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Combinatorial Gene Effects on the Neural Progenitor Pool in Down Syndrome

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1. Introduction

Down syndrome (DS) arises from triplication of genes on chromosome 21 (HSA21) and is characterized by neurological complications including cognitive dysfunction, epilepsy, and early onset Alzheimer's disease (AD). At the histological and cellular level, DS brains show prolongation in the cell cycle length of neural progenitors, increased oxidative stress, and mitochondrial dysfunction. These pathological processes likely contribute to the observed decrease in neurogenesis, increased neuronal cell death, enhanced gliosis in the cortex, and a corresponding decrease in both neuronal glutamatergic and GABAergic expression.

With recent advances in molecular biology and human genetics, many HSA21 genes have been identified, putative functions assigned to these genes and network analyses used to predict interconnecting, functional gene pathways. Interactions at this level are further complicated by gene regulatory mechanisms such as DNA methylation, histone acetylation and microRNA. In this respect, the neurological phenotypes seen in DS are not merely due to genomic imbalance from triplication of HSA21 genes, but also additive effects due to influences on associated genes within a given network or pathway and modification of gene expression due to epigenetic factors.

The development of stem cell technologies has provided the opportunity to isolate human neural progenitors (HNPs) and induced pluripotent stem cells (iPS) from patients with known neurological disorders. These reagents are also used as tools to study molecular mechanisms and pharmacological therapies in various human diseases. They provide a means by which an understanding is established for interactions between various genes causal for a human disorder, especially in contiguous gene syndromes that are difficult to faithfully replicate in animal models. We have focused on the study of DS HNPs in relation to neurological phenotypes in the early onset of DS in these individuals.

In the current review, we summarize the approaches taken, recent progress and insights gained from studies of DS during neural development, incorporating insights gained from the use of human DS neural progenitors.

2. Down syndrome phenotype and genotype during neurodevelopment

The most prominent neurological phenotypes in DS include mental retardation (MR), epilepsy, and dementia (early onset AD). Individuals with DS generally have mild to moderate MR with average intelligence quotient (IQ) among children at 50, as compared

with normal children at an IQ of 100. Children with DS generally exhibit better visual motor skills (such as drawing) than auditory and linguistic skills, which typically lead to a delay in language skill development. MR can also be accompanied by behavioural issues such as attention-deficit disorder (sometimes with hyperactivity) and autistic behaviour, especially in those with severe intellectual disability. The onset of seizures tends to occur in a bimodal distribution. At early ages, individuals with DS are susceptible to infantile spasms and tonic clonic seizures with myoclonus. These epilepsies are associated with MR and likely due to early impairments in brain development. Individuals with DS in the third decade of life are more prone to developing simple partial or complex partial seizures in addition to tonic-clonic seizures. These focal epilepsies are likely associated with the increasing burden of parenchymal damage sustained from the progressive neuronal degeneration. It is estimated that 46% of Down's patients over the age of 50 have a diagnosis of epilepsy. Finally, AD is generally considered a neurodegenerative disorder that affects the elderly at 60 years of age and over. Premature AD is seen in DS in the early 40s (Arya et al., 2011; Contestabile et al., 2010; Lott and Dierssen, 2010; Menendez, 2005)

The clinical phenotypes in DS correlate with functional changes seen at the histological and cellular levels in DS brain. DS cortex shows increased simplified gyral patterning and delayed myelination (Banik et al., 1975; Cragg, 1975; Koo et al., 1992; Wisniewski, 1990; Wisniewski and Schmidt-Sidor, 1989). Neuronal loss has been seen in DS brain both during development, following differentiation and aging (Becker et al., 1991; Benda, 1947; Colon, 1972; Davidoff, 1928; Golden and Hyman, 1994; Guidi et al., 2008; Ross et al., 1984; Wisniewski et al., 1984). Histological evidence of neurofibrillary plaques and tangles can be seen in the DS brain, consistent with the premature onset of AD (Motte and Williams, 1989). Changes in dendritic branching as well as neurotransmitter levels have also been reported in the DS brain tissue and likely correlate with the behavioural and cognitive difficulties seen in these individuals (Reynolds and Warner, 1988; Ross et al., 1984). At the cellular level, DS progenitors have prolonged cell cycle lengths, thereby impairing neurogenesis (Chakrabarti et al., 2007; Contestabile et al., 2007; Guidi et al., 2008). Neuronal numbers further decline presumably through increased levels of reactive oxygen species (ROS), mitochondrial dysfunction and increased cell death in mature neurons (Busciglio et al., 2002; Guidi et al., 2008; Seidl et al., 2001). Following differentiation, neurons also show an imbalance of excitatory-inhibitory neurotransmission (Bhattacharyya et al., 2009), with reduced dendritic branching and spine density (Benavides-Piccione et al., 2004). Overall, these changes could all contribute to the MR, epilepsy and AD phenotypes seen in DS.

Various neurological features in DS have been attributed to over-expression of individual genes on HSA21, and several of these genes might contribute to a final common pathway. For example, APP has been shown to promote neuronal degeneration and oxidative stress. Similar functions have been implicated in other HSA21 genes including BACH1, SOD1, and S100B, raising a possible role in AD. Other HSA21 genes (TIAM1, SYNJ1, ITSN1, DSCR1) have been associated with synaptic dysfunction (suggestive of a pathological role in MR and epilepsy). Individual HSA21 genes with proposed neurological functions are summarized in Table 1.

