Published in final edited form as:
Am J Med Genet C Semin Med Genet. 2006 August 15; 142C(3): 196–205.

The Proteins of Human Chromosome 21

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Abstract

Recent genomic sequence annotation suggests that the long arm of human chromosome 21 encodes more than 400 genes. Because there is no evidence to exclude any significant segment of 21q from containing genes relevant to the Down syndrome cognitive phenotype, all genes in this entire set must be considered as candidates. Only a subset, however, is likely to make critical contributions. Determining which these are is both a major focus in biology and a critical step in efficient development of therapeutics. The subtle molecular abnormality in Down syndrome, the 50% increase in chromosome 21 gene expression, presents significant challenges for researchers in detection and quantitation. Another challenge is the current limitation in understanding gene functions and in interpreting biological characteristics. Here, we review information on chromosome 21-encoded proteins compiled from the literature and from genomics and proteomics databases. For each protein, we summarize their evolutionary conservation, the complexity of their known protein interactions and their level of expression in brain, and discuss the implications and limitations of these data. For a subset, we discuss neurologically relevant phenotypes of mouse models that include knockouts, mutations or overexpression. Lastly, we highlight a small number of genes for which recent evidence suggests a function in biochemical/cellular pathways that are relevant to cognition. Until knowledge deficits are overcome, we suggest that effective development of gene-phenotype correlations in Down syndrome requires a serious and continuous effort to assimilate broad categories of information on chromosome 21 genes, plus the creation of more versatile mouse models.

INTRODUCTION

The development of pharmacological interventions to ameliorate cognitive deficits is a major goal in Down syndrome (DS) research. A rational approach to the design of such therapeutics requires knowledge of the number and function of the genes encoded by chromosome 21 (chr 21). Gene identification, by computational and experimental analysis of human genomic DNA, although still an ongoing process, is probably largely complete for conventional protein coding genes. Comparison with orthologous mouse genomic regions suggests that there are approximately 170 chr 21 genes with clear orthologs coding for proteins in rodents [Gardiner et al 2003]. Other chr 21 genes are not so clearly conserved and many likely do not code for proteins. While this latter group (numbering more than 200) and other non-conventional transcripts [Carninci et al 2005] must be further investigated,

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and indeed may be of critical importance, this article focuses on current information regarding the conserved protein coding genes.

Previous reports have classified chr 21 genes in limited functional categories, such as kinases, transcription factors, those encoding transmembrane domains, etc. [Gardiner 2003; Antonarakis et al 2004]. However, in generating hypotheses regarding a gene's contribution to the DS phenotype, it is reasonable to consider additional classifications and the implications of the resulting information. We have gathered information on chr 21 genes from the literature and from genomics and proteomics databases. For each chr 21 protein, we provide information on their evolutionary conservation in nine organisms from yeast to chimpanzee and the number of their orthologous protein interactions in three organisms, Drosophila, *C. elegans* and *S. cerevisiae*. We also describe the number of known human protein interactions and their relative level of expression in brain. For subsets, we describe the viability and neurological phenotypes of mouse models and participation in pathways involved in learning and memory. These datasets suggest that there are no absolute criteria that allow us to predict a contribution of any chr 21 protein to cognitive deficits in DS. Datasets are incomplete, however, and our biological understanding of their meaning is limited. As data from functional genomics and proteomics projects in model organisms grow, correlations among datasets will emerge and may be expected to lead to helpful insights.

METHODS

The current chr 21 gene list was obtained by merging that of Gardiner et al [2003] with the chr 21 gene lists found in the NCBI Gene database and the H-InvDB database [Imanishi et al 2004]. The gene list, gene structures and lists of orthologous genes in mouse can be found in the Chr 21/DS gene function database (http://chr 21db.cudenver.edu). To identify orthologs, those proteins in other organisms that are most similar to chr 21 proteins were included. Each chr 21-encoded protein sequence was used in BLASTP searches of *S. cerevisiae*, *C. elegans*, Drosophila and zebrafish protein sequences (http://www.ncbi.nlm.nih.gov/BLAST/). A statistically significant match was defined as having an expectation value less than 10^{-3}, identity greater than 20% over at least 30% of the chr 21 protein, and the presence of the same predicted functional domains in both proteins. For each significant match, a second BLASTP search, using the model organism protein against all human proteins, was carried out. If the best match in this reciprocal search was the original chr 21 protein, it was concluded that the model organism protein was the ortholog. If it was not a chr 21 protein, i.e. if there was a non chr 21 human protein with better sequence similarity, then it was concluded that the chr 21 protein is not the ortholog of that organism. In several cases, the chr 21 protein was one of two or more human proteins with essentially equal similarity to the model organism protein. This suggests that the function of the ancestral protein has expanded and/or been split among multiple human proteins, which are termed co-orthologs.

