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Three Phases of DiGeorge/22q11 Deletion Syndrome Pathogenesis during brain development: patterning, proliferation, and mitochondrial functions of 22q11 genes

D.W. Meechan, T.M. Maynard, E.S. Tucker*, and A-S. LaMantia#

Department of Pharmacology and Physiology and GW Institute for Neuroscience, The George Washington University School of Medicine and Health Sciences, 2300 Eye street NW, Washington DC

Abstract

Summary—DiGeorge, or 22q11 Deletion Syndrome (22q11DS), the most common survivable human genetic deletion disorder, is caused by deletion of a minimum of 32 contiguous genes on human chromosome 22, and presumably results from diminished dosage of one, some, or all of these genes—particularly during development. Nevertheless, the normal functions of 22q11 genes in the embryo or neonate, and their contribution to developmental pathogenesis that must underlie 22q11DS are not well understood. Our data suggests that a substantial number of 22q11 genes act specifically and in concert to mediate early morphogenetic interactions and subsequent cellular differentiation at phenotypically compromised sites—the limbs, heart, face and forebrain. When dosage of a broad set of these genes is diminished, early morphogenesis is altered, and initial 22q11DS phenotypes are established. Thereafter, functionally similar subsets of 22q11 genes—especially those that influence the cell cycle or mitochondrial function—remain expressed, particularly in the developing cerebral cortex, to regulate neurogenesis and synaptic development. When dosage of these genes is diminished, numbers, placement and connectivity of neurons and circuits essential for normal behavior may be disrupted. Such disruptions likely contribute to vulnerability for schizophrenia, autism, or attention deficit/ hyperactivity disorder seen in most 22q11DS patients.

22q11DS: a common and variable disorder of development

DiGeorge, or 22q11 Deletion Syndrome (22q11DS) is one of the most common “copy number variant” (CNV) genetic disorders currently known, occurring in an estimated 1/3000 live births (Shprintzen et al., 2005). The dysmorphology common to many 22q11DS phenotypes—cardiovascular, craniofacial, limb malformations as well as thymic dysplasia—suggests that heterozygous deletion of the minimal 1.5MB of chromosome 22 (or the larger, more typical 3MB deletion that includes this region; (Carlson et al., 1997), and resulting diminished dosage of 22q11 genes, disrupts early development. Similarly, the significantly elevated susceptibility of 22q11DS patients for schizophrenia, autism, attention deficit/ hyperactivity disorder (ADHD), language delay, and other behavioral disorders (Murphy et al., 1999, Niklasson et al., 2008), all of which are thought to be “neurodevelopmental

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#Corresponding author (phmasl@gwumc.edu, FAX: 001-202-994-2870, Tel: 001-202-994-3541).

*Current address: Center for Neuroscience, West Virginia University, Morgantown WV

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disorders” that compromise cerebral cortical circuitry (Geschwind and Levitt, 2007, Weinberger, 1987, 1996), suggests that 22q11DS disrupts brain development in parallel with peripheral and visceral morphogenesis.

Despite these obvious signs that diminished 22q11 gene dosage disrupts critical developmental mechanisms, little is known about how such changes in 22q11 gene function result in the constellation of 22q11DS phenotypes. Over the past 10 years, work from this laboratory and several others has defined some functions of individual 22q11 genes, and identified some consequences of diminished 22q11 gene dosage for cardiovascular, limb, craniofacial, and brain development (Merscher et al., 2001, Maynard et al., 2003, Meechan et al., 2009, Sigurdsson et al., 2010). Our work suggests that 22q11 genes operate as functionally related “sets” at distinct times during development. Diminished dosage of a substantial set of 22q11 genes expressed at sites of mesenchymal/ epithelial interaction—which later become the 22q11DS phenotypic sites—likely compromises early morphogenesis in the brain, face, heart and limbs. Subsequently, diminished dosage of a subset of these 22q11 genes—particularly those that regulate the cell cycle—disrupts neurogenesis in the cerebral cortex. Finally, a distinct subset of mitochondrial 22q11 genes may compromise postnatal synaptogenesis. Such combined, recurrent function of 22q11 genes could disrupt differentiation from early embryonic through late postnatal development, especially in the brain. This concatenation of dosage-sensitive changes in development, especially if modified by additional genetic or environmental factors, could explain the variable behavioral pathology, as well as peripheral dysmorphology, associated with 22q11DS.

Genomic mechanisms and consequences of heterozygous 22q11 deletion

Most 22q11DS cases reflect spontaneous deletion of the 1.5 or 3MB region of human chromosome 22 that gives the disorder its name. There are, however, cases in which 22q11-deleted individuals (some lacking clinically obvious phenotypes) pass the deletion to their offspring with consequences from mild to severe (Leana-Cox et al., 1996). This provides additional evidence that genetic modifiers beyond the 22q11 genes themselves play a key role in determining 22q11DS phenotypic severity. The majority of deletions are most likely generated maternally during oogenic meiosis; however, paternal origin is also seen (Leana-Cox et al., 1996, Ryan et al., 1997). Despite speculation on potential maternal effects that modify 22q11DS phenotypes, especially in the brain (Eliez et al., 2001), there is currently no report of weak alleles generating effective nulls at specific loci, nor any evidence for paternal imprinting or allelic biasing that would result in loss of function of single 22q11 genes. Our analysis of parent of origin effects in the 26 contiguous murine orthologues of human 22q11 genes found on mouse chromosome 16 (Figure 1A; (Maynard et al., 2006)) found neither paternal nor maternal imprinting (often conserved between species; (Morison et al., 2005, Paulsen et al., 2001)). Similarly, there are no substantial allelic biases that might render expression levels of 22q11 genes lower than the 50% expected for heterozygous deletion. Thus, the available evidence suggests that the primary molecular consequence of 22q11 deletion is diminished dosage of approximately 50% ((Meechan et al., 2006); Gopalikrishna et al., unpublished observations)) of multiple genes in the region.

The brain, along with other tissues, has diminished expression of most if not all 22q11 genes. We confirmed diminished expression of deleted 22q11 genes at the mRNA or protein level in the brains of mice carrying a deletion that parallels the minimal critical deletion in 22q11DS (*Lgdel* mice; Figure 1B,C, D; (Meechan et al., 2006)). mRNA levels for at least 10 22q11 genes with potentially important developmental functions are reduced by approximately 50% in the brains of late embryonic through adult *Lgdel* mice (Figure 1C). Histological analysis using *in situ* labeled preparations indicates that this change is more

likely due to “per cell” alterations than changes in expression patterns (Figure 1B). Finally, diminished protein levels accompany diminished message levels for 4/ 5 genes for which we were able to quantify protein levels. The fifth gene, *Ufd1l*, shows clear translational dosage compensation, with *Ufd1l* protein levels reaching 100% of wild type in the *Lgdel* embryonic, postnatal and adult brain (Figure 1D). This unusual case of translational dosage compensation is consistent with observations for *Ufd1l* or its orthologues in a broad range of species where 100% dosage is critical, and partial loss of function can be lethal (Johnson et al., 1995). Thus, the apparent immediate consequence of heterozygous 22q11 deletion in mouse models of the human genomic lesion is 50% diminished expression of most if not all 22q11 genes at critical phenotypic sites including limb buds, aortic arches and hearts, craniofacial primordia (branchial arches/ maxillary process) and the frontonasal mass/ forebrain (see below for details), as well as the developing and adult brain thereafter.

How does diminished 22q11 gene dosage compromise development?

I. Altered Mesenchymal/Epithelial (M/E) induction and morphogenesis

The constellation of 22q11DS phenotypes: dysmorphology of the limbs, aortic arches and heart, face and palate, and altered cognitive function—which implies altered forebrain, particularly cerebral cortical, development—suggests that a shared early developmental mechanism might be compromised by diminished dosage of one, some or all of the deleted 22q11 genes at each site. We hypothesized that this mechanism was most likely the interactions between mesenchyme (M) and adjacent epithelia (E) that drive morphogenesis at each site (Creazzo et al., 1998, LaMantia, 1999, Tickle and Eichele, 1994). Accordingly, we first evaluated whether a significant proportion of 22q11 genes from the minimal critical deleted region were expressed selectively or specifically in normal mouse embryos at established sites of M/ E interaction including the developing limb buds, aortic arches (cardiac primordia), branchial arches (facial primordia) and forebrain. A PCR-based screen of the entire set of murine 22q11 orthologues in micro-dissected limb buds, aortic arches/ hearts, branchial arches, and frontonasal mass/ forebrain from embryonic day 10 (E10.5) mouse embryos showed that a large subset of the murine 22q11 genes—22/ 28 orthologues—was expressed at phenotypically compromised sites of M/ E interaction (Figure 2A; (Maynard et al., 2003)). Further analysis using immunohistochemical or *in situ* hybridization in whole embryos showed that most of these genes are specifically or selectively expressed at sites of M/ E interaction compromised in 22q11DS (Figure 2B; (Maynard et al., 2002, Maynard et al., 2003, Meechan et al., 2006)). These observations provide a foundation for our hypothesis that a broad set of 22q11 genes normally act at sites of M/ E interaction to influence initial patterning and morphogenesis, and that diminished dosage of one, some or all of these genes during this epoch of development compromises initial development of each phenotypic site in 22q11DS.