3. Identification of genes and pathways responsible for AD or MR

Although HSA21 is a relatively small chromosome and single candidate genes have been shown to mimic certain DS features, the combinatorial effects of contiguous genes on

HSA21 genes	Downstream targets	Endophenotype	Exophenotype	Ref
NRIP1		Inhibit estrogen and glucocorticoid receptors		(Cavailles et al., 1995; Gardiner and Costa, 2006; Subramaniam et al., 1999; Teyssier et al., 2003)
APP	Ptch1/smo Tau RAGE Tau IL6/gp130 Notch MAP kinases	Abeta deposition, astocytosis, microgliosis, neuronal loss, hyperphosphorylation Tau, decreased proliferation, increased cell death, mitochondrial dysfunction	Dementia, learning and memory deficits	(Abramov et al., 2004; Arancio et al., 2004; Harris-Cerruti et al., 2004; Howlett and Richardson, 2009; Korbel et al., 2009; Kwak et al., 2010; Kwak et al., 2011; Mori et al., 2010; Prasher et al., 1998; Rovelet-Lecrux et al., 2006; Takuma et al., 2009; Trazzi et al.)
BACH1		Increase ROS		(Ferrando-Miguel et al., 2003; Shim et al., 2003)
TIAM1	RAC, NMDAR EphBR	Altered neurite formation, abnormal dendrite spines		(Matsuo et al., 2002; Siddiqui et al., 2008; Tolias et al., 2005; Tolias et al., 2007)
SOD1	APP	Increase ROS, mitochondrial dysfunction	Learning and memory deficits	(Furuta et al., 1995; Harris-Cerruti et al., 2004; Iannello et al., 1999; Lott et al., 2006)
SYNJ1	PtdIns(4,5)P(2)	Synaptic dysfunction, abnormal vesicle trafficking Astrogliagenesis	Learning and memory deficits	(Arai et al., 2002; Chang and Min, 2009; Herrera et al., 2009; Voronov et al., 2008)
Olig2	S100B	Increased inhibitory neurons Increased gliosis Decreased IPSC		(Chakrabarti et al.; Chen et al., 2008; Lu et al., 2002; Tatsumi et al., 2008; Zhou and Anderson, 2002)
Olig1		Increased inhibitory neurons Decreased IPSC		(Chakrabarti et al.; Zhou and Anderson, 2002)
ITSN1		Synaptic dysfunction, abnormal vesicle trafficking		(Chang and Min, 2009; Keating et al., 2006; Yu et al., 2008)
DSCR1	NFAT Calcineurin Tollip ILR1 Caspase9/3	Synaptic dysfunction; abnormal vesicle trafficking, increased neuronal susceptibility to oxidative stress, neuronal apoptosis, mitochondrial dysfunction		(Arron et al., 2006; Chang and Min, 2005; Keating et al., 2008; Lee et al., 2009; Porta et al., 2007; Sun et al.)

HSA21 genes	Downstream targets	Endophenotype	Exophenotype	Ref
SIM2	Drebrin		Learning and memory impairment, reduced exploratory behavior and social interactions, increased tolerance to pain	(Chrast et al., 2000; Ema et al., 1999; Vialard et al., 2000)
DYRK1A	NRSF/REST Calcineurin CREB NFAT Tau Notch P53 APP	↓neuroectodermal progenitor, less neuron with abnormal branched neurites, LTP deficits, hyperphosphorylation Tau and APP, premature neuronal differentiation	Learning and memory deficits, motor defects	(Altafaj et al., 2001; Arron et al., 2006; Canzonetta et al., 2008; Fernandez-Martinez et al., 2009; Guimera et al., 1999; O'Doherty et al., 2005; Olson et al., 2004b; Park et al., 2010; Park et al., 2007; Ryoo et al., 2007; Sago et al., 1998; Siarey et al., 2005; Smith et al., 1997; Woods et al., 2001; Yabut et al., 2010; Yang et al., 2001)
KCNJ6 (GIRK2)	GABAB	Imbalance of excitatory-inhibitory neuronal transmission		(Best et al., 2007; Cramer et al., 2010; Harashima et al., 2006)
ETS2	APP	Increased neuronal apoptosis, increased APP production, mitochondrial dysfunction		(Helguera et al., 2005; Wolvetang et al., 2003a; Wolvetang et al., 2003b)
PCP4	Calmodulin			(Kleerekoper and Putkey, 2009; Thomas et al., 2003; Utal et al., 1998)
DSCAM		Overexpression in Purkinje cells, cortical neurons and senile plaques		(Hattori et al., 2007; Saito et al., 2000; Yamakawa et al., 1998)
PKNOX1	FABP7			(Sanchez-Font et al., 2003)
DNMT3L	DNMT3A/3B HDAC	Transcription repression		(Deplus et al., 2002; Holz-Schietinger and Reich, 2010)
SUMO3	APP NRIP1 ELK	Inhibit glucocorticoid receptors		(Dorval et al., 2007; Gardiner, 2006; Holmstrom et al., 2003)

HSA21 genes	Downstream targets	Endophenotype	Exophenotype	Ref
S100B	RAGE AQP4 JNK GSK3ß Tau P53 ATAD3A	Increased ROS, mitochondrial dysfunction and cell death, GSK3ß and Tau hyperphosphorylation, astrocytosis and neurite proliferation	Premature neurological aging	(Esposito et al., 2008a; Esposito et al., 2008b; Gilquin et al., 2010; Lin et al., 2001; Mori et al., 2010; Reeves et al., 1994)

Table 1. HSA21 genes implicated in neurodevelopment

particular DS phenotypes are not known. Several approaches have been used to ascertain HSA21 genotype phenotype contributions: analyses of gene clusters based on their physical linkage, common function/ interactions and/or temporal-spatial distribution of expression.