Data on chr 21 protein substrates and protein interactions were compiled from the Human Protein reference database HPRD (http://www.hprd.org) by searching the chr 21 protein gene name. Some additional interaction data were obtained from the Biomolecular Interaction Network Database (BIND, http://bind.ca), using GenBank accession numbers from chr 21 mouse or rat orthologs. Expression levels in brain, as sequences per million in brain-derived cDNA libraries, were obtained from UniGene profiles at http://www.ncbi.nlm.nih.gov/UniGene. Protein interaction data for Drosophila, *C. elegans* and yeast were obtained from the General Repository for Interaction Datasets (The GRID, http://biodata.mshri.on.ca/grid/servlet/Index) by searching with the GenBank accession

number or the species-specific gene name for the proteins identified in reciprocal BLASTP searches above.

RESULTS

i) Evolutionary conservation

Approximately 170 chr 21 genes encode open reading frames that are conserved in orthologous regions of mouse chromosomes 16, 17 and 10 [Gardiner et al 2003]. In Supplementary Table I, we summarize conservation of these proteins in additional model organisms, the yeasts, *S. cerevisiae* and *S. pombe*; *C. elegans*, Drosophila, zebrafish, pufferfish, chicken, dog and chimpanzee. The proteins in this table are defined as orthologs of chr 21 proteins based on results of standard sequence similarity searches. For DS research, there are practical implications for this information. Orthologs may retain significant functional characteristics in common with their evolutionary ancestral genes. Results of large scale functional genomics and proteomics projects in progress in many model organisms can help to predict functions of chr 21 proteins and to generate hypotheses regarding their contributions to DS cognitive deficits.

For distantly related organisms, 26 orthologs of chr 21 proteins were identified in *S. cerevisiae*, 45 in *C. elegans*, and 59 in Drosophila. Of *S. cerevisiae* orthologs, most encode enzymes in biochemical pathways. For others, such as *CHAF1B* and *MCM3AP*, which may play roles in chromatin structure and DNA replication, and *PWP2*, described only as a "Periodic Tryptophan Protein", analysis in yeast provides an ideal system to examine otherwise novel protein sequences. For example, systematic knockout of each yeast gene [Giaever et al 2002] has shown that seven chr 21 orthologs (including *MCM3AP*, *PWP2* and the anonymous *DSCR5*) are lethal when knocked out and that 13 are associated with growth abnormalities. For orthologs in *C. elegans* and Drosophila, information is being generated on phenotypes of knockdowns, and decreases in expression by RNAi techniques [Kamath et al 2003; Flockhart et al 2006]. These will provide information related to function. However, directly mimicking the consequences of trisomy, a modest 50% increase in protein expression, based on gene dosage, is more challenging. Trisomy may result in only a modest perturbation of the pathways in which a protein functions, with only subtle consequences for cell or organism viability. One example of this scenario is illustrated by the overexpression of the *DSCR1* ortholog (*nebula*) in Drosophila. In this case, flies demonstrated impairment in spatial learning [Chang et al 2003]. Functional information from knockouts and knockout-downs may help to identify promising candidates on chr 21 for further study.

In considering conservation data from vertebrates, there is an initial caution (with the exception of mouse) that genomic sequence is not yet of the highest quality and may still contain misassembles and gaps that confound gene identification. Annotation for gene content has not been subject to high quality manual curation, and thus, not all orthologs may have been identified.

Large scale experiments in zebrafish designed to identify genes that are embryonic lethal when knocked out have been performed. These experiments found five chr 21 proteins: the splicing factor, *U2AF1*, the mitochondrial ATPase subunit, *ATP50*, *MCM3AP*, *D21S2056E* (*NNP1*) and *c21orf59* [Amsterdam et al 2004]. While none of these proteins are vertebrate-specific, *c21orf59* is of interest because this is the only one with data currently available relating to its function.