To begin to test this hypothesis we first asked whether dosage of a subset of these genes—distributed throughout the region, including those whose function seemed to be likely to contribute significantly to early development—was actually diminished in a mouse model of 22q11DS. In *Lgdel* model embryos, we showed by quantitative PCR in microdissected limb buds, aortic arches and hearts, branchial arches, and frontonasal mass/ forebrains that 22q11 gene expression is indeed diminished by 50% in parallel at each site (Figure 2C; (Meechan et al., 2006)). In addition, we showed that this change was unlikely to reflect morphogenetic alteration in which tissue was lost since expression of the *Snail* transcription factors, which are associated with the neural crest component of the mesenchyme at each site compromised by 22q11DS (Sakai et al., 2006, Sefton et al., 1998) are not changed in expression patterns or levels, despite diminished levels—but not altered patterns—of 22q11 genes (Figure 2D). Even though there are conserved binding sites for Snail transcription factors in many upstream regulatory regions of the 22q11 genes (Meechan et al., 2006), there is little

indication that Snail or any other local transcriptional regulator mediates dosage compensation at the mRNA expression level. In fact, we have only found one example thus far of dosage compensation for 22q11 genes: *Ufd1l* (see Figure 1; (Meechan et al., 2006)). Overall, in *Lgdel* embryos, message levels appear consistently diminished to approximately 50% of wild type at morphogenetic sites compromised in 22q11DS.

Together, these findings suggest, with few exceptions, diminished dosage of multiple 22q11 genes at sites of M/ E interaction can compromise local morphogenesis. Indeed, there is evidence of disrupted morphogenesis in the *Lgdel* mouse aortic arches ((Mersch et al., 2001), as well as skeletal elements in the limb, where we found that proximal and distal bones are modestly, but significantly shorter (p-value is between less than 0.01 and 0.0005) in deleted versus wild type littermates (Figure 3A,B). Nevertheless, there are no significant changes in the overall weight of *Lgdel* adult mice, their brain weights (Figure 3C,D), or gross morphological appearance of the mature *Lgdel* brain. There may be, however, subtle disruptions of patterning in the developing forebrain that parallel dysmorphology seen at other M/ E sites in the *Lgdel* mouse model of 22q11DS, as well as changes in cerebral cortical structure similar to those in 22q11DS patients (see below). Several reports in the literature compare disruptions of development at each 22q11DS phenotypic site with more extreme phenotypes seen in mutants where signaling pathways (Shh, Fgfs, Bmps, and retinoic acid-RA) that mediate M/ E interactions in the limbs, heart, face and forebrain are disrupted (Bachiller et al., 2003, Frank et al., 2002, Garg et al., 2001, Vermot et al., 2003, Washington Smoak et al., 2005). Thus, 22q11 genes might influence normal development either because they are targets of M/ E inductive signals—“downstream” of local signals and transcriptional regulators—or they themselves are “upstream” factors necessary to maintain normal mechanisms of local tissue-tissue interactions that guide morphogenesis and differentiation.

II. Altered neurogenesis and migration in the cerebral cortex

Autism, attention deficit/ hyperactivity disorder, and schizophrenia—the major behavioral disorders currently associated with 22q11 deletion—are believed to reflect altered cortical development, regardless of underlying genetic or etiologic diversity (Arnsten, 2006, McGlashan and Hoffman, 2000, Minshew and Williams, 2007). Imaging studies as well as limited post-mortem analysis of the cerebral cortex in 22q11DS patients suggests that anatomical and cytological changes seen in the cerebral cortex in individuals with a variety of developmental disorders—including areal differences in cortical thickness polymicrogyria, and periventricular heterotopias—are also seen in 22q11DS patients (Bearden et al., 2007, 2009, Kiehl et al., 2009, Schaer et al., 2006, van Amelsvoort et al., 2004). These anomalies are thought to impact normal brain function, and in 22q11DS patients they may reflect sensitivity of cortical development to diminished 22q11 gene dosage. The mechanisms that normally insure appropriate numbers and locations of cortical neurons, including neurogenesis and migration, are now considered likely targets for pathogenesis in neurodevelopmental disorders (Bearden et al., 2009, Mao et al., 2009, Meechan et al., 2009). Thus, we used the *Lgdel* mouse model of 22q11DS to assess consequences of diminished 22q11 gene dosage for cortical neurogenesis and migration. The value of studies of cortical development in this mouse model goes beyond its direct relevance to 22q11DS—the *Lgdel* and 2 other 22q11DS models (Gogos et al., 1999, Long et al., 2006) represent rare opportunities to assess cortical development in model organisms with genomic lesions that directly recapitulate those seen in patients with major neurodevelopmental disorders including schizophrenia, ADHD, and autism.

We began our analysis of potential defects in cortical development by asking whether 22q11 genes continue to be expressed in the cortical rudiment during the last half of gestation in the mouse, when cortical neurogenesis and migration occur. Most 22q11 orthologues in the

1.5MB minimal critical deleted region are expressed in the developing brain from E12 through birth, and many are localized to the cerebral cortex (Maynard et al., 2003, Meechan et al., 2006, Meechan et al., 2009). Thus we evaluated functions of these 22q11 genes, first *in silico*, and subsequently *in vitro* and *in vivo*, to identify those that might influence neurogenesis or migration for further analysis of expression and function. We found a subset that apparently modulate the cell cycle: *Ranbp1*, *Httf9C*, *Cdc45l*, *Hira*, and *Ufd1l*. We recognized that *Ufd1l* was unlikely to make a significant contribution due to translational dosage compensation (see Figure 1D, and (Meechan et al., 2006)); however, the remaining cell cycle genes are all expressed selectively or preferentially in the ventricular and subventricular zone, where cortical neural stem cells are found (Figure 4A). Furthermore, most of these genes are expressed maximally during mid to late gestation—the peak period of cortical neurogenesis—and decline substantially thereafter (Meechan et al., 2006, 2009). The apparent function, localization, and expression dynamics of these genes suggests they might influence cortical neural progenitor proliferation. Thus, we asked whether proliferative characteristics of any of the sub-classes of progenitors in the cortical ventricular zone are disrupted when 22q11 gene dosage is altered.

We found that basal progenitors, a specific class of cortical precursor cells, are compromised by diminished dosage of 22q11 genes during mid-gestation (Figure 4B,C). Basal progenitors are thought to be the transit amplifying cells of the developing cortex (Noctor et al., 2004). They divide rapidly and asymmetrically, and their offspring are primarily projection neurons of cortical layers 2, 3 and 4 (Noctor et al., 2004). Basal progenitor proliferation is diminished in distinct regions of the embryonic *Lgdel* cortex, particularly anterior frontal regions; fewer appear to be in either S or M phase than in wild type littermates (Meechan et al., 2009). Similar changes are not seen in *Prodh* or *Tbx1* mutant mice, despite the suggestion that these two individual 22q11 genes contribute significantly to 22q11DS-related pathology (Gogos et al., 1999, Merscher et al., 2001, Paylor et al., 2006). A consequence of this proliferative deficit in *Lgdel* embryos is, perhaps not surprisingly, diminished frequency of projection neurons in layers 2 through 4 (Figure 5C). Despite this deficit in projection neuron production, neurogenesis ceases at the same time in *Lgdel* and wild type cortex. Thus, diminished 22q11 dosage slows proliferation of basal progenitors initially; these cells never recover their full neurogenic capacity, and they yield fewer cortical projection neurons. We did not detect proliferative changes in the other main class of cortical progenitors, apical progenitors or radial glia, thought to be cortical neural stem cells. This suggests that the basal progenitor pool is generated appropriately; however, once generated these cells fail to progress normally through the series of rapid asymmetric cell divisions that result in appropriate numbers of projection neurons in the supragranular layers of mouse frontal association cortices.