3.1 Structure-based clusters

Genes that are physically linked based on their location within the same region are usually active together and often contribute to the same phenotype. Based on these structure-based clusters, comparison of full trisomy 21 and partial trisomy 21 genotypes provide some insight between contiguous genes and DS phenotypes. One such region includes APP, which is positioned between 27.25 to 27.54Mb on the long arm of HSA21. Five families with various segmental trisomies that range from 0.58 to 6.37Mb in length and contain APP were all shown to have abundant parenchymal and vascular deposits of beta amyloid without MR on brain autopsy, strongly linking APP to AD (Rovelet-Lecrux et al., 2006).
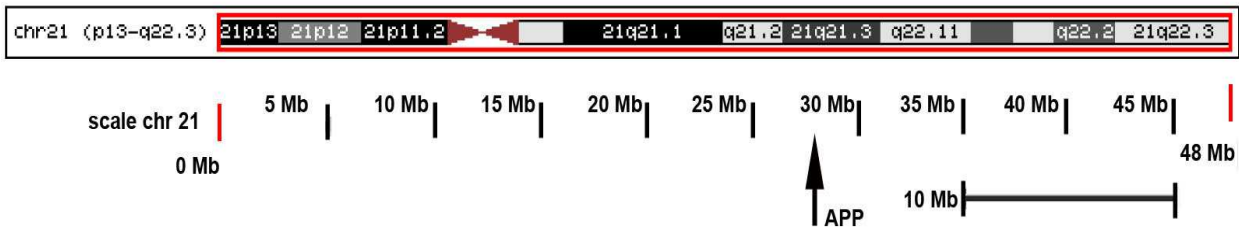

Fig. 1. APP is positioned between 27.25 to 27.54Mb on the long arm of HSA21.

Furthermore, a DS patient with duplication of the distal last 12Mb of HSA21 (a fragment that does not include APP) did not develop typical AD pathology (Prasher et al., 1998). Recently, Korbel et al. analyzed 30 patients carrying rare segmental trisomies of various regions of HSA21 and identified discrete regions between 1.8-16.3Mb in length that were likely to be involved in 8 DS CNS and non-CNS phenotypes including MR and AD (Korbel et al., 2009). They described a patient with a duplication of 18.8 Mb (28.12-telo) without APP, who did not have dementia and had no amyloid accumulation by fMRI. Conversely, several patients with segmental trisomies containing APP did show AD characteristics in the absence of severe MR, which supports the involvement of APP in AD but not MR. These authors were able to narrow down a 1.95Mb interval including APP responsible for the AD phenotype, that excludes genes located distal to 28.12 Mb on HSA21q, including BACH1 and SOD1. These genes were previously suggested to play important roles in the development of AD and MR in DS (Ferrando-Miguel et al., 2003; Lott et al., 2006).

Collectively, these studies conclude a small-segment containing APP as the basis for AD phenotype, with multiple focal regions contributing to MR.

Through segmental trisomy analyses, the primary features of DS have previously been ascribed to the Down syndrome critical region (DSCR). This interval spans about 5Mb around 21q22.12-q22.2 and contains about 33 conserved genes, including DSCR1 (35.88-35.99 Mb), SIM2 (38.07-38.13 Mb), DYRK1A (38.74-38.89), KCNJ6 (38.99-39.29Mb), ETS2 (40.18-40.2Mb), PCP4 (41.24-41.3Mb) and DSCAM (41.38-42.22Mb). The contiguous genes are thought to be sufficient to replicate the DS features including craniofacial abnormalities, short stature, joint hyperlaxity, hypotonia and mental retardation (Delabar et al., 1993; Korenberg, 1990; Korenberg, 1993; McCormick et al., 1989; Olson et al., 2004a; Rahmani et al., 1989). Further studies suggest that DSCR1 and DYRA1A cooperatively destabilize NFATC and induce DS features (Arron et al., 2006; de la Luna and Estivill, 2006). However, several genotype-phenotype correlation studies argue against this hypothesis, given that other patients with partial trisomy 21 that do not include DSCR1, DSCAM or genes within the DSCR have severe DS features including MR (Korbel et al., 2009; Lyle et al., 2009; Ronan et al., 2007).

3.2 Function-based clusters

Microarray expression profiling and sequencing of the human genome have allowed for data analyses and interconnection of functional pathways responsible for various human disorders. By comparing differentially expressed genes between normal and pathological samples, function-based clusters can be generated and attributed to certain disease pathways. We have used this approach in the study of DS human neural progenitors (HNPs) at 18 weeks gestation age (Esposito et al., 2008a). Through pairwise comparison of mRNA expression levels for approximately 54,000 probes in the HU133 plus 2.0 Affymetrix microarray, we observed that some 1900 transcripts (3.8%) were significantly different between WT and DS samples, and that these transcripts were distributed across every chromosome – not just HSA21. Moreover, of these 1900 transcripts, only 330 transcripts (0.07%) were at least 1.5 to 2 fold in excess/deficit on pairwise comparison. These transcripts could then be grouped into specific functional networks, including gene clusters involved in cell cycle (proliferation), cell compromise (cell death, oxidative stress), cell signalling (stress kinases) and cell function and maintenance (differentiation and survival). For example, HSA21 genes such as S100B and amyloid precursor protein (APP) were constitutively over-expressed in DS HNPs along with various stress related kinases and the water channel aquaporin 4 (AQP4). These dysregulated genes comprised a cell signaling functional cluster, whereby HSA21 associated S100B and APP led to increased ROS formation, activation of stress response kinases, and compensatory AQP4 expression. siRNA inhibition of AQP4 resulted in elevated levels of ROS following S100B exposure whereas loss of AQP4 expression led to increased programmed cell death. Results from these studies support the hypothesis that HSA21 gene overdose lead to profound disruption of the entire transcriptome, but presumably in fairly specific functional groups (FitzPatrick et al., 2002; Saran et al., 2003; Tang et al., 2004).