More than 40 proteins were identified with orthologs in fish (zebrafish and/or pufferfish) but not Drosophila, therefore suggesting vertebrate specificity. One hundred chr 21 proteins are potentially restricted in conservation to chicken and mammals (subject to further refinement

of zebrafish genomic sequence annotation). While this is clearly a very broad distinction, it does allow prioritization of such genes as candidates for involvement in higher order organism functions that would include brain development or cognitive function. Interesting examples of bird/mammal specific proteins include the transcription factor BACH1, the transcription regulator, SON, the nuclear hormone receptor inhibitor, NRIP1, two potassium channels, as well as several proteins currently lacking all functional associations.

For defining what is uniquely human in brain development and function, and therefore for identifying a different set of candidates for association with cognitive deficits in DS, comparisons with chimpanzee hold the most promise among model organisms with genome projects. In this case, lack of conservation in chimpanzee was a logical target; however, the recent publication of the complete sequence of chimpanzee chromosome 22 reported no human-specific chr 21 proteins [Watanabe et al 2004]. Subtle protein sequence differences were observed, however, and some are likely functionally significant. The possibility that some represent polymorphisms and not species-specific differences is an important consideration that remains to be investigated. Alternatively, important human-specific features may lie at the level of expression. Two gene products, the coxsackievirus and adenovirus receptor (*CXADR*) and an anonymous protein *C21orf33*, both of completely unknown function, showed higher expression levels in the human cortex versus the chimpanzee cortex [Caceres et al 2003].

ii) Protein-protein interactions

Clearly, no protein functions in isolation. Identifying the interaction partners of chr 21 proteins will help to define the complexes, biochemical pathways or other cellular/tissue processes in which the proteins function. These would be the processes that might be perturbed with overexpression of chr 21 proteins in trisomy. Extensive protein interaction data derived from the whole genome experiments are being carried out in *S. cerevisiae*, *C. elegans* and Drosophila, where literally tens of thousands of pairwise yeast-two-hybrid comparisons are being done to define complete “interactomes” [Tong et al 2004; Li et al 2004; Giot et al 2003]. Supplementary Table I includes the numbers of interaction partners so far reported for each chr 21 ortholog. Identities of each interactor can be found at http://chr 21db.cudenver.edu and at the GRID database (see Methods). Several points regarding these findings are noteworthy.

First, a caution: such large scale approaches and the techniques involved (generally including yeast-two-hybrid) are known to generate false positives [Jansen and Gerstein, 2004]. However, where high confidence data have been obtained, and when verification is carried out, new networks and interaction partners can be identified or predicted involving orthologs of chr 21 proteins. While it cannot be assumed that protein interactions are conserved among such divergent organisms, these data can be used to generate novel and testable hypotheses [Lehner and Fraser 2004]. To aid in this, each model organism interaction network can be “humanized”, by using sequence similarity searches as above to identify the best human match of each component (http://chr 21db.cudenver.edu).

Second, a number of groups have reported that the interaction networks developed from these types of experiments show that a small number of proteins participate in a large number of interactions, while the large majority of proteins participate in only one or a few [Yu et al 2004]. This led to the definition of so-called “hub” proteins and the hypothesis that these will be of critical importance in cell function. Perturbation of their concentration, as in trisomy, may have the most significant consequences. Six Drosophila and five *S. cerevisiae* orthologs have been identified to have ten or more interaction partners. Among these, the protein kinase, DYRK1A, was identified with 52 interactions in Drosophila and 18 in *S.*

cerevisiae. Interaction datasets are far from complete; monitoring their growth will continue to provide new information regarding chr 21 genes.

In humans, while large scale interactome experiments are in progress [Rual et al 2005], considerable data already have accumulated from detailed gene-specific experiments. These data are available from the literature with painstaking searches and from specialized databases where the searches are already completed and manually curated by experts in molecular biology (e.g. HPRD and BIND). Supplementary Table II includes the number of interactions so far identified for each of the 170 chr 21 proteins. The nuclear hormone receptor interacting protein, NRIP1, and the guanine nucleotide exchange factor, TIAM1, participate in more than 20 interactions, and the Alzheimer's disease amyloid precursor protein, APP, participates in as many as 56. Thirteen other proteins have ten or more interaction partners. Analyses are not exhaustive, so the existence of additional interaction partners is assumed. In related information, ten substrates for the ser-thr kinase DYRK1A (mentioned above) have been identified. Overexpression of these well connected proteins has the potential of perturbing, perhaps subtly, numerous processes. Few of the potential "hub" proteins have received significant attention in research specifically directed to DS.

