither the activity of ChAT (Brooksbank et al., 1989) nor the expression of NAChRs was changed in fetal DS brain (Engidawork et al., unpublished observation) indicating the cholinergic impairment which is apparent later in life is not due to initial defect in cholinergic differentiation.

The disease process also affects other neurotransmitter systems. Elevated expression of 5-HT1A receptors was reported in fetal DS brain (Bar-Peled et al., 1991). During development 5-HT1A receptors occur in higher number even in brain regions where it is virtually absent in mature brain suggesting that these receptors play a role in human brain development. Indeed, 5-HT1A receptors located on astrocytes mediate release of S100β that function as a serotonergic neurite extension factor (Whitaker-Azmitia et al., 1990). Thus, the upregulation of these receptors at least in part, could be responsible for the neurodevelopmental abnormalities observed in DS. Glutamate is the major excitatory transmitter in the brain whose actions are subserved by two types of receptors: ionotropic and metabotropic glutamate receptors. Comparable immuno-reactivity of glutamate receptor (GluR), 1, 2/3 and 4, subunits of the alpha-amino-3-hydroxy-5-methyl-4-isoaxolepropionate (AMPA) type ionotropic receptor, was observed in fetal brain. However, only GluR1 subunit was detected in senile plaques of DS brain (Arai et al., 1996). Age related loss of GluR2/3 subunit makes GluR1 subunit to be the principal component of the AMPA receptor complex leading to alteration in cationic permeability of the receptor that likely contributes to neuronal degeneration (Ikonomovic et al., 2000). The increased expression of GluR1 and absence of that of GluR2/3 and GluR4 in adult DS brains thus indicates the potential role of GluR1 in degeneration of neurons and early formation of senile plaques. A similar increase in expression of metabotropic glutamate receptor 5 (mGluR5) in both fetal and adult DS brain (Oka and Takashima, 1999) had also been reported. mGluR5 belongs to the group I metabotropic glutamate receptor and is encoded on chromosome 21 (Hattori et al., 2000). The upregulation of mGluR5 in DS can thus be related to the chromosomal

imbalance. Alternatively, it can also be caused by reactive astrocytes in response to neuronal injury, since reactive gliosis is shown to upregulate mGluR5 (Ferraguti et al., 2001) and is a characteristic feature of the DS brain. mGluR5 has been shown to funnel APP into the non-amyloidogenic pathway (Lee et al., 1995), influence formation and maturation of neurites (Mion et al., 2001), and modulate NMDA receptor-mediated excitotoxicity (Blaabjerg et al., 2001). Considering the above findings, it is plausible that mGluR5 upregulation rather has a protective role in DS neuropathology.

5.2 Neurotransmitter transporters

Two classes of neurotransmitter transporters have been characterized at synapses, the vesicular and plasma membrane transporters. Neurotransmission depends on the regulated release of transmitter molecules and this requires the packaging of the transmitter molecules into specialized secretory vesicles of neurons, a process mediated by specific vesicular transporters. Active transport of neurotransmitters into secretory organelles requires both the presence of a transmembrane H^+ electrochemical gradient, established and maintained by a vacoular-type H^+-ATPase, and a vesicular transporter molecule which catalyzes the exchange of H^+ ions for neurotransmitter. Storage of neurotransmitters in subcellular organelles is important for protecting the neurotransmitter from leakage or intraneuronal metabolism and the neuron from possible toxic effect of the transmitter. In addition, it effectively lowers the concentration gradient across the neuronal membrane and thus acts as an amplification stage for the overall process of uptake (see below). The vesicular transporters include among others, the endocrine specific and neuronal isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2, respectively) and the vesicular acetylcholine transporter (VAChT) (Schuldiner, 1997; Varoqui and Erickson, 1998).