Using a similar strategy, Bhattacharyya et al. compared the differential expression of genes in DS versus WT HNPs at 13 weeks gestation age. Within these earlier aged progenitors, they observed similar functional clusters involved in cell death, cell cycle/ proliferation, and cell fate/ neuronal development. These studies focused on impairments in interneuron neurogenesis, perhaps related to increased expression of gliocentric genes such as Olig1, Olig2, OMG and COUP-TF1/NR2F1 and downregulation of the interneuron related genes DLX1, DLX2 and DLX5.
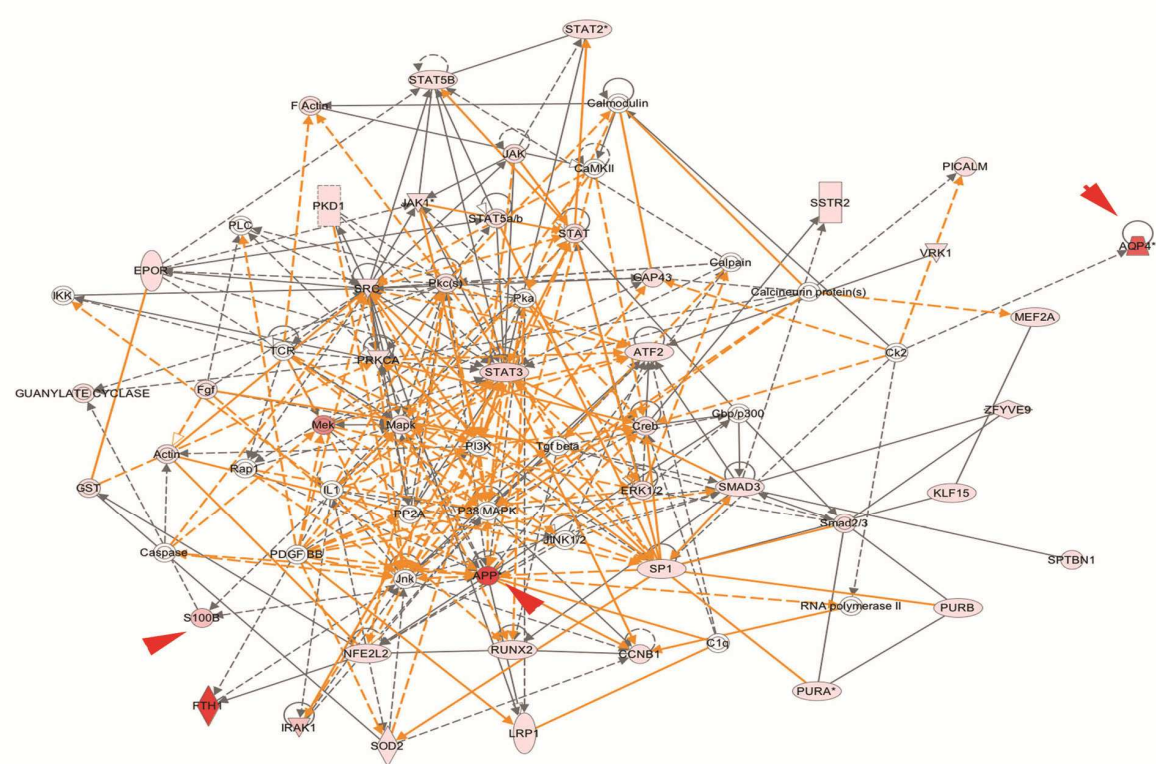
Fig. 2. Constitutively overexpressed genes on HSA21 (S100B & APP) and AQP4 (shown by red arrows) are predicted to form tight interrelationships within known cell signaling pathways shown in the networks pooled out from many differentially expressed genes in DS HNPs with microarray.

In another report, Canzonetta used differential expression analyses following microarray profiling of normal and trsisomy 21 mouse ES cells and pooled the differentially expressed genes into endodermal, mesodermal or ectodermal subgroups. They found reduced expression of stem cell genes controlling pluripotency such as Nanog and Sox2; increased expression of endodermal genes such as Gata4, Gata6 and Foxa2; increased expression of mesodermal genes such as Snail1 and Pitx2 and reduced expression of neuroectodermal genes, such as Nestin, Tubb3, Map2 in trisomy 21 ES cells (Canzonetta et al., 2008). More pervasively, they observed a decrease in Nrsf/Rest, a key regulator of neuronal differentiation and pluripotency. Quantitative linkage analysis (QTL) mapped the REST level change to a 3Mb region of HSA21 containing DYRK1A, suggesting that dysregulation of this HSA21 gene was an early regulator of various embryonic lineages.

3.3 Temporal-spatial mapping-based clusters

Although structure and function cluster approaches provide correlation between DS genotype-phenotypes, temporal and spatial distribution of gene expression further refines the contribution of individual HSA21 genes in DS neural development and later maturation. Previous reports analyzing human trisomy 21 tissue or cells have shown that overexpression of different chromosome 21 genes is cell type and developmental time specific (Ait Yahya-Graison et al., 2007; Chung et al., 2005; Giannone et al., 2004; Gross et al., 2002; Li et al., 2006; Lubec and Sohn, 2003; Mao et al., 2005; Mao et al., 2003; Rozovski et al., 2007). For example, by RNA whole-mount in situ hybridization, gene expression maps of

HSA21 orthologues in various aged embryonic or newborn mouse brain have been reported (Gitton et al., 2002; Reymond et al., 2002). Of the 158 orthologous genes examined in postnatal day 2 mouse brain, 41% were expressed in new cortex, 25% in hippocampus and 25% in cerebellum. The transcripts expressed in neocortex represent a pool of candidate genes for MR of DS, and include DSCAM, SYNJ1 and TIAM1. These genes are implicated in synaptic function, axonal guidance, cell migration and neurite outgrowth (Leeuwen et al., 1997; Saito et al., 2000; Yamakawa et al., 1998).