iii) UniGene expression in brain

Expression profiles for transcripts of the chr 21 protein coding genes conserved in mouse were obtained from UniGene. In human data, most genes appear widely expressed, found in all or most of the 31 tissues represented, which include fetal as well as adult. Sixteen genes showed expression that was limited to brain or to brain plus only 2–5 other tissues. Relative brain expression levels (in number of sequences per million found in dbEST brain cDNA libraries) are listed in Supplementary Table II. Fifteen genes appeared not to be expressed in brain, although expression for each was clearly demonstrable, if not high, in multiple other tissues. *TSPEAR/C21orf29* did not show expression in brain, but only very low levels (less than ten copies per million) in lung, ovary and testes. However, a BLASTN search of dbEST identified a single clone derived from a differentiated neuronal cell line, suggesting that *C21orf29* may indeed be expressed in brain. While not conclusive, this is worth consideration because *C21orf29*, although of unknown function, is vertebrate specific, encodes amino acid motifs common to proteins mutated in epilepsies [Scheel et al 2002] and is within a region recently linked to susceptibility to bi- and unipolar affective disorders [McQuillin et al 2005].

Additional information on brain expression is available for many mouse orthologs. As well as dbEST-derived UniGene profiles, data have been generated on embryonic expression by tissue in situ [Reymond et al 2002] and for multiple tissues by RT-PCR [Lyle et al 2004]. These techniques differ in sensitivity and specificity, and the lack of expression cannot be considered definitive. Low levels of expression and expression restricted in developmental time or cell type remain a challenge to detect and yet may have critical consequences in brain function.

iv) Phenotypic consequences in mouse models

Table I summarizes the phenotypic features of mouse models created for 16 orthologs of chr 21 genes. What such models can and cannot tell us regarding a gene's potential contribution to cognitive deficits in DS is not a trivial issue. First, knockouts that eliminate a chr 21 protein, and mutants that alter its sequence, may inform us about normal protein function from the processes, including learning and memory that are disrupted. This is valuable information but does not allow the prediction of some "opposite" phenotype in trisomy, i.e. a process that is inhibited in a knockout of a gene is not predicted to be enhanced by increased expression of the same gene in trisomy. Second, overexpressing transgenics

generated from cDNA constructs lack normal regulation of expression in time and place and normal protein isoforms produced by alternative splicing. Most often, they also lack the modest 50% increase in protein level expected in DS, and instead show increases of many fold, preventing direct extrapolation to DS. Third, overexpressing transgenics generated from genomic constructs, e.g. BAC clones, overcome the problems of cDNAs, but still lack another important feature: the genomic context of DS where many genes are overexpressed simultaneously. If chr 21 proteins mutually interact, directly or indirectly, and many do (see below), the effects of overexpression of these interactions will not be recapitulated in single gene transgenics. With these caveats in mind, data on mouse models of several chr 21 proteins is worthy of consideration with respect to DS cognitive deficits.

Knockouts of six genes in Table I are lethal. Of these, the phosphoinositol phosphatase, *SYNJ1*, and the protein kinase, *DYRK1A*, are conserved down to yeast and have 18 and 13 interactions/substrates, respectively, so far identified. [Slepnev and De Camilli, 2000;Cremona et al 1999;Fotaki et al 2002].*OLIG1* and *OLIG2*, basic helix-loop-helix transcription factors induced by Sonic Hedgehog, are conserved only among vertebrates [Ross et al 2003]. Knockouts of *Olig1* die at P14. In brain, they lack all expression of myelin specific genes and develop progressive axonal degeneration [Xin et al 2005]. Knockouts of *Olig2* die at birth. They lack oligodendrocytes and motorneurons in the spinal cord, and instead show expression of the astrocyte marker S100B (a chr21 gene) in neuroepithilial cells [Takebayashi et al 2002]. One can speculate that *OLIG1* and *OLIG2* may play a role in the delayed central myelination that has been observed by both neuropathologic analysis and magnetic resonance imaging (MRI) in the brains of children with DS [Koo et al., 1992]. *ADARB1* (*ADAR2*) is a pre-mRNA adenosine deaminase that carries out site-specific editing of the pre-mRNAs for the serotonin 2C receptor, a potassium channel and several ionotropic glutamate receptors, as well as several other less well studied proteins and numerous non-coding sequences [reviewed in Valente and Nishikura, 2005;Levanon et al 2005]. Although knockouts of *Adar2* suffer seizures and die between P0 and P20 [Higuchi et al 2000], lethality can be rescued by an appropriately edited version of the GRIA2/GluRB glutamate receptor subunit alone. Lastly, the mammalian transcription factors, *SIM1* (a chromosome 6 gene) and *SIM2* (chr 21), are orthologs of the Drosophila *single-minded* gene. In flies, loss of *single-minded* produces a drastic neurological phenotype characterized by a complete loss of CNS midline cells [Nambu et al 1990]. The mouse knockout of *Sim2* displays craniofacial abnormalities and dies by P3 from lung and breathing defects [Shamblott et al 2002;Goshu et al 2002]; abnormal neurological features have not been described.