Removal of the transmitter from the synaptic cleft constitutes one mechanism by which termination of a signal is effected and usually occurs by its reuptake into the presynaptic terminal or into glial elements by a sodium-dependent plasma membrane transporter. Sodium dependent high affinity neurotransmitter transporters comprise a small family of integral membrane proteins that couple the uptake of small molecules (e.g., noradrenaline, dopamine, serotonin, glutamate) to the movement of ions. This process assures constant and high levels of neurotransmitters in the neuron and low concentrations in the cleft. The intraneuronal transmitter is then taken-up by the storage vesicles for further rounds of release (Tate, 1998). To gain an understanding whether the neurotransmitter deficits observed in DS (Godridge et al., 1986; Seidl et al., 1999c) are attributable to transporter expression and to add to the body of evidence that neurotransmitter changes do not occur at the early stage of human development, we investigated expression of vesicular as well as plasma membrane transporters in DS brain. In fetal DS brain, no apparent alteration was detected in expression of VMAT2, VAChT, and SERT (serotonin transporter) (Lubec et al., 2001) confirming

that prenatal life starts with normal neurotransmitter systems. However, increased SERT expression was noted in adult DS brain (Gulesserian et al., 2000). Non-cognitive behavioral symptoms are considered as cardinal feature of functional decline in adults with DS and are typically presented as depression, compulsive behavior with or without aggression and self-injury (Gedye, 1990, 1991; Geldmacher et al., 1997). The behavioral symptoms are largely ascribed to the disorder of serotonergic function, as serotonergic deficit was described in DS (Godrige et al., 1987; Seidl et al., 1999c; Yates et al., 1986) and symptoms are ameliorated with serotonergic diet and selective serotonin-reuptake inhibitors (Gedye, 1990, 1991; Geldmacher et al., 1997). The upregulation of SERT may therefore explain the serotonergic deficit reported in adult DS.

5.3 Synaptic proteins

Synaptic transmission can be achieved through vesicle-mediated secretion of the transmitter. This process requires vesicle docking and fusion to the plasma membrane effected by proteins of the exocytotic machinery in response to a rise in intracellular calcium generated by the action potential. The machinery consists of synaptosomal proteins, including N-ethylmaleimide sensitive factor (NSF) attachment proteins (SNAPs) present as three isoforms (α, β, and γ), synaptosomal associated protein 25 (SNAP25) and synaptotagmin as well as vesicular proteins (Clary et al., 1990; Staple and Catsicas, 1997). Synaptic pathology is central in AD, but in DS, although early synaptic dysgenesis had been reported (Becker et al., 1991; Petit et al., 1984), information available on synaptic proteins was scanty. This formed the rationale to investigate synaptic proteins in fetal cerebral cortex as well as different brain regions of adults with DS. In fetal brain, α-SNAP and SNAP 25 were significantly reduced in DS, whereas channel associated protein of synapse 110 (Chapsin 110) and β-SNAP were comparable between control and DS, (Weitzdoerfer et al., 2001b). Moreover, drebrin, a marker for dendritic spines, also displayed a marked reduction (Weitzdoerfer et al., 2001b) providing explanation, at least in part, for early cortical dysgenesis in DS. In adult brain, the results revealed decreased expression of β-SNAP in temporal cortex, synaptotagmin I in thalamus, and SNAP-25 in all regions studied (Greber-Platzer et al., 1999a; Yoo et al., 2001a). Furthermore, deranged expression of voltage-dependent anion selective channel proteins 1 & 2 (VDAC 1&2) (Yoo et al., 2001b) in adult DS brain adds to the existing body of evidence for the disrupted synaptic function in DS brain. The data obtained from adult DS brain compliment that of the fetal brain and point to the fact that proteins (e.g., β-SNAP) that appear to be normal early during development can be affected with disease progression (for e.g., with appearance of neuronal loss). Evidence for this comes from the observation that greater reduction of β-SNAP was observed in DS compared to AD (Yoo et al., 2001a), as neuronal loss is more severe in the former than the latter (Cairns, 1999). Based on these and other findings it is tempting to speculate that impaired synaptogenesis and dedifferentiation of DS brain

neurons (Kim et al., 2000) may underlie the shortened frontal-occipital length of DS brain. In addition, the involvement of synaptotagmin in endocytosis as well as membrane recycling (Zhang et al., 1994), suggests that not only exocytosis, but also membrane retrieval is affected in DS.

Based upon the above findings neurotransmitter system deficit in DS may result from altered expression of proteins involved in neurotransmission. The lack of change in some neurotransmitter markers in fetal brain probably indicates that the intensity of the insult in fetal brain is at subthreshold level and becomes apparent in adult life with the slowly developing AD pathology.