While several studies have used human cortical progenitors to identify changes in expression profiling, the findings of gene expression levels vary between studies. The temporal-spatial variations in DS HNPs may account for these differences. For example, the REST/NRSF transcription factor was previously reported to be selectively repressed in the DS HNPs (Bahn et al., 2002). These changes were not seen in our studies or those of others (Bhattacharyya et al., 2009; Esposito et al., 2008a). However, this discrepancy could be due to the fact that the gestational ages of the euploid and DS fetal tissues used in these differential expression studies were not the same. Similarly, our observations of increased S100B and aquaporin 4 levels in 19 week gestational age DS HNPs were not appreciated in 13 week gestational age HNPs. This difference would be consistent with the previous notion that HNPs mimic normal developmental patterns such that later generated or propagated HNPs generate fewer neurons than HNPs harvested at an earlier age. Given that S100B and AQP4 are associated with more gliocentric phenotypes, upregulation of these genes would be expected in later gestational aged HNPs. Similar differences appear to exist with other HSA21 genes such as Olig1/2 and DNMT3L (unpublished observations).

4. Characterization of endophenotypes for AD or MR

Endophenotypes are pathological changes observed at the histological, cellular or molecular levels, which provide an association between candidate genes and the clinical symptoms. Clinical neurological symptoms of DS include early onset AD, mental retardation and seizures. Histological changes such as increased cell death, gliosis, demyelination, loss in neuronal numbers, and gyral simplification are thought to contribute to AD and/or MR in DS brain. At the cellular level, DS cells show impairments in mitochondrial function, increased levels of oxidative stress, and changes in proliferative rates. An imbalance in excitatory-inhibitory neurotransmission, expression changes of genes that affect cell fate/differentiation, proliferation/cell cycle and cell injury have been observed at the molecular level.

4.1 Mitochondrial dysfunction and apoptosis

Oxidative stress is increased in DS brain and the imbalance of reactive oxygen species (ROS) metabolism may be the key component giving rise to DS pathogenesis- namely neuronal degeneration (Brooksbank and Balazs, 1984; Busciglio and Yankner, 1995). The mitochondrial respiratory system is the primary source of ROS, and dysregulation of the redox states may induce sequential apoptosis. Several lines of evidence implicate multiple HSA21 genes in mitochondrial dysfunction and DS/AD neuropathology. APP is a membrane protein associated with neuronal survival and overexpression of APP and its products Abeta has been attributed to neurodegeneration in DS and AD (Conti et al., 2010; Isacson et al., 2002; Salehi et al., 2006). APP and Abeta are located in mitochondria (Devi and Anandatheerthavarada, 2010; Manczak et al., 2006). Abeta peptides induce mitochondrial

dysfunction and oxidative stress in astrocytes leading to cell death in neurons (Abramov et al., 2004). Impairments in mitochondrial function in astrocytes also alter metabolism and secretion of neuroprotective APP and increase neuronal vulnerability (Busciglio et al., 2002). Mitochondrial dysfunction, however, does not require aberrant APP and Abeta processing specifically. The Ts1Cje DS mouse model, whose partial trisomy 16 does not include the APP gene, also exhibits diminished mitochondrial activity (Shukkur et al., 2006). These mice show decreased mitochondrial membrane potential and ATP production, increased ROS, and increased GSK3ß and JNK/SAPK activities. GSK3ß preserves mitochondrial function, and inhibits Ca(2+)-induced mitochondrial permeability (Mio et al., 2009). In addition to GSK3ß, several other genes within the Ts1Cje trisomic segment are thought to be involved in mitochondrial function. DSCR1 has been reported to be located in the mitochondria and to play a critical role in mitochondrial function (Chang and Min, 2005). DYRK1A can phosphorylate tau and APP (Park et al., 2007; Woods et al., 2001), and the transcription factor ETS-2 increases APP production and promotes the activation of a mitochondrial death pathway in DS neurons (Helguera et al., 2005; Wolvetang et al., 2003a; Wolvetang et al., 2003b). S100B is another HSA21 gene that can increase ROS, it is a calcium binding protein that affects calcium-dependent signals, targeting mitochondrial proteins such as p53 and ATPase ATAD3A, thereby assisting the cytoplasmic processing of proteins for proper folding and subcellular localization. S100B overexpression can also lead to p53 inactivation (Donato, 2003; Gilquin et al., 2010; Leclerc et al., 2010; Lin et al., 2001; Mihara et al., 2003). Finally, similar mechanisms involving mitochondrial dysfunction have been proposed with other HSA21 genes including SOD1 and BATCH1 (Arbuzova et al., 2002; Ferrando-Miguel et al., 2003; Furuta et al., 1995; Harris-Cerruti et al., 2004; Iannello et al., 1999; Lott et al., 2006; Shim et al., 2003). The added, combinatorial effects of these various HSA21 genes in oxidative stress, apoptosis in neurons and neuronal progenitors are not known.

While mitochondrial dysfunction, oxidative stress, and subsequent apoptosis have been well appreciated in neurons, only recently changes have been reported in DS HNPs. We have shown that upregulation of glial-associated proteins such as S100B lead to tau and GSK3ß hyperphosphorylation, suggesting loss of mitochondrial function (Esposito et al., 2008a; Esposito et al., 2008b). HNPs over-express the HSA21-localized S100B, leading to increased ROS formation such as hydrogen peroxide and nitric oxide, activation of stress response kinase pathways (JNK/STAT), and the upregulation of the glial-associated water channel AQP4 (Esposito et al., 2008a). This same functional network also predicts an added contribution through HSA21 localized APP. Both APP and S100B are upregulated in DS HNPs, they reciprocally enhance the expression of one another, have an additive effect on increasing hydrogen peroxide, decreasing mitochondrial membrane potential and increasing apoptosis (unpublished data). The co-localization of APP and S100B within the same HNPs along VZ of cortex may make progenitors even more vulnerable to oxidative stress, which is supported by the fact that increased progenitor cell death is seen at nano or pico level of S100B or Abeta stimulation (unpublished observations). These same doses are neuroprotective in more mature neurons (Lambert et al., 1998; Van Eldik and Wainwright, 2003; Yankner and Lu, 2009). The additive effects from S100B and APP may depend upon their common activation through the extracellular RAGE receptor which has been shown to regulate intracellular mitochondrial function (Devi and Anandatheerthavarada, 2010; Donato, 2003; Leclerc et al., 2010; Manczak et al., 2006). The *in vitro* observations from DS HNPs are consistent with *in vivo* observations in polytransgenic mice. S100B/APP double transgenic (Tg2576/APP-huS100B) mice display augmented reactive astrocytosis and