As basal progenitors are failing to produce normal numbers of cortical projection neurons in discrete neocortical areas in the *Lgdel* mouse, tangential migration of embryonic interneurons from the basal forebrain into the same cortical areas is also disrupted (Figure 5A,B). As with embryonic basal progenitors, this developmental disruption results in a specific defect in the postnatal brain: in this case, altered distribution of parvalbumin immunoreactive interneurons between cortical layers (Figure 5D), although total numbers of parvalbumin-labeled cells were not diminished. Parvalbumin cells make up a large cohort of molecularly distinct interneuron sub-types in the adult rodent and primate cortex (Conde et al., 1994, Tamamaki et al., 2003). Post mortem studies of schizophrenic brains have indicated reductions in parvalbumin positive cortical cell numbers (Beasley et al., 2002) or diminished parvalbumin gene expression in the cortex (Hashimoto et al., 2003) indicating that the integrity of this class of interneuron may be compromised as part of schizophrenia pathogenesis (Benes, 2000, Lewis et al., 2005). Thus, in the *Lgdel*/22q11DS model, the initial migration and subsequent cortical distribution of a class of interneurons implicated in

schizophrenic pathology is altered. Remarkably, the most substantial change in distribution of parvalbumin interneurons is a reduction in their frequency in cortical layer V (and apparent increase in layer II-IV). Layer V parvalbumin corresponds to a population of basket interneurons that heavily innervate layer 2-4 pyramidal cells (Kisvarday, 1992). The coincident reduction of layer 2-4 projection neurons as well as altered placement of interneurons in the *Lgdel* mouse model indicates that cortical circuit development is disrupted in multiple locations by diminished 22q11 gene dosage. Such disruptions may contribute to behavioral phenotypes reported in the *Lgdel* mouse model (Long et al., 2006; and see below), and suggest that developmental changes may underlie at least some of the vulnerability for psychiatric and behavioral disorders in 22q11DS.

The basal progenitor phenotype in *Lgdel* mice, the accompanying interneuron migratory deficit, and their potential consequences for cortical circuit development and function, provide new opportunities to further define potential *developmental* pathogenic mechanisms that contribute to diseases of cortical connectivity associated with 22q11DS. 22q11 gene dosage most likely influences a network of cell cycle regulators and other factors that insure appropriate numbers of cortical projection neurons as well as migratory targeting of GABAergic interneurons. Neither *Tbx1* nor *Prodh*, both suggested as causal genes for cardiovascular or neural phenotypes in 22q11DS (Gogos et al., 1999, Merscher et al., 2001, Lindsay et al., 2001, Paylor et al., 2006) contribute to the basal progenitor proliferative defect nor its sequelae seen in the *Lgdel* mouse (Meechan et al., 2009). Among the remaining 22q11 genes, *Cdc45l* and *Ranbp1* are attractive candidates for further study: both are robustly and specifically expressed in proliferative zones and their levels decline by 50% in the developing cortex of the *Lgdel* mouse. Furthermore, they have defined roles in proliferation: Cdc45l protein is a critical component of the *Go Ichi Ni San*-mini chromosome maintenance complex (GINS-MCM) that facilitates chromatin unwinding and polymerase activity during DNA replication, and Ranbp1 protein plays an important role in centrosomal cohesion and chromosomal alignment/ segregation during mitosis (Guarguaglini et al., 1997, Moyer et al., 2006, Tedeschi et al., 2007). Analysis of proliferative and migratory phenotypes in mice heterozygously deleted for *Cdc45l*, *Ranbp1*, or both, will clarify their contributions to disrupted cortical neurogenesis in the *Lgdel* 22q11DS mouse model.

Signaling molecules available in the developing cortex are also likely to contribute to the basal progenitor regulatory network that includes 22q11 genes. The generation, proliferation and maintenance of basal progenitors most likely rely on the integrity of Fgf signaling pathways (Kang et al., 2009, Sahara and O'Leary, 2009). Deletions distal to the 3MB 22q11 interval have been associated with basal progenitor defects via ERK/ FGF signaling (Samuels et al., 2008). Thus there may be a cluster of genes in the 22q11 region important for—and sensitive to—signaling pathways that influence basal progenitors. Fgf signaling molecules and receptors are selectively expressed in regions of the developing cortex. Accordingly, inappropriate regional expression or activity of these molecules due to diminished 22q11 gene dosage may disrupt basal progenitor proliferation. Such disruptions may actually begin during patterning of the anterior/ ventral forebrain—the source of cortical GABAergic interneurons—which relies upon local Fgf8, Fgf15 and Fgf17 signaling and the integrity of Fgf receptors (Borello et al., 2008, Cholfin and Rubenstein, 2007, Faedo et al., 2010, Storm et al., 2006). We have shown that the expression and patterning of Fgf8, as well as Fgf signaling activity, depends upon M/ E interactions between the frontonasal mesenchyme and the ventral forebrain (Tucker et al., 2008). Thus, it is possible that the altered cortical GABAergic migration in the *Lgdel* model of 22q11DS reflects not only dysregulation of cortical development during late gestation, but anomalous patterning and inductive signaling at earlier stages of embryonic development.

Our current data provides a new outline of how a genomic lesion associated with vulnerability for autism, ADHD, schizophrenia, and other affective disorders might disrupt the initial generation and placement of key elements of cortical circuits during development. Nevertheless, several questions remain: We do not yet know whether the basal progenitor proliferative defect or interneuron migratory defect is cell autonomous. We do not know whether interneurons beyond the parvalbumin subpopulation have similarly altered migration and position, or perhaps are also modified in frequency. Finally, we do not know the functional consequences of the proliferative and migratory changes we have found. These cell biological changes might contribute to some of the subtle behavioral, synaptic and network changes reported recently in several 22q11DS mouse models (Mukai et al., 2004, 2008, Stark et al., 2008; Sigurdsson et al, 2010). Additional electrophysiological analysis in distinct regions of the postnatal cortex, paired with critical behavioral analyses that probe relevant mouse behaviors will be needed to determine whether localized abnormalities in neurogenesis and migration contribute to functional deficits relevant for the behavioral disorders that accompany 22q11DS.

III. Altered mitochondrial function and synaptogenesis

The last phase of cortical circuit development—following neurogenesis and migration—encompasses dendritic and axonal growth, trophic-mediated competitive interactions that regulate cell survival, as well as establishment and activity-dependent stabilization of synaptic connections. The 22q11 minimal critical region lacks genes generally thought of as mediators of axonal growth, development, or survival; there are no ligands or receptors for neurotrophic factors, neural cell adhesions molecules, nor genes directly associated with process growth or apoptosis. A few 22q11 genes have indirectly been associated with these processes. *Septin5* interacts with the SNARE complex at neuronal synapses (Beites et al., 2005), and over-expression apparently disrupts synaptic communication (Son et al., 2005). Initial reports, however, suggest that null mutation of *Septin5* in mouse models has no identifiable consequences in the nervous system (Peng et al., 2002), although more recent reports suggest subtle, strain specific anomalies (Suzuki et al., 2009). Thus, if 22q11 genes influence synapse formation and processing capacity in cortical circuits, they most likely act indirectly, perhaps by regulating essential cellular mechanisms that impact synaptogenesis rather than adult synaptic function.

Our data indicates that 22q11 genes may influence the ubiquitous but essential role of mitochondrial function during cortical circuit development and maintenance, especially during activity dependent synaptogenesis. Synaptogenesis requires substantial metabolic support (Kazama et al., 2008), as does the ongoing maintenance of synaptic function. Mitochondrial activity in the rodent brain peaks at birth (Nakai et al., 2000), corresponding with a peak period in synapse formation. There is a similar peak of synapse formation in the early postnatal primate brain, including humans, with maximal synaptic density occurring just after birth, followed by a progressive refinement (and net loss) of synapses during adolescence (Huttenlocher, 1979, Rakic et al., 1986). Thus, our identification and characterization of 6 mitochondrial 22q11 genes—nearly 1/3 of the full set of 22q11 genes known to be expressed in the developing or mature brain—suggests that heterozygous 22q11 deletion might negatively influence mitochondrial function necessary for synaptogenesis. This hypothesis is further supported by the expression dynamics of the 22q11 mitochondrial genes: most are maximally expressed in the brain at or around birth, and several decline thereafter (Meechan et al., 2006). Accordingly, disruption of bioenergetic homeostasis during synapse formation and refinement due to diminished dosage of mitochondrial 22q11 genes might provide a final insult to an already disrupted history of neural development in 22q11DS.