6 Apoptosis related proteins

Apoptosis is a genetically programmed mode of cell death that facilitates the survival of the organism by the controlled elimination of damaged or superfluous cells (Wellington and Hyden, 2000). It was first described as a phenomenon associated with developmental events. Cells, especially during development depend on trophic support normally provided by the surrounding cells. Interaction thus often occurs between responsive cells that are competing for trophic support. Cells that compete successfully survive, while the others undergo apoptosis (Becher et al., 1998; Bredesen et al., 1998). Consequently, apoptosis is considered as a mechanism to match the neuron population to its target fields and adjust the initial progenitor pool needed for proper morphogenesis of the nervous system (Kuan et al., 2000). Recently, apoptosis has got a wider scope and is recognized as a mechanism of cell death in neurodevelopmental and neurodegenerative disorders, including DS, AD, stroke, and Parkinson's disease (Anderson et al., 2000, Copani et al., 2001; Mochizuki et al., 1997). In these disorders, apoptotic death might be triggered by a specific insult, such as oxygen or glucose deprivation, amyloid beta or ROS (Copani et al., 2001; Iannello et al., 1999). The apoptotic process, as a mechanism of cell death, is activated by mitochondria, death receptors of the tumor necrosis factor (TNF) superfamily and the endoplasmic reticulum stress pathways (Daniel, 2000). To substantiate the role of apoptosis in DS, a variety of apoptosis related proteins were investigated in DS.

6.1 Caspases and their activator/inhibitors

The cell death machinery is conserved throughout evolution and is composed of activators, inhibitors, and effectors (Chinnaiyan and Dixit, 1996). The effector arm of the cell death pathway is represented by a novel family of cysteinyl aspartate specific proteases related to the *Caenorhabditis elegans* cell death product CED-3, termed caspases (Alnemri et al., 1996). Caspases are synthesized as zymogens containing a distinct protein module, such as death domain (DD), death effector domain (DED) or caspase activation and recruitment domain (CARD), which links them to the rest of the death signaling machinery. Caspases cleave specific aspartate containing sites in many proteins, including caspase themselves to initiate a proteolytic cascade. So far, at

least 14 family members have been identified and several of them are implicated in apoptosis (Cohen, 1997). To assess the role of caspases in DS, we investigated expression of caspase protein with immunoblotting. The results obtained in adult brain supported the view that apoptosis is a mechanism of cell death in DS, as revealed by decreased expression of procaspase-3 and -8, and a slight reduction of procaspase-9 (Gulesserian et al., 2001b). This finding was further supported by altered expression of caspase regulators, such as FLIP (Flice like inhibitory proteins), DFF (DNA fragmentation factor)-45 (Gulesserian et al., 2001b) and neuronal apoptosis inhibitory proteins (NAIP) (Seidl et al., 1999a). What's interesting is cytochrome c and apoptosis activating factor-1 (Apaf-1), which are responsible for activation of procaspase-9 to execute the death program through the mitochondrial loop, (Li et al., 1997) did not show any alteration (Gulesserian et al., 2001b). However, the death receptor CD95 that signals apoptosis via its cytoplasmic death domain was increased (de la Monte et al., 1998; Seidl et al., 1999b). This finding suggests that the mitochondrial pathway may be dispensable for apoptosis in DS. The same pattern of expression was found in AD brain (Engidawork et al., 2001g) indicating that death receptor-mediated apoptosis is a common element in DS and AD.