microgliosis, increased levels of pro-inflammatory cytokines and enhanced apoptosis (Mori et al., 2010). The double transgenic (mutant APP (mAPP)/RAGE) mice demonstrate increased activation of stress pathways (phosphorylation of p38 and JNK) and altered expression of markers of synaptic plasticity (MAP kinases), leading to early abnormalities in spatial learning/memory (Arancio et al., 2004).

4.2 Gliosis and inflammatory changes

Increased astrocytes and gliosis have previously been reported in DS brain (Guidi et al., 2008). Altered redox states and mitochondrial compromise leading to cell death are thought to promote these inflammatory changes in mature DS brain (Griffin et al., 1989). In DS the increase in the glial marker S100B could be explained, in part, by a gene dosage effect and in part by reactive gliosis (Selinfreund et al., 1991).

A shift in the DS progenitor pool may contribute to the neuronal reduction and increase in glial cells within the mature trisomy 21 brain. Some studies have suggested impairments in interneuron neurogenesis within the DS HNP pool, possibly due to over-expression of the transcription factor COUP-TF1/NR2F1 (Bhattacharyya et al., 2009). Increased levels of COUP-TF1/NR2F1 cause increases in the generation of earlier born neurons and depletion of later born interneurons. Alternatively, other reports suggest that HSA21 associated DYRK1A causes a skewed ratio of primitive endoderm at the expense of neuroectodermal progenitors, leading to a reduction in neurogenesis (Canzonetta et al., 2008). Mensah also reported an inhibition of neuroectodermal differentiation in a teratoma model transplanted with mouse ES cells injected with HSA21 (Mensah et al., 2007). Our prior studies of DS HNPs had demonstrated a constitutive increase in the glial and HSA21 associated S100B protein, which in turn, enhanced expression of another glial-associated protein, the water channel AQP4. Aquaporins can partially mitigate damage due to ROS, such that glial progenitors are more resistant to oxidative damage (Esposito et al., 2008a). In this setting endogenous overexpression of a glial-associated gene S100B might drive gliogenic progenitors, while the inflammatory changes from oxidative stressors would further promote a gliocentric progenitor pool. Other candidate genes such as HSA21-localized Olig1 and Olig2 are basic helix-loop-helix (bHLH) transcription factors essential for development of oligodendrocytes (Jakovcevski and Zecevic, 2005; Lu et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002) or astrocytes (Cai et al., 2007; Marshall et al., 2005; Ono et al., 2008); and SYNJ1 could increase astrogliosis (Herrera et al., 2009). The number of Olig1/Olig2(+) progenitors increases in the injured CNS (Arnett et al., 2004), and Olig2(+) cells preferentially differentiate into GFAP-expressing astrocytes, the main contributors to glial scars which further secrete S100B (Chen et al., 2008; Tatsumi et al., 2008). S100B also contributes to oligodendrocyte progenitor cells (OPC) differentiation in response to demyelinating insults (Deloulme et al., 2004). In addition to transcription factors, epigenetic regulation can also control the cell fate changes within progenitors. One possible candidate gene in DS is HSA21 associated DNMT3L. DNMT3L is a DNA methyltransferase like gene playing a role in DNA methylation by activating DNMT3A or DNMT3B (Jia et al., 2007; Ooi et al., 2007; Suetake et al., 2004). It also represses transcription by recruiting histone deacetylase (HDAC) (Aapola et al., 2002; Deplus et al., 2002). Hypomethylation in neural progenitors leads to precocious astroglial differentiation and perturbed neuronal maturation in DNMT1 or DNMT3A knockout mice (Fan et al., 2005; Hutnick et al., 2009), whereas HDAC activation increases oligodendrocytes differentiation (Hsieh et al., 2004; Liu et al.,

2009; Liu and Casaccia, 2010; Shen et al., 2005; Ye et al., 2009). Again the combinatorial effects from these various HSA21 genes associated with cell fate changes are not known.

4.3 Prolonged cell cycle and reduced proliferation

Proliferation changes during development have been thought to contribute to the decreased neuronal numbers and reduced cortical volume in DS brains. Recent studies demonstrated impairments in proliferation within both hippocampus and neocortical germinal matrix of 17 weeks to 21 weeks gestation age fetal DS brains (Contestabile et al., 2007; Guidi et al., 2008). The DS progenitor cell cycle is prolonged with more cells remaining in G2 phase, thereby causing a reduction in neurogenesis and presumptive increase in astrocytosis. Similar endophenotypes are also found in animal models, which show a reduced progenitor pool and neurogenesis in ventricular zone or dentate gyrus (Contestabile et al., 2009; Haydar et al., 2000; Lorenzi and Reeves, 2006; Moldrich et al., 2009). The molecular mechanisms behind G2 phase prolongation are not known although many genes on HSA21 can cause proliferation changes. Overexpression of APP would antagonistically compete with the APP-BP1, which is required for the cell cycle progression from G1 to S phase. This change would lead to a predicted slowing in proliferation but at the G1 to S phase (Joo et al., 2010). Overexpression of S100B could promote p53 nuclear accumulation and inhibit proliferation (Scotto et al., 1999). Olig2 overexpression has been shown to increase CDK inhibitor p27, leading to decreased proliferation (Tabu et al., 2006). Presumably, various HSA21 genes may directly act in a combinatorial fashion to affect progenitor proliferation.

