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The 22q11.2 microdeletion: fifteen years of insights into the genetic and neural complexity of psychiatric disorders

Liam J. Drew[a], **Gregg W. Crabtree**[a], **Sander Markx**[b], **Kimberly L. Stark**[a,b], **Florence Chaverneff**[a], **Bin Xu**[a,b], **Jun Mukai**[a], **Karine Fenelon**[a], **Pei-Ken Hsu**[a,c], **Joseph A. Gogos**[a,d], and **Maria Karayiorgou**[b,e]

[a]Department of Physiology and Cellular Biophysics, Columbia University, New York, New York 10032, USA

[b]Department of Psychiatry, Columbia University, New York, New York 10032, USA

[c]Integrated Program in Cellular, Molecular, and Biophysical Studies, Columbia University, New York, New York 10032, USA

[d]Department of Neuroscience, College of Physicians and Surgeons, Columbia University, New York, New York 10032, USA

[e]New York State Psychiatric Institute, New York, New York 10032, USA

Abstract

Over the last fifteen years it has become established that 22q11.2 deletion syndrome (22q11DS) is a true genetic risk factor for schizophrenia. Carriers of deletions in chromosome 22q11.2 develop schizophrenia at rate of 25–30% and such deletions account for as many as 1–2% of cases of sporadic schizophrenia in the general population. Access to a relatively homogeneous population of individuals that suffer from schizophrenia as the result of a shared etiological factor and the potential to generate etiologically valid mouse models provides an immense opportunity to better understand the pathobiology of this disease. In this review we survey the clinical literature associated with the 22q11.2 microdeletions with a focus on neuroanatomical changes. Then, we highlight results from work modeling this structural mutation in animals. The key biological pathways disrupted by the mutation are discussed and how these changes impact the structure and function of neural circuits is described.

Keywords

Schizophrenia; 22q11.2; psychiatric genetics; Prodh; Dgcr8; Zdhhc8; Comt; copy number variant; microRNAs; palmitoylation; neurodevelopment

1 Introduction

One approach to understanding how schizophrenia is caused in general is to focus on specific cases where the disease is caused by a known factor. Although rare, there are now a

Correspondence: Maria Karayiorgou (mk2758@columbia.edu) or Joseph A. Gogos (jag90@columbia.edu).

number of instances where the primary genetic causative factor appears to have been identified (Karayiorgou et al., 1995; Millar et al., 2000; Xu et al., 2009). Here, we discuss the example of genomic microdeletions in the chromosomal region 22q11.2 that may account for as many as 1–2% of cases of schizophrenia (Karayiorgou et al., 2010) and is, to date, the only confirmed recurrent structural mutation responsible for the introduction of sporadic cases of schizophrenia.

The identification, fifteen years ago, of 22q11.2 microdeletions as a causative factor for schizophrenia was the first demonstration that deletions, or duplications, of chromosomal regions may play an important role in schizophrenia etiology in many cases of the disease (Karayiorgou et al., 1995). Such mutations, known as copy number variations (CNVs), result in altered gene dosage and a rapidly expanding literature shows a high prevalence of such mutations in schizophrenia patients (International Schizophrenia Consortium, 2008; Stefansson et al., 2008; Xu et al., 2008; Walsh et al., 2008; Xu et al., 2009). By allowing the generation of etiological valid animal models, identification of highly penetrant mutations such as these offer unprecedented opportunities to determine the key neural changes that can lead to psychotic illness (Arguello and Gogos, 2006). Here we argue that the 22q11.2 deletion can act as prototype for this type of investigation. That is to say, the rigorous definition of the pathway from mutation to disease phenotype in models of this mutation will provide invaluable insights into the etiology and pathophysiology of schizophrenia as a whole.

2 22q11.2 Deletion Syndrome

Chromosomal microdeletions at region q11.2 of human chromosome 22 occur at a frequency of around 1 in every 4,000 live births, making it the most common known interstitial deletion in humans (Scambler, 2000). Substantial stretches of low copy repeats in this region make it particularly susceptible to deletions during meiosis (Edelmann et al., 1999; Kurahashi et al., 2000; Bittel et al., 2009) and it is of note that duplications at this locus are also relatively common (Portnoï, 2009). The majority of the 22q11.2 microdeletions (~90%) are 3 megabases (Mb) in size (containing approximately 60 known genes, see Tables 1 and 2), while ~8% are 1.5Mb in size (containing approximately 28 known genes, see Table 1) (Edelmann et al., 1999; Shaikh et al., 2000), and the remainder are atypical deletions (containing only a small number of genes) (Urban et al., 2006). It has been argued that the 1.5Mb deletions contain all key genes responsible for the syndrome (Carlson et al., 1997) and, in particular, for the increased risk of psychiatric illness (Karayiorgou et al., 1995).