Increased incidence of seizures, which is also seen in children with DS, was observed in knockouts of three other genes: the inwardly rectifying potassium channel subunit gene, *Kcnj6/Girk2* [Signorini et al 1997], the thiol-protease inhibitor gene, cystatin B, *Cstb* [Pennacchio et al 1998], and a Ca- and Zn-binding protein and a marker for astrocytes, S100B [Dyck et al 2002].

For many genes, the phenotype of a knockout is difficult to predict from the complexity of protein interactions or depth of conservation. A knockout of *App*, mutated in some forms of Familial Alzheimer's Disease (AD), participating in 56 protein interactions, and conserved in Drosophila and *C. elegans*, resulted in a relatively mild phenotype. Mice were viable and described as largely normal, but showed abnormalities in the long-lasting synaptic plasticity phenomenon known as long-term potentiation (LTP) in some assays and impairment on tests of conditioned avoidance and spatial learning [Dawson et al 1999; Fitzjhon et al 2000; Herber et al 2000]. The aspartyl protease, *Bace2*, is of interest because it can participate in the processing of APP. As a single gene knockout, it showed no significant phenotype, but in a double knockout with *Bace1*, the primary β-site cleavage enzyme for APP, it increased the frequency of the *Bace1* knockout neonatal lethality [Dominguez et al 2005]. A knockout

of the glutamate receptor subunit gene, *Grik1/Glur5*, was also viable and largely healthy [Mulle et al 2000], however, phenotypic features include a reduced sensitivity to pain [Ko et al 2005]. *Grik1* is present in an extra copy in the DS mouse model, Ts65Dn, which similarly has been reported to have reduced pain sensitivity [Martinez-Cue et al 1999]. Furthermore, although individuals with DS are not insensitive to pain, they express pain or discomfort more slowly and less precisely than the general population [Hennequin et al., 2000].

Knockouts of other genes associated with large numbers of interactions and/or high evolutionary conservation so far lack assessment of neurological phenotype. These include (i) the guanine nucleotide exchange factor specific for Rac, *Tiam1*, which is conserved in Drosophila and has 23 documented interactions in human; TIAM1 has been studied largely in tumor development [Malliri et al 2002], (ii) the nuclear hormone receptor inhibitor, *Nrip1*, has no orthologs beyond mammals; NRIP1 participates in 21 protein interactions in human, but has been studied largely in relation to fertility [White et al 2000], and (iii) the inhibitor of calcineurin, *Dscr1* (calcipressin), which is conserved down to yeast has been analyzed in relation to heart function [Vega et al 2003].

Of the genes listed in Table I, only five have been examined in transgenic mice that overexpress the chr 21 protein or its mouse ortholog. The *APP* gene has been extensively studied because of its association with AD, but few experiments have been designed to examine overexpression of a normal protein; instead, mutated proteins have been studied. Perhaps the clearest demonstration of the importance of APP in DS is from studies of the Ts65Dn mouse model. This mouse is trisomic for 94 chr 21 conserved protein coding genes, including *App*. The Ts65Dn exhibits abnormal early endosomes, in particular in the basal forebrain, a region which subsequently develops age-related loss of functional markers, features of both AD and DS. Cataldo et al [2003] showed that the abnormal endosomes were eliminated by removal of the extra copy of *App*. Amelioration of learning/memory deficits has not been described.