To investigate the possible role of mitochondrial 22q11 genes in synapse development or other neuronal functions, we confirmed or established that protein products of 22q11 transcripts predicted to be mitochondrial by bioinformatic-based analysis were actually localized to mitochondria (Figure 6A). We next asked where these genes are expressed in the developing and mature brain—particularly in cortical neurons (Maynard et al., 2008). We found that each of this distinct subset of six mitochondrial 22q11 genes—*Mrp140*, encoding a mitochondrial ribosomal subunit protein; *Prodh*, a key enzyme for amino acid catabolism as well as metabolism; *Slc25a1*, a mitochondrial citrate transporter; *Txnrd2*, a mitochondrial thioredoxin reductase; *T10* which encodes a protein of unknown function, and *Zdhhc8*, whose localization and function includes mitochondrial regulation of cell survival as well as potential palmitoyl transferase activity—is robustly expressed in the developing and mature cortex, some with distinctive laminar distributions (see Figure 1). Moreover, many are detected biochemically in mitochondria from brain synaptosome fractions (Figure 6B). *Zdhhc8* protein, in particular, is present in synaptic mitochondria, particularly those in glutamatergic synaptic terminals (Figure 6C). All 6 mitochondrial genes reach or maintain maximal expression around birth (P0)—concurrent with the period of peak synaptogenesis in the mouse—and most decline thereafter (Meechan et al., 2006). Reduced expression of 22q11 genes in the *Lgdel* mouse appears to influence ongoing function of cortical mitochondria as well: during early postnatal life (when the energetic demand is highest, as is the number of synapses), there is a dysregulation of normal mitochondrial gene expression, with several mitochondrial genes expressed at significantly higher levels in the cerebral cortex of the mouse model of 22q11DS (Figure 7). This dysregulation may represent compensation for reduced expression of 22q11 mitochondrial genes. The increased expression of other redox complex subunits may help to increase the overall metabolic output of mitochondria compromised by the genetic lesion that defines 22q11DS.

Of these six mitochondrial 22q11 genes, *Prodh* and *Zdhhc8* have been most frequently implicated in 22q11-related behavioral phenotypes based on human genome-wide association studies (Chen et al., 2004, Kempf et al., 2008, Liu et al., 2002, Mukai et al., 2004) as well as analysis of mature mutant mice (Gogos et al., 1999, Mukai et al., 2004, 2008). There is little definitive data on the cellular function of *Prodh* protein, beyond an apparent role in proline metabolism and mitochondrial redox regulation (Gogos et al., 1999, Phang et al., 1980). *Zdhhc8*, in contrast, may have multiple functions including palmitoyl transferase activity, cell cycle regulatory capacity, and dosage dependent roles in mitochondrial-regulated cell survival (Maynard et al., 2008, Mukai et al., 2004, 2008). *Zdhhc8* contains a short “DHHC” protein domain that is strongly conserved among a large (>20 member) protein family. Some DHHC domain-proteins are involved in palmitoylation: catalytic addition of palmitic acid residues that can modify the activity or localization of substrate proteins. Based upon homology to the first identified palmitoyl transferase, yeast ERF2 (Lobo et al., 2002), the DHHC domain has been confirmed as a catalytic site for mammalian palmitoyl transeferases (Huang et al., 2009, Iwanaga et al., 2009). Thus, *Zdhhc8*, which has palmitoyl transferase activity *in vitro*, is assumed to have similar activity *in vivo*. Nevertheless, *Zdhhc8* has also been identified as a modulator of cell cycle progression at the G2/ M checkpoint in response to DNA damage (Sudo et al., 2007). Furthermore, we identified a potential biochemical interaction between *Zdhhc8* and *Uqcrc1*, a mitochondrial core complex 1 subunit, and found that over expression of GFP- or HA-tagged *Zdhhc8* in multiple cell lines rapidly leads to Bcl2-regulated apoptosis mediated by the intact DHHC domain (Figure 7) (Maynard et al., 2008). Finally, *Zdhhc8* null mice have altered behavior and synaptic communication (Mukai et al., 2008), including decreased open field behavior, sensory gating deficits—surprisingly in females only—and decreased sensitivity to the NMDA/ glutamate receptor antagonist MK801 (Mukai et al., 2004, 2008). These disruptions have been associated with decreased ability of *Zdhhc8* mutant neurons to form dendrites and spines *in vitro*. It is not yet clear, however, how *Zdhhc8* might influence

dendritic differentiation, or whether *Zdhhc8* is required for maintenance of postsynaptic dendritic domains.

These diverse and uncertain functional associations suggest that *Zdhhc8* protein may have distinct roles in neuronal development and subsequent homeostasis under different cellular conditions. *Zdhhc8* may interact with electron transport machinery in mitochondria to influence bioenergetic capacity, cell growth or survival. The nature of the putative interaction between *Zdhhc8* and *Uqcrc1* has yet to be confirmed in neuronal cells. It is possible, however, that this interaction influences electron transport efficiency by modifying *Uqcrc1* function and may thus modulate metabolic abilities of neurons to cope with energy demands of activity dependent synapse formation and stabilization. The role of palmitoylation in brain development and function has only begun to be explored. In addition to a presumed role in subcellular targeting, palmitoylation has been implicated in apoptosis (Guo et al., 2007, Peterson et al., 2008), apparently by directly modulating the generation of ceramides (Turpin et al., 2006). One possible explanation for multiple functions of *Zdhhc8* is suggested by the nature of the palmitoyl transferase activity of ERF2, the DHHC orthologue in yeast. ERF2 apparently regulates palmitoylation of the yeast RAS homologue, resulting in translocation of RAS from the endoplasmic reticulum to the plasma membrane. In the absence of a third protein, however, ERF4, which directly interacts with ERF2, this palmitoylation does not occur (Zhao et al., 2002). *Zdhhc8*'s cellular function may similarly depend upon its interactions with other cellular proteins. *Zdhhc8* may have different functions when it interacts with GCP16 (a presumptive functional ERF4 homologue; Swarthout et al., 2005) in the endoplasmic reticulum than when it interacts with mitochondrial proteins like *Uqcrc1*. Changes in expression levels of interacting proteins in distinct cell classes, therefore, could mediate the apparent functional distinctions observed for *Zdhhc8*.

Aside from *Zdhhc8*, there are few specific indications of potential mechanistic roles for the remaining 22q11 mitochondrial genes during synaptic development. Indeed, one of these genes, *T10*, encodes a protein with no apparent homology to other known proteins. Nevertheless, the developmental modulation of expression levels, the localization of the 22q11 mitochondrial proteins to synapses, and the transient altered expression of additional mitochondrial genes in the *Lgdel* model of 22q11DS all indicate that 22q11 gene dosage—especially that of the mitochondrial 22q11 genes—can modulate mitochondrial function and metabolic capacity during early postnatal synaptogenesis.

Disrupted cortical development and disrupted behavior: are they related?

It is not clear if disrupted cortical development in the *Lgdel* mouse results in specific behavioral deficits. Nevertheless, aberrant behaviors commonly associated with altered forebrain circuitry are seen in the *Lgdel* as well as other 22q11DS mouse models. The *Lgdel* as well as the *Df(16)* mouse, another 22q11DS genomic model (Stark et al., 2008), have deficits in pre-pulse inhibition (PPI), which reflects disrupted “sensory gating” (Long et al., 2008, Stark et al., 2008). Similar inability to “gate” or filter unnecessary sensory information are a hallmark of psychiatric illnesses including schizophrenia (Braff and Geyer, 1990). Accordingly, PPI deficits in *Lgdel* and *Df(16)* mice are consistent with the hypothesis that diminished 22q11 gene dosage results in altered cortical circuitry associated with behavioral pathology seen in schizophrenia. The *Df(16)* mouse also has deficits in conditioned fear responses, open field exploration, and t-maze tasks (Stark et al., 2008; Sigurdsson et al., 2010); however, conditioned fear and open field deficits are not seen in the *Lgdel* mouse (Long et al., 2008). Conditioned fear and spatial working memory tasks such as the t-maze rely upon coordinated activity of hippocampal and cortical circuits, and reduced hippocampal/ cortical oscillatory synchrony in *Df(16)* mice (Sigurdsson et al., 2010) suggests parallel disrupted synaptic or circuit function; nevertheless, the precise

relationship between the behavioral deficits and the physiological disruption has not yet been defined. Variable deficits in *Lgdel* and *Df(16)* lines, however, highlight challenges in correlating genomic lesions, physiological changes, and behavior in mice which modest genetic and environmental variability influences performance. It is possible that more precise behavioral tasks that assess the murine equivalent of “executive function” (Bussey et al., 2001), which is thought to be a primary behavioral target of schizophrenia pathogenesis (Greene et al., 2008), may provide more stable, reproducible insight into specific deficits caused by diminished 22q11 gene dosage. Finally, caution must be applied when comparing behavioral deficits present in mice and disruptions in working memory, executive function, affect, and social interaction seen in patients with schizophrenia.