22q11.2 microdeletions cause a complex syndrome that is typically categorized as DiGeorge syndrome or velo-cardio-facial (VCF) syndrome (Scambler, 2000) or 22q11.2 deletion syndrome (22q11.2DS). The first two terms refer, respectively, to the physician who originally delineated the condition in the mid-1960s (Dodson et al., 1969) and to the types of symptoms that normally lead to clinical presentation (velo refers to the palate of the mouth, cleft palates being a common manifestation) (Shprintzen et al., 1978). In this review we will refer specifically to 22q11.2DS to specify a syndrome definitively caused by a mutation at this locus.

The phenotype associated with the 22q11.2 microdeletions is highly variable and involves multiple organ systems, however there is no correlation between the severity of the phenotype and the size of the deletion (Carlson et al., 1997). Phenotypic variability can be caused by breakpoint heterogeneity as well as other genetic, environmental, and stochastic factors. The phenotype includes craniofacial and cardiovascular abnormalities,

immunodeficiency, hypocalcaemia, short stature and cognitive impairments (Shprintzen et al., 1978; Ryan et al., 1997; Scambler, 2000; Kobrynski and Sullivan, 2007).

22q11.2 microdeletions account for up to 1–2% of sporadic cases of schizophrenia (Karayiorgou et al., 1995; Xu et al., 2008). Moreover, it has been shown that at adolescence or early adulthood, up to one third of 22q11.2 deletion carriers develop schizophrenia or schizoaffective disorder, which is approximately 25–31 times that of the general population (Pulver et al., 1994; Murphy et al., 1999). This bidirectional association makes the 22q11.2 microdeletion the strongest known risk factor for developing schizophrenia. In addition, since all but the most severe cardiovascular and immune defects associated with 22q11.2DS can now be treated and almost all affected individuals survive into adulthood, long-term medical care and prenatal screening are increasingly being directed toward the recognition and treatment of cognitive and psychiatric phenotypes.

In this review we focus on the now well-established link between 22q11.2 DS and psychotic illnesses, with reference to cognitive dysfunction. We begin with an outline of these psychiatric conditions and cognitive impairments found in 22q11.2 deletion carriers and discuss studies of brain structure and function that have used neuroimaging techniques. Subsequent sections will focus on the complex genetic make-up of this syndrome and how we believe that the creation of mouse models are an essential means of investigation for understanding the syndrome. First we will review behavioral, cell biological and neurophysiological studies in various mouse models of the syndrome, before focusing on what we believe may be the key deleted genes in this region. Finally, we discuss future directions for this work, including the use of induced pluripotent stem cells for creating human neuronal models.

2.1 Psychiatric Phenotype of the 22q11.2 Deletion Syndrome

2.1.1 Susceptibility to Schizophrenia—The most prevalent psychiatric disorder in adults with 22q11.2DS is schizophrenia (Karayiorgou et al., 1995; Murphy et al., 1999; Pulver et al., 1994; Xu et al., 2008). Although cases of childhood onset schizophrenia have been described (Usiskin et al., 1999), most carriers develop schizophrenia in early adulthood, as is the case with schizophrenia in general (Shprintzen et al., 1992; Murphy et al., 1999). Overall, the literature suggests that schizophrenia in 22q11.2 deletion carriers is indistinguishable from sporadic forms of the illness (Bassett et al., 1998; 2003). This high association has prompted researchers to look for early psychiatric signs in this ‘at risk’ population before patients develop a full-blown psychotic disorder. Sub-threshold symptoms indicative of psychosis (*i.e.*, disturbances of social and adaptive functioning prior to the onset of characteristic symptoms of psychosis) were identified in approximately one-third to one1 half of children afflicted with 22q11.2DS (Feinstein et al., 2002; Baker et al., 2005a; Debbane et al., 2006a). One longitudinal study of patients with 22q11.2DS, following patients from childhood and adolescence to young adulthood, identified similar sub-threshold symptoms as a major risk factor for later development of schizophrenia (Gothelf et al., 2007a).

2.1.2 Cognitive Dysfunction—Cognitive dysfunction is common in patients with 22q11.2DS (Chow et al., 2006; De Smedt et al., 2007). Detailed analysis of the domains in which such changes are prevalent is important for pinpointing which neural circuits are likely disrupted by the deletion and additionally to determine which, if any, cognitive changes are specifically associated with development of schizophrenia. Schizophrenia is itself increasingly recognized as a disorder in which cognitive deficits are a core feature.