6.2 Bcl-2 family proteins

Aberrant expression of apoptosis-related gene in DS brain is not limited to caspases, but also includes the Bcl-2 family of proteins. Originally found in a translocation in human follicular lymphoma, Bcl-2 and its homologues have been implicated in regulating a diverse range of apoptotic programs (Rowen and Fisher, 1997). The family consists of approximately 16 cellular members and 5 viral homologues (Schendel et al., 1998) and shares homology with ced-9 at one or more Bcl-2 homology (BH) domains, BH1-BH4. The family can be divided into anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) groups. In adult DS brain, Bax message (Sawa et al., 1997) as well as Bax protein (Engidawork et al., 2001e) were unchanged, but another member of the proapoptotic group, Bim/BOD protein was increased (Engidawork et al., 2001e). Bcl-2 was either increased (Engidawork et al., 2001e), decreased (Sawa et al., 1997) or unchanged (Nagy and Esiri, 1997). In addition, Bcl-2 was shown to increase in abnormal myleopoiesis associated with DS (Yoshika et al., 2000). The unchanged Bax levels give further support to the view that the death receptor apoptotic pathway is the likely operative pathway in DS. The levels of Bcl-2 appear to be discrepant (see above) which may well be attributed to differences in methodology, brain regions used or severity of disease at the time of death. Otherwise, the findings generally indicate that the Bcl-2 protein exhibits alteration as a result of dysregulated apoptosis.

6.3 Protein kinases

Death receptor-mediated apoptosis not only triggers a death domain cascade that communicates with the downstream effectors, such as the caspases, but

also a protein kinase cascade. Several Ser/Thr protein kinases are specifically involved in apoptosis. Dlk/ZIP (death associated protein-like/Zipper interacting protein) kinase (Page et al., 1999) and RICK (receptor interacting protein-like interacting caspase-8-like apoptosis related protein kinase) (Inohara et al., 1998) are among the protein kinases involved in apoptosis. In adult DS brain, ZIP kinase did not show any detectable change (Engidawork et al., 2001e), whereas RICK was increased (Gulesserian et al., 2001b). Interestingly, ZIP kinase was increased in AD (Engidawork et al., 2001f) indicating that although apoptosis is thought to be common for both diseases, there are subtle differences in the way the process is executed. RICK is involved in the promotion of CD95-dependent cell death pathways (Inohara et al., 1998) and its elevation is consistent with activation of the death receptor pathway. Although we did not determine the expression of the DD containing RIP (receptor interacting protein) kinase directly, we measured the levels of RAIDD (RIP associated ICH-1/CED-3 homologous protein with death domain), an apoptotic effector and adaptor domain that interacts with RIP kinase. RAIDD levels were unaltered in DS brain (Engidawork et al., 2001e) and this finding was consistent with reports that RAIDD is dispensable for death receptor-mediated apoptosis in non-neuronal cells (Villunger et al., 2000). These findings may indicate that the involvement of RIP kinase in DS apoptosis is less likely.

Deregulation of cyclin dependent kinase 5 (Cdk5) activity, a protein homologous to mitotic Cdks, has been shown to cause cytoskeletal dysruption and apoptosis in cultured primary neurons (Patrick et al., 1999). This is achieved through binding of p25, a proteolytic cleavage product of p35, which is a regulatory subunit required for activation of Cdk5. The correlation between accumulation of p25 and increased Cdk5 activity in neurons from brains of AD patients led to the assumption that p25-mediated deregulation may account for neurodegeneration in AD brain (Patrick et al., 1999). Since DS has a neurodegenerative component at later age, we were interested to see if there was a similar accumulation of p25 as reported for AD. Surprisingly, p25 was decreased in both DS, as well as AD brain and p35 was comparable to controls (Yoo and Lubec, 2001). This report makes the proposed neurodegeneration-promoting role of p25 doubtful and its exact role is still an open question.

Using primary neurons obtained from fetal DS brain and age matched controls, Busciglio and Yankner (1995) showed that while cortical neurons obtained from both groups differentiate normally in culture, DS neurons subsequently degenerate and undergo apoptosis. Moreover, DS neurons exhibited a three-to four-fold increase in intracellular ROS that preceded neuronal death. This report provided direct evidence for the link between brain abnormalities and increased neuronal death in development, as well as aging in DS. The findings in adult DS brain mentioned above are also in line with this report. The results obtained from autopsy fetal DS brain, however, are discordant with this view. Caspase-3, a terminal caspase that plays a critical role in developmental apoptosis (Kuan et al., 2000), was unaltered. In addition, other apoptosis-related proteins, including CD95, Bcl-2, and annexin V

(a marker of apoptosis) were similarly unaffected casting doubt on the presence of enhanced apoptosis in fetal DS brain (Engidawork et al., 2001b).