22q11DS: A genetic disease of neural development

The correlation of 22q11DS with schizophrenia vulnerability, first reported in the early 1990's, provided one of the earliest associations between a consistent genomic lesion, and a behavioral disorder believed to reflect disordered cortical circuitry. In parallel with this genetic discovery—based upon completely independent arguments—several investigators elaborated the “neurodevelopmental hypothesis” of schizophrenia pathogenesis (Weinberger, 1987, 1996), and this hypothesis has since been broadened to include additional behavioral disorders—including autistic spectrum and ADHD (Geschwind and Levitt, 2007)—seen in 22q11DS patients. Thus, when considering the relationship between 22q11DS and behavioral disorders, one must ask whether schizophrenia, autism, or ADHD-like pathology in 22q11DS patients truly reflects aberrant brain development. As summarized above, a small number of studies of complete loss of function, or heterozygous deletion of individual genes from the 22q11 minimal (1.5MB) or typical (3MB) deleted region have shown anomalies in mouse behavior, circuit activity or synaptic function (Mukai et al., 2004, 2008, Stark et al., 2008). It is not clear from these studies, however, whether the anomalies are due to developmental changes, or acute change in neuronal function that impacts circuitry and behavior in the adult brain. The data from our laboratory summarized in this review indicates that diminished dosage of subsets of 22q11 genes can modify initial forebrain patterning, subsequent cortical neurogenesis and migration, and finally, mitochondrial support of activity-dependent synapse formation and elimination in the early postnatal brain. Each successive assault on normal developmental mechanisms might compound damage done previously. Thus, the cortex of 22q11DS patients may have neurons with altered identity due to aberrant specification of early precursors, altered frequency due to deficits in neurogenesis, altered positions due to disrupted migration, and altered connections due to dysregulation of mitochondrial function. Whether these departures from development underlie the behavioral disorders found in 22q11DS patients remains to be determined. Nevertheless, the coincidence of disrupted forebrain development, altered cortical circuitry and elevated incidence of schizophrenia, autism and ADHD— all considered to be “neurodevelopmental disorders”— argues strongly that 22q11DS is indeed a genetic disorder of neural development.

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References

- Arnsten AF. Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. *J Clin Psychiatry*. 2006; 67(Suppl 8):7–12. [PubMed: 16961424]

- Bachiller D, Klingensmith J, Shneyder N, Tran U, Anderson R, Rossant J, De Robertis EM. The role of chordin/Bmp signals in mammalian pharyngeal development and DiGeorge syndrome. *Development*. 2003; 130:3567–3578. [PubMed: 12810603]
- Bearden CE, van Erp TG, Dutton RA, Lee AD, Simon TJ, Cannon TD, Emanuel BS, McDonald-McGinn D, Zackai EH, Thompson PM. Alterations in midline cortical thickness and gyrification patterns mapped in children with 22q11.2 deletions. *Cereb Cortex*. 2009; 19:115–126. [PubMed: 18483006]
- Bearden CE, van Erp TG, Dutton RA, Tran H, Zimmermann L, Sun D, Geaga JA, Simon TJ, Glahn DC, Cannon TD, Emanuel BS, Toga AW, Thompson PM. Mapping cortical thickness in children with 22q11.2 deletions. *Cereb Cortex*. 2007; 17:1889–1898. [PubMed: 17056649]
- Beasley CL, Zhang ZJ, Patten I, Reynolds GP. Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biol Psychiatry*. 2002; 52:708–715. [PubMed: 12372661]
- Beites CL, Campbell KA, Trimble WS. The septin Sept5/CDCrel-1 competes with alpha-SNAP for binding to the SNARE complex. *Biochem J*. 2005; 385:347–353. [PubMed: 15355307]
- Benes FM. Emerging principles of altered neural circuitry in schizophrenia. *Brain Res Brain Res Rev*. 2000; 31:251–269. [PubMed: 10719152]
- Borello U, Cobos I, Long JE, McWhirter JR, Murre C, Rubenstein JL. FGF15 promotes neurogenesis and opposes FGF8 function during neocortical development. *Neural Dev*. 2008; 3:17. [PubMed: 18625063]
- Braff DL, Geyer MA. Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry*. 1990; 47:181–188. [PubMed: 2405807]
- Bussey TJ, Saksida LM, Rothblat LA. Discrimination of computer-graphic stimuli by mice: a method for the behavioral characterization of transgenic and gene-knockout models. *Behav Neurosci*. 2001; 115:957–960. [PubMed: 11508736]
- Carlson C, Sirotkin H, Pandita R, Goldberg R, McKie J, Wadey R, Patanjali SR, Weissman SM, Anyane-Yeboah K, Warburton D, Scambler P, Shprintzen R, Kucherlapati R, Morrow BE. Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. *Am J Hum Genet*. 1997; 61:620–629. [PubMed: 9326327]
- Chen WY, Shi YY, Zheng YL, Zhao XZ, Zhang GJ, Chen SQ, Yang PD, He L. Case-control study and transmission disequilibrium test provide consistent evidence for association between schizophrenia and genetic variation in the 22q11 gene ZDHHC8. *Hum Mol Genet*. 2004; 13:2991–2995. [PubMed: 15489219]
- Cholfin JA, Rubenstein JL. Patterning of frontal cortex subdivisions by Fgf17. *Proc Natl Acad Sci U S A*. 2007; 104:7652–7657. [PubMed: 17442747]
- Conde F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA. Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. *J Comp Neurol*. 1994; 341:95–116. [PubMed: 8006226]
- Creazzo TL, Godt RE, Leatherbury L, Conway SJ, Kirby ML. Role of cardiac neural crest cells in cardiovascular development. *Annu Rev Physiol*. 1998; 60:267–286. [PubMed: 9558464]
- Eliez S, Antonarakis SE, Morris MA, Dahoun SP, Reiss AL. Parental origin of the deletion 22q11.2 and brain development in velocardiofacial syndrome: a preliminary study. *Arch Gen Psychiatry*. 2001; 58:64–68. [PubMed: 11146759]
- Faedo A, Borello U, Rubenstein JL. Repression of Fgf signaling by sprouty1-2 regulates cortical patterning in two distinct regions and times. *J Neurosci*. 2010; 30:4015–4023. [PubMed: 20237272]
- Frank DU, Fotheringham LK, Brewer JA, Muglia LJ, Tristani-Firouzi M, Capecchi MR, Moon AM. An Fgf8 mouse mutant phenocopies human 22q11 deletion syndrome. *Development*. 2002; 129:4591–4603. [PubMed: 12223415]
- Garg V, Yamagishi C, Hu T, Kathiriyi IS, Yamagishi H, Srivastava D. Tbx1, a DiGeorge syndrome candidate gene, is regulated by sonic hedgehog during pharyngeal arch development. *Dev Biol*. 2001; 235:62–73. [PubMed: 11412027]
- Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol*. 2007; 17:103–111. [PubMed: 17275283]