Increased expression of the protein kinase DYRK1A seems to be clearly implicated in learning/memory; transgenic mice constructed from genomic and cDNA constructs display deficits in spatial learning, plus molecular abnormalities predicted from known substrates [Smith et al 1997; Branchi et al 2004; Altafaj et al 2001]. Phenotypic features of mice overexpressing S100B include hyperactivity, lack of habituation to novel environments, impairment in spatial learning, and increased expression of markers associated with neuropathological aging [Nishiyama et al 2002; Shapiro et al 2004]. S100B is expressed at highest levels in astrocytes and is believed to function in cellular energy metabolism, cell proliferation and differentiation. In the transgenic mice, S100B is expressed at levels of 5–8 fold greater than normal, so it is not an ideal model for DS. In vitro, low levels of S100B enhance neuronal survival, while high levels induce apoptosis [reviewed in Rothermundt et al 2003]. Mice overexpressing the transcription factor, SIM2, displayed deficits in the Morris Water Maze test of spatial learning [Ema et al 1999] and decreased sensitivity to pain [Chrast et al 2000]. Of the several models overexpressing SOD1 that have been reported, an interesting study combined increased SOD1 with increased APP [Harris-Cerruti et al 2004]. The double transgenics showed an age-related deterioration in working memory.

v) Pathways involved in learning and memory

Chr 21 proteins are predicted to have direct and indirect effects on pathways and individual proteins required for learning and memory.

Estrogen and glucocorticoid signaling—Estrogen has been shown in some studies to protect against AD [Dhandapani and Brann 2002]. The chr 21 protein, NRIP1, a nuclear receptor inhibitor protein, inhibits the estrogen receptor [Cavailles et al 1995; Teyssier et al

2003] and has been shown to be overexpressed in hippocampi from aged individuals with DS [Gardiner, 2005]. Effects of normal levels of estrogen may therefore be diminished in persons with DS, possibly contributing to early menopause and associated cognitive decline. NRIP1 also inhibits the glucocorticoid receptor (GR) [Subramanian et al 1999]. Mouse models with genetic modifications that decrease GR levels display hippocampal abnormalities consistent with DS [Oitzl et al 2001]. A second chr 21 protein, SUMO3, also inhibits GR [Holmstrom et al 2003], possibly compounding the negative effects of increased NRIP1 activity.

MAP kinase and calcineurin signaling—Phosphorylation and dephosphorylation are mechanisms to control protein function without altering protein levels. The Mitogen Activated Protein kinase (MAPK) pathway is a cascade pathway that phosphorylates proteins and calcineurin is a phosphatase that dephosphorylates a partially overlapping set of proteins. Ras, ERK1/2, Creb, GR and Elk are examples of MAPK and calcineurin targets for which altering the phosphorylation levels, either by genetic or pharmacological manipulation, has been shown to affect learning and memory [Sweatt, 2004; Mansuy, 2003]. Multiple chr 21 proteins impact components of these pathways [for reviews see Gardiner et al 2004; Gardiner, 2005]. Chr 21 proteins TIAM1 and ITSN1 indirectly perturb MAPK activity, via Rac, Ras and JNK. If MAPK activity is altered, activation levels of ERK1/2 and targets Creb, Elk and GR also will be altered. DSCR1 is a direct inhibitor of calcineurin [Rothermel et al 2000], and PCP4 and SOD may indirectly alter calcineurin activity by modulating calmodulin (required for calcineurin activity) and by producing hydrogen peroxide (an inducer of DSCR1), respectively [Putkey et al 2003; Lin et al 2003]. DYRK1A directly phosphorylates Creb [Yang et al 2001]. In addition to calcineurin, MAPK and SUMO3 (mentioned above) demonstrate dynamic and competitive activation/inactivation of Elk [Yang et al 2003].

Adult neurogenesis—In the adult brain, neurogenesis occurs in the olfactory bulb and the hippocampal dentate gyrus, and several studies suggest that it is required for at least some hippocampal-dependent learning [Snyder et al 2005; Zhou et al 2002]. Recent studies in the adult olfactory bulb have shown that the chr 21 encoded transcription factor OLIG2, discussed above in knockout mice, specifies the fate of transit-amplifying precursor cells. Importantly, it opposes the generation of neuronal progenitors promoted by PAX6 and promotes oligodendrogliogenesis [Hack et al., 2005]. Perturbation of relative concentrations of PAX6 and OLIG2 therefore may alter the balance of neuron vs. oligodendrocyte production. Related observations were reported by Lu et al [2001]. where ectopic expression of Olig1 decreased neuronal survival. Thus, pathways in adult neurogenesis may be perturbed in DS. Consistent with this, Rueda et al [2005] have shown a significant deficit in hippocampal neurogenesis in aged (13–15 months) Ts65Dn mice compared to euploid controls. These findings have been extended to young adult Ts65Dn mice (2–5 months) [Clark et al 2006]. These results may provide the biological basis for the previously reported hypocellularity in the dentate gyrus of Ts65Dn mice [Insausti et al., 1998] and low hippocampal volume in children and adults with DS [Pinter et al., 2001; Aylward et al 1999]. Many factors, including diet, exercise and drug treatments, can influence adult neurogenesis [reviewed in Ming and Song, 2005]. It is noteworthy that Clark et al [2006] observed that chronic treatment with the serotonin selective reuptake inhibitor (SSRI), fluoxetine, increased neurogenesis in the Ts65Dn mice to levels comparable to euploid animals, augmenting both cell proliferation and survival.