7 DNA associated proteins

Several proteins are involved in the process of duplication or copying of information contained in the genome to maintain the survival of the species and organism, respectively. Among these proteins, transcription factors and DNA repair proteins whose expression is studied in DS are considered below.

7.1 Transcription factors

We realize from neuropathological findings that the abnormal molding of brain in DS is a molecular misconception resulting from a serious deterioration of the concerted action of factors normally modeling and wiring the brain. The major factors for the complex structuring of the brain are thousands of transcription factors, which wax and wane during different phases of brain development and wiring, thus enabling the required multitude of information necessary for histoarchitectonics. Transcription factors are not only key regulators of building the brain, but also are central in cross-talks among cells and signaling molecules. Transcription factors execute these functions by serving as trans-acting factors that regulate gene transcription by binding directly or through an intermediate protein to a particular DNA sequence, called a cis-regulatory region. There are several types of transcription factors, which are grouped into different families based on sequence similarities in their protein interaction and/or DNA binding domain. For example, transcription factors with the POU domain, helix-loop-helix motifs, leucine-zippers, forkhead genes and homebox genes determine proliferation, programmed cell death and differentiation of neurons and glial cells and thus mainly control and contribute to forming the brain. (Arenander et al., 1994; He and Rosenfeld, 1991).

Since differential gene expression underlies the unique phenotypic response during development and adult plasticity, studies on expression of transcription factors might help to explain the DS brain pathology, particularly if that transcription factor is encoded on the affected chromosome. We therefore investigated the expression of ETS-2, a proto-oncogene and transcription factor ubiquitously expressed and involved in organogenesis, encoded on chromosome 21. The expression of ETS-2 was first determined by RT-PCR in several brain regions of adult controls and DS patients as well as in heart biopsies of children obtained following surgery (Greber-Platzer et al., 1999b, c). ETS-2 was not overexpressed in brain as well as heart, as predicted from the gene dosage effect. Rather, it was significantly decreased in DS temporal and frontal cortex, but comparable in heart. We next determined expression of ETS-2 protein by immunoblotting in fetal brain (Engidawork et al., 2001a). Comparable levels of ETS-2 was found between

controls and DS, adding further evidence that overexpression of chromosome 21 genes can not fully explain the DS phenotype. We performed gene hunting using subtractive hybridization in fetal DS brain and found a downregulated sequence highly homologous to Jun D. Subsequent immunoblot analysis of Jun D in different brain regions of adult DS patients revealed reduced levels of Jun D in frontal and temporal cortex as well as in cerebellum (Labudova et al., 1998). Monomers of different transcription factors can interact to form either homodimers and heterodimers and these types of interaction provide a major source of regulatory complexity. Indeed, ETS, Jun, and Fos family members appear to require co-operation for their activity (Gutman et al., 1991; Wasylik et al., 1990). Hence, altered expression of one of the partner could lead to loss of such a combinatorial control on differential gene expression and may account for abnormal wiring of the DS brain (Epstein, 2001).

p53, a tumour suppressor protein considered as "guardian of the genome", is a transcription factor essential for cell cycle arrest and induction of apoptosis in response to DNA damage, metabolic deprivation or expression of oncogenes (Evan and Littlewood1998; Hansen and Oren, 1997). The growth arrest appears to depend on transcriptional induction of p21 and that of apoptosis on Bax and CD95 (Bennett et al., 1998; Hansen and Oren, 1997). Increased expression of p53 was found in adult DS brain (de la Monte et al., 1998; Seidl et al., 1999b) and this was accompanied by a similar increase in CD95 (de la Monte et al., 1998; Seidl et al., 1999b) as well as p21 (Engidawork et al., 2001e). This shows that p53 may, at least in part, be responsible for activation of the CD95 apoptotic signaling pathway and for premature aging via upregulation of p21, a biochemical marker of senescence. p53 is eliminated by ubiquitination through the ubiquitin-proteasome proteolytic pathway. Its upregulation in DS may be associated with increased expression of isopeptidase T, an ubiquitin specific protease, that could deubiquitinate p53 marked for degradation, thereby increasing the half-life of the protein (Engidawork et al., 2001c). Such an upregulation may permit p53 to effect its negative modulatory role in growth and development. Another TF of profound importance is nuclear factor kappa B (NF-κB). The redox sensitive NF-κB designates a family of transcription factors participating in the activation of a wide range of genes crucially involved in immune and inflammatory functions. The contribution of NF-κB family members to cell death and cell survival pathways is an intriguing and debated issue (Grilli and Memo, 1999; Mattson et al., 2000). Nevertheless, a large body of evidence indicates that NF-κB prevents apoptosis at least, in acute neurodegenerative conditions (Kaltschmidt et al., 1999; Mattson et al., 2000). Analysis of NF-κB in adult DS brain did not show any noticeable change compared to controls. However, a 32 kDa protein that appeared to be its truncated product, as there is in vitro evidence for proteolytic processing of NF-κB (Franzoso et al., 1994), exhibited a significant increase (Engidawork et al., 2001e). It is possible that the increased levels of the truncated product may have resulted from activation of the proteolytic processing of NF-κB by p53 or any other proapoptotic protein to attenuate it's antiapoptotic property.