Studies from human DS HNPs raise another potential explanation for altered progenitor proliferative rates. Consistent with previous reports, we have also found reduced proliferation in HNPs, localized along the ventricular zone of DS cortex, and within DS HNPs cultured *in vitro*. However, characterization of these progenitors have shown that these progenitors, while retaining neural progenitor markers, are more gliocentric in their expression profile. Some preliminary studies suggest that the DS HNPs have reduced voltage gated, outward rectifying potassium channel activity, which correlated to decreased proliferation. Increased potassium channel activity has been correlated with increased proliferation, whereas decreased channel activity coincides with oligodendrocyte differentiation (Chittajallu et al., 2002). Taken in this context, changes in progenitor cell fates (due to such HSA21 genes as S100B, APP, and Olig2) may in part explain the reduction in cell proliferation.

4.4 Imbalance of excitatory-inhibitory neurotransmission

The balance of neurotransmission is important for maintaining normal physiology and behavior. Imbalance of excitatory-inhibitory neurotransmission could contribute to the MR phenotypes seen in DS. Prior studies have shown decreased excitatory and increased inhibitory activities in DS neurons or Trisomy 16 mice. The excitatory neurotransmitter glutamate and inhibitory neurotransmitter GABA are both decreased in DS fetal brain (Reynolds and Warner, 1988; Risser et al., 1997; Smigielska-Kuzia et al., 2010; Whittle et al., 2007). Ts65Dn mice show increased inhibitory synapses and decreased excitatory synapses in the hippocampus (Belichenko et al., 2009; Belichenko et al., 2004; Kurt et al., 2000; Perez-Cremades et al., 2010). Excessive GABA inhibition also alters LTP in the hippocampus (Costa and Grybko, 2005; Kleschevnikov et al., 2004; Siarey et al., 1997). Likewise, Ts1Cje mice show a shortened long term potentiation and an increase in long term depression, as

well as a decrease in the evoked field excitatory postsynaptic potentials- all consistent with impairments in short and long term plasticity (Siarey et al., 2005).

Various HSA21 genes could theoretically affect synaptic function and thereby alter memory and learning. TINM1, SYNJ1 and ITSN1 are thought to play some role in synaptic plasticity, KCNJ6, KCNJ15, KCNE1 and KCNE2 would alter channel activity, whereas NRIP1, ETS2, PCP4, DSCR1, DYRK1A, S100B and APP have some role in affecting neurotransmitter receptors or intracellular signaling. Another more closely scrutinized HSA21 gene GIRK2 (KCNJ6), an inwardly rectifying K channel, could contribute to synaptic inhibition. GIRK2 channels are overexpressed in Ts65Dn hippocampus and frontal cortex (Harashima et al., 2006). The overexpression leads to a significant increase in GABAB-mediated GIRK current in primary cultured hippocampal neurons, and likely affects the balance between excitatory and inhibitory neuronal transmission (Best et al., 2007). Pharmacological treatment of the trisomy 16 mice with GABAA antagonists at non-epileptic doses causes a persistent post-drug recovery of cognition and LTP (Fernandez et al., 2007). Interestingly, treatment of these same mice with the uncompetitive NMDAR antagonist memantine rescues the performance deficits on a fear-conditioning test (Costa et al., 2008). Memantine mimics calcineurin action and the HSA21 DSCR1 gene inhibits calcineurin activity. This raises the hypothesis that DSCR1 overexpression produces a pathological increase in NMDAR activity.

Earlier developmental changes in DS HNPs could also contribute to the imbalance of excitatory and inhibitory neurotransmission. Alterations in cell fate through genes such as Olig1 and Olig2 could lead to an abnormal ratio or distribution of excitatory and inhibitory neurons. Overexpression of HSA21 located Olig1 and Olig2 have been shown to increase GABAergic interneuron neurogenesis in Ts65Dn mice, and the dysregulation of excitatory-inhibitory imbalance could be rescued by knocking down the extra copy of these two genes (Chakrabarti et al., 2010). This phenotype contrasts with our observations and those from other groups, who have observed a reduction of interneurons in DS HNPs at the expense of increasing oligodendrocytes (Bhattacharyya et al., 2009). We see a shift from neuronal to gliocentric progenitors with overexpression of S100B (Esposito et al., 2008a) and APP (unpublished observations), which would potentially further compromise the interneuron population. This decline in interneuron neuronal production is consistent with the loss in GABA-ergic interneurons, as opposed to total neuronal numbers, reported in the DS cortex, further reiterating differences between the mouse and human disorders (Golden and Hyman, 1994; Ross et al., 1984; Weitzdoerfer et al., 2001).

5. Combinatorial gene effects in DS HNPs contribute to the DS phenotype

HSA21 associated genes which are implicated in cell death and oxidative stress could contribute to the later endophenotypes seen in DS. To identify functional modules of gene expression and interacting partners relevant to DS HNPs, we had applied network-based analyses through the Ingenuity Pathways Knowledge Base (IPA) (Calvano et al., 2005). Among 1902 genes that were significantly different on pairwise comparison between WT-DS expression profiling of HNPs, 334 genes were shown to interact within 46 functional networks (Esposito et al., 2008a). The top four interactive networks incorporated 93 dysregulated genes involved in cell cycle, cell death, oxidative stress and several canonical signal transduction pathways. These findings would suggest that genes involved in these primary functional clusters might later contribute to the observed DS endophenotypes.