DISCUSSION AND FUTURE DIRECTIONS

The datasets discussed here will continue to improve. Monitoring functional genomics/ proteomics information on chr 21 orthologs in model organisms provides opportunities to

develop insights into protein function and protein interactions. These, in turn, will help to identify the pathways and cellular processes in which chr 21 proteins function, and therefore those which may be perturbed in trisomy. In particular, when multiple chr 21 proteins function within a single pathway, examples of which were described above, targeting drug discovery towards those pathways will be more effective than targeting individual chr 21 proteins. Two additional challenges in identifying chr 21 proteins that contribute to DS cognitive deficits should be mentioned.

i) Challenges in gene-phenotype correlations – predicting, detecting and quantifying a phenotype

Predicting the actual perturbation produced by trisomy of a chr 21 gene, even one thought to act as a molecular hub, is one of the major challenges in DS research. First, even with experimental data on the role of the gene in various pathways, increased expression at the protein level must be demonstrated. Second, assumptions still must be made about the stoichiometry of protein complexes and/or co-regulation of expression of interaction partners. For example, the chr 21-encoded transcription factor, GABPA has DNA binding capability, but for transcriptional activation of target genes, it must interact with GABPB and be phosphorylated by MAP kinase [Fromm and Burden 2001]. In the Ts65Dn mouse model of DS, GABPA protein levels are increased in response to gene dosage, but only in brain and muscle [O'Leary et al 2004]. If GABPB levels are not similarly increased and/or if MAPK activity is perturbed, it is possible that transcription of target genes may actually be inhibited due to increased GABPA promoter binding in the absence of transcriptional activation capability. Stoichiometry may be an important consideration for the chr 21 glutamate receptor subunit, GRIK1. GRIK1 forms heteromeric channels with other kainate receptor subunits (GRIKs2,3 and KA1,2), and altering proportions of the subunits may alter overall channel composition and biochemical properties [Mulle et al 2001; reviewed in Ozawa et al 1998]. In a slightly different scenario, the chr 21 potassium channel subunit GIRK2 forms a heteromeric channel with GIRK1. If GIRK1 levels are not increased in DS when GIRK2 is increased, there may be no consequences for increased GIRK2.

Another issue not commonly considered is that it must be determined that expression of the chr 21 gene in disomic cells is not already at saturating levels in terms of its physiological function, such that any increase in trisomy is then irrelevant. This was demonstrated for the chr 21 protein GIRK2. Ehrengruber et al.[1997] showed that *in vitro* overexpression of *Girk1* and *Girk2* together inhibited the excitability of hippocampal neurons and that this inhibition increased with increased levels of GIRK1 and GIRK2, thus showing that normal GIRK2 levels are not saturating. Lastly, another example from GIRK2 shows that the direct perturbation produced by a 50% overexpression can be difficult to quantify, but may still have enough impact at the physiological level to produce a phenotypic component of DS. GIRK activation produces both a hyperpolarization and an increased conductance in hippocampal neurons [Ehrengruber et al 1997]. In Ts65Dn mice, the GIRK conductance will be 50% larger than in control animals, producing a difference of only a few mV and only a slight decrease in depolarization response, difficult to demonstrate conclusively with state of the art patch-clamp techniques. However, they may also translate into a decreased excitability of Ts65Dn neurons, which may have more easily measurable physiological consequences, such as a decreased rate of action potential firing. Thus, searching for gene-phenotype correlations may require broad thinking about where and how to detect the consequences of perturbations.

ii) Challenges in gene-phenotype correlations – the need for diverse mouse models

Design of DS relevant mouse models needs critical consideration. As in examples discussed above, multiple chr 21 genes may mutually interact or impact the same pathway or process.