7.2 DNA repair proteins

Elaborate systems for DNA repair have evolved in order to maintain the integrity of the genome. Consequently, the dysregulation of repair genes can be expected to be associated with significant, detrimental health effects, which can include an increased prevalence of birth defects, enhancement of cancer risk, and accelerated rate of aging (Ronen and Glickman, 2001). Over 125 genes directly involved in DNA repair have now been identified in humans, and their cDNA sequence established. These genes function in a diverse set of pathways that involve the recognition and removal of DNA lesions, tolerance to DNA damage, and protection from errors of incorporation made during DNA replication or DNA repair (Ronen and Glickman, 2001). DS lymphocytes exhibit accelerated DNA repair in response to ionizing radiation (Chiricolo et al., 1993). We reported increased expression of nucleotide excision repair genes, such as ERCC (excision repair cross complementing)2, ERRC3, and XRCC (X-ray repair cross complementing)1 in DS brain (Fang-Kircher et al., 1999; Hermon et al., 1998). These findings indicate the occurrence of extensive DNA modifying and processing secondarily to ROS inflicted DNA damage and may account for increased incidence of leukemia as well as premature aging.

8 Molecular chaperones

Molecular chaperones are proteins that play a critical role in mediating folding of certain polypeptides and their subsequent assembly into oligomeric structures. The prominent chaperones include heat shock protein (HSP) 70 family, HSP 40 family, HSP 90 family, the small HSPs and the chaperonins (Agashe and Hartl, 2000; Ellis, 2000). The recognition of AD as a protein folding disease and the consistent manifestation of AD-pathology in DS, as well as the apoptosis modulatory role of molecular chaperones attracted our interest and we investigated their expression in DS brain. Interestingly, molecular chaperones displayed brain region specific expressional aberrance reflecting that folding abnormalities do exist in adult DS brain (Yoo et al., 2001d). The intriguing thing is that all molecular chaperones did not show the same type of alterations. Some were either increased (HSP70.1) or decreased (HSC71) and others were unchanged (HSP60), which probably indicates how cells use differential expression to respond to different demands. The increase in glucose regulated protein (GRP) 78 noted in DS brain suggests that the endoplasmic reticulum (ER) loop of the apoptotic pathway may be operative in DS brain. The ER loop is activated by the unfolded protein response and accompanied by increased expression of the ER chaperones. This loop activates caspase-12 to initiate the caspase cascade (Nakagawa et al., 2000). The chaperone t-complex polypeptide 1 (TCP-1) ε subunit on the other hand was reduced providing a tentative explanation for the neurochemical changes of cytoskeletal structures in DS brain. In fetal DS brain, while majority of the chaperones investigated were comparable, TCP-1 α and β subunits were decreased (Yoo et al., 2001c). The possible implication of this finding is that

abnormalities in cytoskeletal structures may have paramount importance in the developing brain.

9 Signaling proteins

Cells are constantly receiving extracellular messages that are either favorable or deleterious for their survival. These signals are transduced intracellularly by using proteins that serve as a molecular switch and are localized in the cytosol, nucleus, or on specific membranes. Indeed, in recent years, many signal transduction pathways have been unveiled that control cell proliferation, differentiation, and apoptosis, although understanding the intricate mechanisms that regulate these pathways remains a daunting challenge.