- Gogos JA, Santha M, Takacs Z, Beck KD, Luine V, Lucas LR, Nadler JV, Karayiorgou M. The gene encoding proline dehydrogenase modulates sensorimotor gating in mice. *Nat Genet.* 1999; 21:434–439. [PubMed: 10192398]
- Greene CM, Braet W, Johnson KA, Bellgrove MA. Imaging the genetics of executive function. *Biol Psychol.* 2008; 79:30–42. [PubMed: 18178303]
- Guarguaglini G, Battistoni A, Pittoggi C, Di Matteo G, Di Fiore B, Lavia P. Expression of the murine RanBP1 and Htf9-c genes is regulated from a shared bidirectional promoter during cell cycle progression. *Biochem J.* 1997; 325(Pt 1):277–286. [PubMed: 9224656]
- Guo W, Wong S, Xie W, Lei T, Luo Z. Palmitate modulates intracellular signaling, induces endoplasmic reticulum stress, and causes apoptosis in mouse 3T3-L1 and rat primary preadipocytes. *Am J Physiol Endocrinol Metab.* 2007; 293:E576–586. [PubMed: 17519282]
- Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci.* 2003; 23:6315–6326. [PubMed: 12867516]
- Huang K, Sanders S, Singaraja R, Orban P, Cijssouw T, Arstikaitis P, Yanai A, Hayden MR, El-Husseini A. Neuronal palmitoyl acyl transferases exhibit distinct substrate specificity. *FASEB J.* 2009; 23:2605–2615. [PubMed: 19299482]
- Huttenlocher PR. Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res.* 1979; 163:195–205. [PubMed: 427544]
- Iwanaga T, Tsutsumi R, Noritake J, Fukata Y, Fukata M. Dynamic protein palmitoylation in cellular signaling. *Prog Lipid Res.* 2009; 48:117–127. [PubMed: 19233228]
- Johnson ES, Ma PC, Ota IM, Varshavsky A. A proteolytic pathway that recognizes ubiquitin as a degradation signal. *J Biol Chem.* 1995; 270:17442–17456. [PubMed: 7615550]
- Kang W, Wong LC, Shi SH, Hebert JM. The transition from radial glial to intermediate progenitor cell is inhibited by FGF signaling during corticogenesis. *J Neurosci.* 2009; 29:14571–14580. [PubMed: 19923290]
- Karayorgou M, Simon TJ, Gogos JA. 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci.* 11:402–416. [PubMed: 20485365]
- Kazama H, Ichikawa A, Kohsaka H, Morimoto-Tanifuji T, Nose A. Innervation and activity dependent dynamics of postsynaptic oxidative metabolism. *Neuroscience.* 2008; 152:40–49. [PubMed: 18242000]
- Kempf L, Nicodemus KK, Kolachana B, Vakkalanka R, Verchinski BA, Egan MF, Straub RE, Mattay VA, Callicott JH, Weinberger DR, Meyer-Lindenberg A. Functional polymorphisms in PRODH are associated with risk and protection for schizophrenia and fronto-striatal structure and function. *PLoS Genet.* 2008; 4:e1000252. [PubMed: 18989458]
- Kiehl TR, Chow EW, Mikulis DJ, George SR, Bassett AS. Neuropathologic features in adults with 22q11.2 deletion syndrome. *Cereb Cortex.* 2009; 19:153–164. [PubMed: 18483005]
- Kisvarday ZF. GABAergic networks of basket cells in the visual cortex. *Prog Brain Res.* 1992; 90:385–405. [PubMed: 1631306]
- LaMantia AS. Forebrain induction, retinoic acid, and vulnerability to schizophrenia: insights from molecular and genetic analysis in developing mice. *Biol Psychiatry.* 1999; 46:19–30. [PubMed: 10394471]
- Leana-Cox J, Pangkanon S, Egan KR, Curtin MS, Wulfsberg EA. Familial DiGeorge/velocardiofacial syndrome with deletions of chromosome area 22q11.2: report of five families with a review of the literature. *Am J Med Genet.* 1996; 65:309–316. [PubMed: 8923941]
- Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci.* 2005; 6:312–324. [PubMed: 15803162]
- Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, Pramparo T, Jurecic V, Ogunrinhu G, Sutherland HF, Scambler PJ, Bradley A, Baldini A. Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature.* 2001; 410:97–101. [PubMed: 11242049]
- Liu H, Abecasis GR, Heath SC, Knowles A, Demars S, Chen YJ, Roos JL, Rapoport JL, Gogos JA, Karayiorgou M. Genetic variation in the 22q11 locus and susceptibility to schizophrenia. *Proc Natl Acad Sci U S A.* 2002; 99:16859–16864. [PubMed: 12477929]

- Lobo S, Greentree WK, Linder ME, Deschenes RJ. Identification of a Ras palmitoyltransferase in *Saccharomyces cerevisiae*. *J Biol Chem*. 2002; 277:41268–41273. [PubMed: 12193598]
- Long JM, Laporte P, Merscher S, Funke B, Saint-Jore B, Puech A, Kucherlapati R, Morrow BE, Skoultchi AI, Wynshaw-Boris A. Behavior of mice with mutations in the conserved region deleted in velocardiofacial/DiGeorge syndrome. *Neurogenetics*. 2006; 7:247–257. [PubMed: 16900388]
- Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, Biechele T, Petryshen TL, Moon RT, Haggarty SJ, Tsai LH. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3 β /beta-catenin signaling. *Cell*. 2009; 136:1017–1031. [PubMed: 19303846]
- Maynard TM, Haskell GT, Bhasin N, Lee JM, Gassman AA, Lieberman JA, LaMantia AS. RanBP1, a velocardiofacial/DiGeorge syndrome candidate gene, is expressed at sites of mesenchymal/epithelial induction. *Mech Dev*. 2002; 111:177–180. [PubMed: 11804793]
- Maynard TM, Haskell GT, Peters AZ, Sikich L, Lieberman JA, LaMantia AS. A comprehensive analysis of 22q11 gene expression in the developing and adult brain. *Proc Natl Acad Sci U S A*. 2003; 100:14433–14438. [PubMed: 14614146]
- Maynard TM, Meechan DW, Dudevoir ML, Gopalakrishna D, Peters AZ, Heindel CC, Sugimoto TJ, Wu Y, Lieberman JA, Lamantia AS. Mitochondrial localization and function of a subset of 22q11 deletion syndrome candidate genes. *Mol Cell Neurosci*. 2008; 39:439–451. [PubMed: 18775783]
- Maynard TM, Meechan DW, Heindel CC, Peters AZ, Hamer RM, Lieberman JA, LaMantia AS. No evidence for parental imprinting of mouse 22q11 gene orthologs. *Mamm Genome*. 2006; 17:822–832. [PubMed: 16897343]
- McGlashan TH, Hoffman RE. Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Arch Gen Psychiatry*. 2000; 57:637–648. [PubMed: 10891034]
- Meechan DW, Maynard TM, Wu Y, Gopalakrishna D, Lieberman JA, LaMantia AS. Gene dosage in the developing and adult brain in a mouse model of 22q11 deletion syndrome. *Mol Cell Neurosci*. 2006; 33:412–428. [PubMed: 17097888]
- Meechan DW, Tucker ES, Maynard TM, LaMantia AS. Diminished dosage of 22q11 genes disrupts neurogenesis and cortical development in a mouse model of 22q11 deletion/DiGeorge syndrome. *Proc Natl Acad Sci U S A*. 2009; 106:16434–16445. [PubMed: 19805316]
- Merscher S, Funke B, Epstein JA, Heyer J, Puech A, Lu MM, Xavier RJ, Demay MB, Russell RG, Factor S, Tokooya K, Jore BS, Lopez M, Pandita RK, Lia M, Carrion D, Xu H, Schorle H, Kobler JB, Scambler P, Wynshaw-Boris A, Skoultchi AI, Morrow BE, Kucherlapati R. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell*. 2001; 104:619–629. [PubMed: 11239417]
- Minshew NJ, Williams DL. The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch Neurol*. 2007; 64:945–950. [PubMed: 17620483]
- Morison IM, Ramsay JP, Spencer HG. A census of mammalian imprinting. *Trends Genet*. 2005; 21:457–465. [PubMed: 15990197]
- Moyer SE, Lewis PW, Botchan MR. Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc Natl Acad Sci U S A*. 2006; 103:10236–10241. [PubMed: 16798881]
- Mukai J, Dhillia A, Drew LJ, Stark KL, Cao L, MacDermott AB, Karayiorgou M, Gogos JA. Palmitoylation-dependent neurodevelopmental deficits in a mouse model of 22q11 microdeletion. *Nat Neurosci*. 2008; 11:1302–1310. [PubMed: 18836441]
- Mukai J, Liu H, Burt RA, Swor DE, Lai WS, Karayiorgou M, Gogos JA. Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. *Nat Genet*. 2004; 36:725–731. [PubMed: 15184899]
- Murphy KC, Jones LA, Owen MJ. High rates of schizophrenia in adults with velocardio-facial syndrome. *Arch Gen Psychiatry*. 1999; 56:940–945. [PubMed: 10530637]
- Nakai A, Taniuchi Y, Asakura H, Oya A, Yokota A, Koshino T, Araki T. Developmental changes in mitochondrial activity and energy metabolism in fetal and neonatal rat brain. *Brain Res Dev Brain Res*. 2000; 121:67–72.