To recapitulate the phenotypic consequences in DS, all interacting genes must be present in three copies. Segmental trisomy mouse models of DS that include the Ts65Dn, Ts1Cje and Ts1Rhr [reviewed in Davisson and Costa, 1999; Sago et al 1998; Olson et al 2005], are trisomic for regions of mouse chr16 containing 94, 70 and 33 of the genes listed in Supplemental Table I and Supplemental Table II. These mice will continue to be informative but their limitations must be recognized. Genomically contiguous segmental trisomies frequently will not (and indeed do not) contain the set of genes required to recapitulate all the trisomic interactions present in DS, and thus may lack phenotypes associated with DS or generate phenotypes that are not relevant to DS. A more diverse set of multi-gene trisomies needs to be generated to include additional segmental trisomies, custom trisomies generated by co integration of multiple gene-specific BAC clones, and combinations of each of these with additional single gene transgenics. As functional information on chr 21 proteins continues to emerge, design of mouse models will be refined.

Summary

As illustrated by the discussion of GIRK2, DS researchers potentially must deal with subtle direct phenotypes that are difficult to quantify. Looking downstream of the initial perturbations or combining mouse models may result in amplified phenotypes more amenable to quantitation. Lastly, a more thorough understanding of the functions of chr 21 proteins will come from analysis of other model organisms. For example, the functional consequences of human/chimpanzee protein sequence and expression differences may be particularly relevant to neurological development and higher order cognitive function. Phenotypes of knockouts, as illustrated by the lethality of the *C21orf59* knockout in zebrafish, also may suggest important new candidate genes for further study. Researchers will be aided by mechanisms that facilitate integration and communication of a constant stream of such broad categories of new data. DS is an important medical and social challenge. While it is perhaps uniquely complex, it is tractable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the Fondation Jerome Lejeune and the National Institutes of Health (HD49460).

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Table I

Mouse models and neurological phenotypes

Chr 21 Gene	GenBank ID	Description	Comments	#I/#S	Phenotype of mouse models	Z,D,C,Y
NRIP1	8204	Inhibitor of estrogen, Glucocorticoid, etc receptors		21	(KO) mild	
APP	351	Amyloid precursor	Alzheimer's disease; abnormal endosomes	56	(KO) mild; (Mut, Tg) AD-like	Z,D,C
GRIK1	2897	Glutamate receptor subunit (kainate)	Target of pre-mRNA editing	5+	(KO) reduced pain perception	Z,D,C
TIAM1	7074	Rho-GEF for RAC	Neurite outgrowth; Inhibits myc apoptosis	23	(KO) normal neurological; resistant to skin tumors	Z,D
SOD1	6647	Superoxide dismutase	Production of H_2O_2	4	(KO) spatial learning deficits	Z, D,C,Y
SYNJ1	8867	Phosphoinositol phosphatase	Endocytosis	18	(KO) lethal, neurological	Z,D,C,Y
OLIG2	10215	Oligodendrocyte transcription factor	Neurogenesis	1	(KO) lethal, neurological	Z
OLIG1	116488	Oligodendrocyte transcription factor	Neurogenesis	/4	(KO) lethal, neurological	Z
SIM2	6493	Transcription factor	Repressor	1/1	(KO) mild; (tg) learning impaired	Z,D
DSCR1	1827	Inhibitor of calcineurin	Cognitive deficits in Drosophila	1	(KO) mild; some Ts65Dn	Z,D,C,Y
DYRK1A	1859	Ser-thr protein kinase	Cognitive deficits in mouse	3/10	(KO) lethal (embryonic); (Tg) learning impaired Ts65Dn	Z,D,C,
KCNJ6	3763	K channel	Weaver mutation in mouse	8	(KO) hyperactive, seizures; (Mut) Weaver	Z,D,C
BACE2	25825	Aspartyl protease	APP cleavage		(KO) no neurological	
CSTB	1476	Thiol protease inhibitor	Mutation in epilepsy	4	(KO) neurological atrophy; epilepsy	Z
ADAR2	104	Pre-mRNA adenosine deaminase	Targets include glutamate, serotonin receptors; K channel	/7	(KO) lethal, neurological	Z,D, C
S100B	6285	Ca, calmodulin binding		16	(KO) seizures; (Tg) premature neurological aging	Z

#I/#S, number of identified protein interactors or substrates/targets (blank, no information). Z, D, C, Y: protein conserved in zebrafish, Drosophila, *C elegans*, *S cerevisiae*. KO, homozygous knockout mouse; Mut, mutation; Tg, transgenic mouse model carrying and overexpressing an extra copy of the chromosome 21 ortholog. References are found in the text.