14-3-3 proteins are a group of multifunctional homo- or heterodimer adaptor proteins that are primarily, but not exclusively expressed in neurons and bind to and modulate the function of a wide array of cellular proteins. Several isoforms of 14-3-3 protein have been identified based on their elution profile following reverse phase high performance liquid chromatography. These isoforms participate in the regulation of diverse biological processes, including neuronal development, signal transduction, cell growth and cell death through interaction with their effector proteins (Fu et al., 1998; Skoulakis and Davis, 1998). We have shown increased expression of 14-3-3 epsilon and gamma isoforms in different brain regions of adults with DS (Fountoulakis et al., 1999), whereas levels of alpha/beta and zeta/delta were comparable and that of gamma decreased in fetal DS brain (Peyrl et al., submitted). The decrease of gamma isoform in fetal DS may have relevance for impaired development and differentiation of the brain, as deletion of the 14-3-3 epsilon sequence is associated with cortical disorganization (Fu et al., 2000). Moreover, the unchanged levels of other isoforms may indicate that different isoforms are engaged in distinct signal transduction pathways and not all 14-3-3 mediated pathways are affected in fetal brain. The reversed pattern of alteration in 14-3-3 isoforms in adult brain may, however, be linked more to apoptosis than any other cellular activity. This is consistent with the observation that 14-3-3 proteins are highly enriched in areas of massive death of adult neurons caused by pathological processes (Skoulakis and Davis, 1998). Not only 14-3-3 proteins, but also other signaling proteins such as receptor for activated C-kinase (RACK-1) (Peyrl et al., submitted), nucleoside diphosphate kinase B (NDK B), Rab GDP-dissociation inhibitor 2 (GDI-2), and histidine triad nucleotide binding protein (Weizdoerfer et al., 20001c) were found to be decreased in fetal DS brain. These findings present evidence for the signal transduction defect early in fetal life, which may lead to the suboptimal mental functioning then and later in life. Further support for the aberrant signaling pathway in DS comes from the observation that overexpression of Down syndrome critical region 1(DSCR1) message in fetal DS that inhibits calcineurin mediated signaling pathways (Fuentes et al., 2000; Rothermel et al., 2000). In addition, increased expression of cyclooxygenase 2 (COX-2) (Oka and Takashima, 1997), an enzyme that produces biomessenger

molecules from arachidonic acid, and decreased stathmin levels (Cheon et al., 2001b) were reported in adult DS brain. Stathmin plays a key role in signal transduction pathways involved in cell proliferation and differentiation, and is a major substrate for cyclin dependent kinases (cdk) and protein kinase A (PKA), which were shown to be remarkably decreased in DS brain (Bernert et al., 1996; Kim et al., 2001b). The concurrent reduction of these three proteins reflects dysfunction of the relay function that integrates diverse intracellular signaling pathways involved in regulation of cell proliferation and differentiation. Taken together altered expression of signaling proteins in DS may provide a tentative explanation for deranged wiring and building of the brain in DS owing to their role in neural differentiation, migration, and synaptic transmission as well as transcription.

10 Concluding remarks

In conclusion, the data presented so far suggest that the DS phenotype can not be explained by overexpression of genes residing on chromosome 21 alone (Table 1). Rather, it appears that the chromosomal imbalance may affect the coordinated regulation of expression and interaction of proteins that have relevance to normal brain development and function. One also should note that lack of correlation between mRNA and the corresponding protein is not an uncommon phenomenon, which in fact underscores the need for the genomic data to be complemented by proteomic data in order to get a complete picture of gene expression. The other fact that deserves mentioning is age-related changes in protein expression. Proteins that are dysregulated in fetal DS brain (Table 1) may be proteins that have importance for normal developmental processes and could be responsible for neurodevelopmental disorder of DS. APP could be an exception, as there is another mode of accumulation of soluble Aβ. By contrast, proteins unchanged in fetal DS brain but altered in adults may reflect age-related/neurodegenerative changes. Absence of alteration in fetal brain and its presence in adults may be because the insult is at subthreshold level to bring about any significant changes at earlier stages or alternatively the changes are associated with progressive development of AD-like pathologies later in life.