Most school-aged children who have a 22q11.2 microdeletion fall into the category of ‘borderline intellectual functioning’ with a Full-Scale IQ (FSIQ) of 71–85, followed by a

smaller proportion of children that falls into the 'mild intellectual disability' range (FSIQ 55–70), and a small percentage in the 'low-average intelligence range' (FSIQ>85) (De Smedt et al., 2007). More severe levels of mental retardation are relatively uncommon (Chow et al., 2006). It is important to emphasize however, that a large number of children with 22q11.2DS present with a cognitive profile that is characterized by a relative sparing of Verbal IQ (VIQ) compared with Performance IQ (PIQ) (Moss et al., 1999; Wang et al., 2000; De Smedt et al., 2007). During school years, reading, spelling, and verbal memory scores are in the low average to superior range (Swillen et al., 1999; Wang et al., 2000; Woodin et al., 2001) and patients consistently score higher on verbal memory than visual-spatial memory (Bearden et al., 2001; Lajiness-O'Neill et al., 2005; Sobin et al., 2005a).

It has been suggested that impairment in attention could be a major contributor to the cognitive profile in 22q11.2DS as attention is required for many nonverbal cognitive functions. For example, studies have suggested that children with 22q11.2DS have difficulty in identifying and interpreting salient spatial and temporal information (Simon et al., 2005a; 2005b; Bish et al., 2007). This suggests that these patients are less able to focus their attention by navigating space, guiding vision, and selecting and integrating goal-relevant information. Counting ability (which requires spatial memory) is also specifically impaired in children with 22q11.2DS. Furthermore, both children and adults with 22q11.2DS demonstrate impairments in 'magnitude comparison' and 'time duration comparison', further pointing to spatial, temporal, and numerical impairments (Debbane et al., 2005; Simon et al., 2005a; Simon et al., 2008). In combination, these cognitive deficits indicate dysfunction in both parietal and frontal cortical neurocircuitry (Posner et al., 1990; Corbetta et al., 2002).

Attention also plays a role in inhibiting the processing of irrelevant information. Several studies have reported impairments in this ability in children with 22q11.2DS (Gratton et al., 1991; Sobin et al., 2004; Bish et al., 2005; Takarae et al., 2009). Sensorimotor gating, a form of preattentive inhibition – in this case attenuation of the acoustic startle response when a loud noise is preceded by a predictive tone, the so called pre-pulse inhibition (PPI) paradigm – is also reduced and associated with attentional deficits in patients with 22q11.2DS (Sobin et al., 2004; Sobin et al., 2005b). Many patients with schizophrenia have been shown to have abnormal PPI, and several lines of evidence suggest that PPI may represent a heritable endophenotype. Such gating deficits have been hypothesized to cause problems with the 'screening out' or suppression of irrelevant thoughts and sensory information from conscious awareness in patients with schizophrenia (Hasenkamp et al., 2010).

Adolescents with 22q11.2DS have also been shown to have deficits in assays of mismatch negativities (MMN), another measure of preattentive processing (Baker et al, 2005b). In electroencephalographical studies a prominent negative component of the evoked-related potential is typically evoked by an unexpected, or "oddball", stimulus in a train of otherwise identical stimuli. It is well established that patients with idiopathic schizophrenia commonly have deficits in MMN and it has been reported that such deficits are predictive of poor functional outcome in the disease (Light and Braff, 2005). Interestingly, Baker et al. found deficits specifically in the frontocortical recording site but not from temporal cortical electrodes (see Section 3.1.4).

2.2 Neuroanatomical Changes in Patients with 22q11.2DS

There have been a large number of neuroimaging studies on patients with 22q11.2DS that report a wide range of abnormalities in both brain structure and function. However, despite extensive investigation, this literature remains relatively inconsistent regarding changes in specific neuronal regions or structures. Furthermore, direct functional correlates of these structural changes have not yet been firmly established, which is largely due to the use of

different measurement protocols in studies with relatively small but differing samples (Karayiorgou et al., 2010). Nevertheless, when only looking at findings that have been replicated by at least two independent research groups in at least two separate and independent studies, a pattern of neuroanatomical abnormalities appears to emerge in patients with 22q11.2DS, both with and without psychosis (Karayiorgou et al., 2010). Moreover, some abnormalities show similarity to those found in non-22q11.2DS-associated schizophrenia. Table 3 provides a summary of the main neuroanatomical findings in 22q11.2DS.