- Niklasson L, Rasmussen P, Oskarsdottir S, Gillberg C. Autism, ADHD, mental retardation and behavior problems in 100 individuals with 22q11 deletion syndrome. *Res Dev Disabil.* 2008; 31:185–194. [PubMed: 19815377]
- Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci.* 2004; 7:136–144. [PubMed: 14703572]
- Paulsen M, Takada S, Youngson NA, Benchaib M, Charlier C, Segers K, Georges M, Ferguson-Smith AC. Comparative sequence analysis of the imprinted *Dlk1-Gtl2* locus in three mammalian species reveals highly conserved genomic elements and refines comparison with the *Igf2-H19* region. *Genome Res.* 2001; 11:2085–2094. [PubMed: 11731499]
- Paylor R, Glaser B, Mupo A, Ataliotis P, Spencer C, Sobotka A, Sparks C, Choi CH, Oghalai J, Curran S, Murphy KC, Monks S, Williams N, O'Donovan MC, Owen MJ, Scambler PJ, Lindsay E. *Tbx1* haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. *Proc Natl Acad Sci U S A.* 2006; 103:7729–7734. [PubMed: 16684884]
- Peng XR, Jia Z, Zhang Y, Ware J, Trimble WS. The septin *CDCrel-1* is dispensable for normal development and neurotransmitter release. *Mol Cell Biol.* 2002; 22:378–387. [PubMed: 11739749]
- Peterson JM, Wang Y, Bryner RW, Williamson DL, Alway SE. Bax signaling regulates palmitate-mediated apoptosis in C(2)C(12) myotubes. *Am J Physiol Endocrinol Metab.* 2008; 295:E1307–1314. [PubMed: 18840766]
- Phang JM, Downing SJ, Yeh GC. Linkage of the HMP pathway to ATP generation by the proline cycle. *Biochem Biophys Res Commun.* 1980; 93:462–470. [PubMed: 6892988]
- Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science.* 1986; 232:232–235. [PubMed: 3952506]
- Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, Schuffenhauer S, Oechsler H, Belohradsky B, Prieur M, Aurias A, Raymond FL, Clayton-Smith J, Hatchwell E, McKeown C, Beemer FA, Dallapiccola B, Novelli G, Hurst JA, Ignatius J, Green AJ, Winter RM, Brueton L, Brondum-Nielsen K, Scambler PJ, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet.* 1997; 34:798–804. [PubMed: 9350810]
- Sahara S, O'Leary DD. *Fgf10* regulates transition period of cortical stem cell differentiation to radial glia controlling generation of neurons and basal progenitors. *Neuron.* 2009; 63:48–62. [PubMed: 19607792]
- Sakai D, Suzuki T, Osumi N, Wakamatsu Y. Cooperative action of *Sox9*, *Snail2* and PKA signaling in early neural crest development. *Development.* 2006; 133:1323–1333. [PubMed: 16510505]
- Samuels IS, Karlo JC, Faruzzi AN, Pickering K, Herrup K, Sweatt JD, Saitta SC, Landreth GE. Deletion of *ERK2* mitogen-activated protein kinase identifies its key roles in cortical neurogenesis and cognitive function. *J Neurosci.* 2008; 28:6983–6995. [PubMed: 18596172]
- Schaer M, Schmitt JE, Glaser B, Lazeyras F, Delavelle J, Eliez S. Abnormal patterns of cortical gyrification in velo-cardio-facial syndrome (deletion 22q11.2): an MRI study. *Psychiatry Res.* 2006; 146:1–11. [PubMed: 16388934]
- Sefton M, Sanchez S, Nieto MA. Conserved and divergent roles for members of the *Snail* family of transcription factors in the chick and mouse embryo. *Development.* 1998; 125:3111–3121. [PubMed: 9671584]
- Shprintzen RJ, Higgins AM, Antshel K, Fremont W, Roizen N, Kates W. Velocardio-facial syndrome. *Curr Opin Pediatr.* 2005; 17:725–730. [PubMed: 16282778]
- Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA. Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. *Nature.* 2010; 464:763–767. [PubMed: 20360742]
- Son JH, Kawamata H, Yoo MS, Kim DJ, Lee YK, Kim S, Dawson TM, Zhang H, Sulzer D, Yang L, Beal MF, Degiorgio LA, Chun HS, Baker H, Peng C. Neurotoxicity and behavioral deficits associated with Septin 5 accumulation in dopaminergic neurons. *J Neurochem.* 2005; 94:1040–1053. [PubMed: 16092945]

- Stark KL, Xu B, Bagchi A, Lai WS, Liu H, Hsu R, Wan X, Pavlidis P, Mills AA, Karayiorgou M, Gogos JA. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat Genet.* 2008; 40:751–760. [PubMed: 18469815]
- Storm EE, Garel S, Borello U, Hebert JM, Martinez S, McConnell SK, Martin GR, Rubenstein JL. Dose-dependent functions of Fgf8 in regulating telencephalic patterning centers. *Development.* 2006; 133:1831–1844. [PubMed: 16613831]
- Sudo H, Tsuji AB, Sugyo A, Imai T, Saga T, Harada YN. A loss of function screen identifies nine new radiation susceptibility genes. *Biochem Biophys Res Commun.* 2007; 364:695–701. [PubMed: 17964541]
- Suzuki G, Harper KM, Hiramoto T, Sawamura T, Lee M, Kang G, Tanigaki K, Buell M, Geyer MA, Trimble WS, Agatsuma S, Hiroi N. Sept5 deficiency exerts pleiotropic influence on affective behaviors and cognitive functions in mice. *Hum Mol Genet.* 2009; 18:1652–1660. [PubMed: 19240081]
- Swarthout JT, Lobo S, Farh L, Croke MR, Greentree WK, Deschenes RJ, Linder ME. DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. *J Biol Chem.* 2005; 280:31141–31148. [PubMed: 16000296]
- Tamamaki N, Yanagawa Y, Tomioka R, Miyazaki J, Obata K, Kaneko T. Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J Comp Neurol.* 2003; 467:60–79. [PubMed: 14574680]
- Tedeschi A, Ciciarello M, Mangiacasale R, Roscioli E, Rensen WM, Lavia P. RANBP1 localizes a subset of mitotic regulatory factors on spindle microtubules and regulates chromosome segregation in human cells. *J Cell Sci.* 2007; 120:3748–3761. [PubMed: 17940066]
- Tickle C, Eichele G. Vertebrate limb development. *Annu Rev Cell Biol.* 1994; 10:121–152. [PubMed: 7888174]
- Tucker ES, Segall S, Gopalakrishna D, Wu Y, Vernon M, Polleux F, Lamantia AS. Molecular specification and patterning of progenitor cells in the lateral and medial ganglionic eminences. *J Neurosci.* 2008; 28:9504–9518. [PubMed: 18799682]
- Turpin SM, Lancaster GI, Darby I, Febbraio MA, Watt MJ. Apoptosis in skeletal muscle myotubes is induced by ceramides and is positively related to insulin resistance. *Am J Physiol Endocrinol Metab.* 2006; 291:E1341–1350. [PubMed: 16849630]
- van Amelsvoort T, Daly E, Henry J, Robertson D, Ng V, Owen M, Murphy KC, Murphy DG. Brain anatomy in adults with velocardiofacial syndrome with and without schizophrenia: preliminary results of a structural magnetic resonance imaging study. *Arch Gen Psychiatry.* 2004; 61:1085–1096. [PubMed: 15520356]
- Vermot J, Niederreither K, Garnier JM, Chambon P, Dolle P. Decreased embryonic retinoic acid synthesis results in a DiGeorge syndrome phenotype in newborn mice. *Proc Natl Acad Sci U S A.* 2003; 100:1763–1768. [PubMed: 12563036]
- Washington Smoak I, Byrd NA, Abu-Issa R, Goddeeris MM, Anderson R, Morris J, Yamamura K, Klingensmith J, Meyers EN. Sonic hedgehog is required for cardiac outflow tract and neural crest cell development. *Dev Biol.* 2005; 283:357–372. [PubMed: 15936751]
- Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry.* 1987; 44:660–669. [PubMed: 3606332]
- Weinberger DR. On the plausibility of “the neurodevelopmental hypothesis” of schizophrenia. *Neuropsychopharmacology.* 1996; 14:1S–11S. [PubMed: 8866738]
- Zhao L, Lobo S, Dong X, Ault AD, Deschenes RJ. Erf4p and Erf2p form an endoplasmic reticulum-associated complex involved in the plasma membrane localization of yeast Ras proteins. *J Biol Chem.* 2002; 277:49352–49359. [PubMed: 12379641]

Figure 1. 22q11 genes, 22q11 deletion, and the brain

A. A schematic of the 32 genes in the minimal critical deleted region associated with 22q11 Deletion Syndrome (22q11DS) on human chromosome 21, and the 26 murine orthologues of these genes found on mouse chromosome (mmChr) 16. The lines indicate either colinearity between orthologues, or reversal of 5' and 3' position on the chromosome. **B.** Brain expression of representative 22q11 genes in the large deletion mouse model (*Lgdel*) of 22q11DS or wild type controls, shown by *in situ* hybridization. There is widespread regional and cellular expression of 22q11 genes in the adult mouse brain. **C.** mRNA levels for multiple 22q11 orthologues are diminished by 50% in the adult (post natal day 60) *Lgdel*

mouse brain. **D.** Protein levels of a subset of 22q11 genes are also diminished by 50% in the embryonic (shown here) as well as adult brain, in parallel with mRNA, except for Ufd1L, for which there is translational dosage compensation—protein levels return to 100% of wild type, despite a 50% decrement in message levels.