The protein expression profile reviewed here can also be of some help for designing therapeutic strategies. Based on the cholinergic hypothesis of cognitive decline, we reported the promising effect produced by nicotinic agonists (Seidl et al., 2000). Increased expression of 5-HT1A receptors and SERT, which act in concert to regulate synaptic concentration of serotonin may also provide a rationale for evaluation of 5-HT1A antagonists and selective serotonin reuptake inhibitors. Given the role of caspases in mediating cell death and apoptosis being the major mechanism of neuronal loss in DS, caspase inhibitors may also prove to be useful in producing beneficial effects. Last but not least, stem cell therapy is one strategy that holds great promise in diseases, such as DS that are difficult if not impossible to treat by conventional means.

Table 1. Changes in expression of some representative proteins in fetal and adult brain with DS

Protein	Fetus	Adult	Reference
1. Chromosome 21 proteins			
Amyloid precursor protein	—	↑	Engidawork et al., 2001a; Griffin et al., 1998, Rumble et al., 1989
S100β	↑	↑	Griffin et al., 1998
Superoxide dismutase I	—	↑	Gulesserian et al., 2001a, c
Down syndrome cell adhesion molecule	↑	↑	Saito et al., 2000
Collagen (VI) α1 chain precursor	↓	n.d	Engidawork et al., 2001a
Metabotropic glutamate receptor 5	↑	↑	Oka and Takashima, 1999
ETS-2	—	n.d	Engidawork et al., 2001a
2. Other proteins			
Catalase	—	n.d	Gulesserian et al., 2001a
Peroxiredoxin I & II	↓	↑	Gulesserian et al., 2001a; Kim et al., 2001a
Dihydrpyrimidinase related protein 2	↓	↓	Lubec et al., 1999; Weitzdoerfer et al., 2001a
Nicotinic acetylcholine receptor α3	—	↑	Engidawork et al., 2001d, unpublished observation
Nicotinic acetylcholine receptor α4	n.d	—	Engidawrok et al., 2001d
Nicotinic acetylcholine receptor α7	n.d	↓	Engidawork et al., 2001d
Nicotinic acetylcholine receptor β2	—	↑	Engidawork et al., 2001d, unpublished observation
5-HT1A	↑	n.d	Bar-Peled et al., 1991
Glutamate receptor 1	—	↑	Arai et al., 1996
Vesicular monamine transporter 2 & vesicular acetylcholine transporter	—	n.d	Lubec et al., 2001
Serotonin transporter	—	↑	Gulesserian et al., 2000; Lubec et al., 2001
Synaptic associated protein (SNAP 25)	↓	↓	Greber-Platzer et al., 1999a, Weitzdoerfer et al., 2001b
Drebrin	↓	n.d	Weitzdorfer et al., 2001b
Synaptic associated protein (β-SNAP)	—	↓	Weitzdorfer et al., 2001b; Yoo et al., 2001a
Procaspase-3	—	↓	Engidawork et al., 2001b; Gulesserian et al., 2001b
CD95	—	↑	de la Monte et al., 1998; Engidawork et al., 2001b; Seidl et al., 1999b
Bcl-2	—	↑, —	Engidawork et al., 2001b, e; Nagy and Esri, 1997
p25	n.d	↓	Yoo and Lubec, 2001
jun D	n.d	↓	Labudova et al., 1998
p53	n.d	↑	de la Monte et al., 1998, Seidl et al., 1999b
14-3-3 gamma	↓	↑	Fountoulakis et al., 1999; Peyrl et al., submitted
Protein kinase A	n.d	↓	Kim et al., 2001b
Glucose related protein 78	—	↑	Yoo et al., 2001d
Stathmin	—	↓	Cheon et al., 2001b

(*n.d* not determined; —, no change; ↓, decreased; ↑, increased)

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Authors' address: Prof. Dr. Gert Lubec, CChem, FRSC (UK), Department of Pediatrics, University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria, Fax: +43-1-40400 3194, E-mail: Gert.Lubec@akh-wien.ac.at

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