To understand the role of HSA21 genes in progenitor cell death and changes in redox state, we first focused on HSA21 genes APP and S100B. These molecules were included in one of the top functional clusters and were associated with other cell signaling molecules, including JAK/STAT and MAPK, which have been implicated in the stress response pathway, inflammation and potentially gliosis. Using this potential pathway, we found that upregulation of S100B activated the JAK/STAT MAPK pathway through the RAGE receptor. RAGE receptor activation induced mitochondrial disruption and oxidative stress. Activation of this pathway enhanced progenitor cell death and likely gliosis, as suggested by increased expression of glial associated water channel AQP4 (Esposito et al., 2008a). We have also been able to observe a S100B-mediated induction of tau protein hyperphosphorylation via Dickopff-1 up-regulation and disruption of the Wnt pathway, suggesting a shared common final pathway with APP (Esposito et al., 2008b).

Although APP and S100B have previously been implicated in oxidative stress and cell death, constitutive over-expression of these and other HSA21 genes would also predict upregulation of inflammatory responses (astrocytosis), leading to a skewed gliocentric progenitor phenotype. The ongoing cell death and inflammation from APP and S100B promote reactive astrocytosis and astrocytes secrete S100B, causing further cell injury and death (Li et al., 2011). This same mechanism of injury in the mature brain could also take place within the progenitor population during development. Additionally, the transcription factors Olig1/2 increase oligodendroglial progenitor numbers and would likely augment any gliocentric shift. Similarly, the HSA21 DNA methylthansferase like DNMT3L gene recruits HDACs, which compete with beta catenin to regulate TCF4 dependent transcriptional inhibition of neuronal progenitor proliferation and activation of oligoprogenitor phenotypes (Aapola et al., 2002; Deplus et al., 2002; Liu et al., 2009; Ye et al., 2009).

A gliocentric shift in the progenitor pool could alter rates of proliferation and create an imbalance in excitatory-inhibitory transmission. Gliocentric progenitors would be predicted to demonstrate reduced voltage gated, outward rectifying potassium channel activity, and consequent slower rates of proliferation. Moreover, the ongoing neural progenitor cell death from oxidative stress enhances gliocentric progenitor characteristics at the expense of neuronal progenitor phenotypes. This shift becomes more prominent later in cortical development when the cyclical and synergistic roles played by such inflammatory mediators such as S100B and APP become more pronounced. Under this paradigm, the loss in neuronogenic progenitors would manifest later in development and thereby affect interneuron production.

These observations raise a hypothetical paradigm whereby several specific HSA21-localized genes promote a deleterious, cyclical pathway involving ROS, hypersecretion of S100B, APP overproduction and proliferation of gliocentric progenitors. A gliocentric shift in the progenitor pool would provide a potential explanation for the predominant endophenotypes seen in the DS brain.

6. Pharmacological approach using DS HNPs

From a translational standpoint, human neurally-derived reagents will be useful to identify pathological mechanisms and address the efficacy of pharmacotherapeutics. Given that DS is a contiguous gene syndrome, multiple genes on HSA21 will interact, share substrates, and/or influence the same processes or pathways relevant to neural function. Our ongoing work demonstrates that some genes such as S100B and APP are over-expressed from

development into adulthood and would therefore have potentially cumulative effects over time. In this respect, treatment directed toward correcting aberrant pathways regulated by these genes may require early and ongoing management to achieve efficacy. Other genes which might also contribute to gliocentric phenotypes and which reside on HSA21 such as Olig1/2 appear to be more temporally restricted (personal observations, Lu and Sheen) and therefore might be more amenable to a finite pharmacological approach. It remains to be seen to what extent the observations made from various treatment modalities for trisomy mouse models and/or DS HNPs are faithfully replicated in actual individuals with DS. That said, the use of DS HNPs would provide another measure of validation for pharmocotherapies devised in treatment of these disorders.

7. Conclusion

DS is a contiguous gene syndrome giving rise to MR, dementia, and seizures. These clinical outcomes are mirrored by endophenotypes including mitochondrial dysfunction, oxidative stress, cell death, gliosis, inflammation, prolonged cell cycle, reduction in proliferation and imbalances in excitatory and inhibitory neurotransmission. While these characteristics have largely been observed in neurons in the mature brain, function based cluster analyses of pair-wise comparisons between normal and DS human neural progenitors suggest that similar changes are ongoing during development. Moreover, these endophenotypes likely arise from the integration of various genetic and epigenetic factors on chromosome 21 such as APP, S100B, Olig1/2 and DNMT3L. The ongoing disruption of mitochondrial redux states and inflammation promote early gliocentric phenotypes in the progenitor pool, which would alter progenitor proliferative rates and contribute to a decline in interneuron production. Overall, early developmental changes in the progenitor population could promote many of the deleterious changes seen later in the mature DS brain.

8. Acknowledgment

9. References

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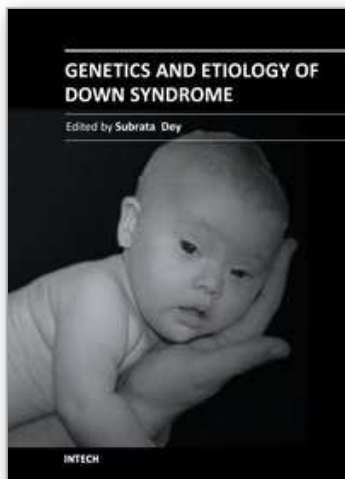
Genetics and Etiology of Down Syndrome

Edited by Prof. Subrata Dey

This book provides a concise yet comprehensive source of current information on Down syndrome. Research workers, scientists, medical graduates and paediatricians will find it an excellent source for reference and review. This book has been divided into four sections, beginning with the Genetics and Etiology and ending with Prenatal Diagnosis and Screening. Inside, you will find state-of-the-art information on: 1. Genetics and Etiology 2. Down syndrome Model 3. Neurologic, Urologic, Dental & Allergic disorders 4. Prenatal Diagnosis and Screening Whilst aimed primarily at research workers on Down syndrome, we hope that the appeal of this book will extend beyond the narrow confines of academic interest and be of interest to a wider audience, especially parents and relatives of Down syndrome patients.

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