Figure 2. 22q11 genes and mesenchymal/epithelial (M/E) interactions

A. A PCR array evaluating multiple 22q11 genes from the 1.5 MB minimal critical deleted region in microdissected samples of four regions from E10.5 mouse embryos where M/E interactions drive morphogenesis: the frontonasal mass and forebrain (fnm/ fb), the branchial arches (ba), the aortic arches and heart (aah) and the forelimb bud. At the far right, four genes that are differentially associated with each region are included as controls to validate the identity of the microdissected material. **B.** Immuno- (brown label) or *in situ* hybridization localization of 5 22q11 genes showing selective or specific expression in the forelimb bud, the heart or aortic arches, the branchial arches, and the frontonasal mass/ forebrain. **C.** *At left:* littermate wild type and *Lgdel* mutant E10.5 embryos hybridized for *Prodh* (these two embryos were labeled simultaneously in the same reaction vials, and genotyped subsequently) show distinct diminished expression levels of 22q11 genes following heterozygous deletion, without appreciable change in patterned expression in the

fnm/ fb (1), the ba (2), the aah (3) or the forelimb bud (4). **At right:** qPCR analysis of expression of 10 representative 22q11 genes in microdissected fnm/ fb from wild type and *Lgdel*/E10.5 embryos shows consistent 50% decrease in mRNA levels. Ggt, a 22q11 gene in human that is not located on mmChr.16 with other 22q11 orthologues, is not diminished in dosage. **D.** Coincidence of 22q11 gene expression with neural crest-related genes in the mesenchyme at sites of M/ E interactions. **At left:** a wild type E10.5 embryo hybridized for mRNA for *Snail2*, which is selectively expressed in mesenchymal neural crest. **Center:** E10.5 section in situ preparations from the fnm/ fb and flb of wild type (+/ +) and *Lgdel* (+/-) embryos showing the coincidence of *Snail2* and *ProdH2*, a 22q11 gene, in the mesenchyme. At right: *Snail2* mRNA levels in *Lgdel* embryos, measured using qPCR, approximate those seen in wild type embryos.

Figure 3. Morphometric assessment of brain and body phenotypes in the *Lgdel* mouse model of 22q11DS

A. There are visible differences in the lengths of major limb bones from the *Lgdel* young adult mouse compared to wild type littermates. **B.** The lengths of all of the major bones of the forelimb are significantly reduced compared to wild type littermates. **C.** Body weight does not differ significantly in *Lgdel* mouse. **D.** There is a trend for diminished brain weight; however this difference does not reach statistical significance.

Figure 4. 22q11 genes are robustly expressed in the proliferating zones of the embryonic cortex and proliferation is specifically disrupted in the svz of the embryonic *Lgdel* cortex

A. In-situ hybridization of wild-type embryonic mouse forebrain for the 22q11 genes, *Ranbp1*, *Cdc45l*, *Htf9c*, *Ufd1l*, *Hira* and *Sept5*. Signal intensity is most intense in the cortical proliferative zone for *Ranbp1* and *Cdc45l*. Expression is more broadly distributed for *Htf9c*, *Ufd1l* and *Hira*. Finally, *Sept5* expression is absent from the proliferative zone and prominent in the cortical plate. **B:** Cartoon and immunohistochemical images indicate that the mitotic marker PH3 (phosphohistone 3) is reduced in the subventricular zone of embryonic day 13.5 *Lgdel* cortex but not in the ventricular zone. **C.** Similarly, the DNA synthesis mitotic marker, BrdU, is less frequently observed in subventricular zone cells (Tbr2+) of the *Lgdel* cortex. However, non-subventricular BrdU + mitotic cells are not disrupted (right panels).

Figure 5. Embryonic interneurons are altered in their location during migration and mature interneurons and projection neurons have altered laminar location/ frequency in the postnatal cortex

A. Staining for the interneuron marker, calbindin, in E13 *Lgdel* and wild-type cortex shows that these cells are less frequently observed in the *Lgdel* at dorsal cortical locations. **B.** By marking 5 equidistant Bins in the cortex (see A), we show that calbindin cells are less frequently observed in more dorsal cortical locations. **C.** Upper layer projection neurons (Cux1 +) are less frequent in postnatal *Lgdel* cortex compared to wild-type. **D.** In postnatal day 21 cortex, the distribution of the interneuron marker, parvalbumin, is altered in *Lgdel* animals.

Figure 6. A subset of 22q11 genes is associated with synaptic mitochondria

A. Six 22q11 gene products are localized to mitochondria. The localization of five proteins (Mrpl40, Prodh, Slc25a1, T10, Txnrd2) are illustrated by co-transfection of a GFP-labeled expression construct with a mitochondrially-targeted mCherry construct. Co-localized products appear yellow in merged images. Localization of Zdhhc8 is illustrated by immunostaining with anti-Zdhhc8 antiserum (green) and anti-Uqcrc1 antiserum (red). **B.** Mitochondrial localization of Zdhhc8 and Txnrd2 was also demonstrated by fractionating cortical tissue extracts to enrich for free mitochondria and synaptosomal mitochondria; western blot analysis shows an abundance of both proteins in synaptic mitochondrial fractions. **C.** Zdhhc8 is present in synapses, particularly those in glutamatergic synaptic terminals (which are recognized based on immunoreactivity for the vesicular glutamate transporter, Vglut1). The dense neuropil of the glomeruli of the olfactory bulb, which include both excitatory glutamatergic and inhibitory GABAergic terminals provide a clear illustration of the restricted localization of Zdhhc8 in the Vglut1 labeled glutamatergic terminals (presumably from olfactory receptor neuron axons) within the glomeruli. In contrast, Zdhhc8 is largely absent from the Gad67 and Parvalbumin labeled GABAergic subset of glomerular synaptic terminals.

Figure 7. Mitochondrial function of 22q11 genes

A. Controlled expression of HA-tagged Zdhhc8 protein in a tetracycline-inducible 3T3 cell line shows that high level overexpression of Zdhhc8 causes substantial apoptosis by 18 hours. **B.** Mitochondrial localization and induction of cell death by Zdhhc8 require the C-terminus of the protein, which includes the DHHC domain. **C-E.** Zdhhc8 over-expression induced cell death (shown in **C**) appears to be apoptotic, as it is robustly inhibited both by co-expression of the anti-apoptotic factor Bcl2 (**D**) and by the addition of an oxygen radical-scavenging agent PBN (**E**). **F.** A yeast two-hybrid analysis indicates that the Zdhhc8 tail domain (AA 215-762) can interact with the Uqcrc1 protein. Full length Uqcrc1 (AA 23-480)

was co-transfected into yeast with deletion fragments of *Zdhhc8*. Right columns show growth of reporter yeast transformed with both plasmids on selective (-His) or non-selective (+His) growth media (-Leu/ -Trp). **G.** Reduced expression of 22q11 genes in the *Lgdel* mouse may influence ongoing function of cortical mitochondria. In the cortex of the *Lgdel* mouse, the expression of 22q11 genes, including *Zdhhc8*, is reduced by approximately 50%; however, several mitochondrial genes that are not on 22q11 show increased expression levels in the neonatal cortex. This increased expression of other mitochondrial genes (particularly *Uqcrc1* and *Uqcrc2*, components of mitochondrial Complex III) may represent compensation for reduced expression of one or more 22q11 mitochondrial genes.

Figure 8. A summary of critical stages of brain development compromised by diminished dosage of 22q11 genes, based upon data summarized in this review

Our evidence suggests that 22q11 genes act normally to regulate each major step of neural development. Heterozygous deletion leads to altered dosage and function of critical subsets of 22q11 genes, some with maximal function during particular neurodevelopmental events. The consequence of these changes due to 22q11 deletion include altered frequency of cortical projection neurons in layers 2/3, particularly in anterior/ frontal association cortices, and parallel disruption of position of GABAergic interneurons.