2.2.1 Qualitative Abnormalities in Children and Adults with 22q11.2DS—Several studies have identified developmental abnormalities that affect midline structures of the brain in patients with 22q11.2DS with or without psychosis. These include cavum vergae (*i.e.*, a horizontal cleft formed between the commisura fornicis and the corpus callosum when the two commissural plates failed to fuse completely during fetal development) or cavum septum pellucidum (*i.e.*, a septum pellucidum that has a separation between its two leaflets) in up to 30–45% of patients in certain samples (Chow 1999; van Amelsvoort et al., 2001; Shashi et al., 2004). Similar midline developmental defects have also been found in patients with non-22q11.2DS-associated schizophrenia (Nopoulos et al., 1997; Kwon et al., 1998). Furthermore, studies have described polymicrogyria in a small number of patients with 22q11.2 microdeletions. Polymicrogyria is a brain malformation involving abnormal neuronal migration, leading to many abnormally small gyri that do not follow the normal gyral pattern (Sztriha et al., 2004; Robin et al., 2006). Finally, some studies have reported on white matter hyperintensities on T2-weighed magnetic resonance (MRI) imaging in patients with 22q11.2DS (Chow et al. 1999; van Amelsvoort et al., 2001). White matter hyperintensities are non-specific abnormalities sometime found on MRI that may reflect abnormalities in myelination and increased white matter water content and have been associated with ageing, demyelinating disease, and cerebrovascular disease (Chow et al., 1999).

2.2.2 Volumetric Abnormalities in Patients with 22q11.2DS—Tan et al. (2009) published a systematic review and meta-analysis of volumetric magnetic imaging studies of region of interest (ROI) studies comparing 22q11.2DS patients with healthy controls. They reported a pattern of volumetric abnormalities that includes global brain volumetric reduction, including widespread cortical reductions in frontal, temporal, parietal, and occipital lobes – as well as a decreased volume of both the cerebellum and hippocampus. A similar pattern of volumetric reductions of cortical and subcortical regions has also been found in patients with non-22q11.2DS-associated schizophrenia (Shelton and Weinberger 1986; Pfefferbaum and Zipursky 1991). In addition to this, Tan et al. (2009) also reported a volumetric increase of the corpus callosum. Many of the volumetric neuroimaging studies used for the meta-analysis report a different pattern for children and adolescents versus adults with 22q11.2DS. We will therefore review these two groups separately below.

2.2.2.1 Children and Adolescents: Studies have consistently shown enlarged ventricles and a decrease in total brain volume in children and adolescents with 22q11.2DS compared with age-matched, normal controls (Eliez et al., 2000; Kates et al., 2001), with white matter affected more than gray matter. After correcting for global brain reduction, research has demonstrated a relative sparing of frontal lobe volume (Eliez et al., 2000; Kates et al., 2001; Kates et al., 2004; Simon et al., 2005a) whereas parietal lobe volume was significantly reduced (Eliez et al., 2000; Kates et al., 2001; van Amelsvoort et al., 2001). These studies, hence, indicate a possible neuroanatomical substrate for certain specific neuropsychological findings often found in patients with 22q11.2DS, such as deficits in visuo-spatial processing and arithmetic reasoning (Gothelf et al., 2008). Furthermore, after adjusting for total brain

volume, a relative frontal lobe enlargement in children with 22q11.2DS has been associated with a preserved borderline IQ compared with children with 22q11.2DS without this enlargement (Eliez et al., 2000).

These findings are consistent with a rostro-caudal gradient of severity in neuroanatomical abnormalities (*i.e.*, greater reductions in posterior compared with anterior regional volumes) in the brains of 22q11.2 DS patients (Gothelf et al., 2008). Such a gradient has also been observed within specific brain structures of children who carry the 22q11.2 microdeletion. For example, the caudate nucleus (Eliez et al., 2002; Kates et al., 2004; Campbell et al., 2006), the thalamus (Bish et al., 2004), corpus callosum (Antshel et al., 2005; Machado et al., 2007), and fusiform gyrus (Glaser et al., 2007) have all been reported to be more severely reduced in posterior regions, and at times to show enlargement in their anterior regions (Eliez et al., 2002; Gothelf et al., 2008). Another caudal brain structure that appears to be significantly reduced in size in patients with 22q11.2DS is the cerebellum (Chow et al., 1999; Sashi et al., 2010) – specifically the vermis, anterior lobes, and neocerebellum (Eliez et al., 2001b, Bish et al. 2006, Kates et al., 2006).

Furthermore, reduction in hippocampal volume has been identified in some studies (Debbane et al., 2006b; Deboer et al., 2007), although others did not demonstrate volumetric differences when compared to controls (Eliez et al., 2001a; Kates et al., 2006; Gothelf et al., 2007b). In patients with 22q11.2DS, reduction of the hippocampal volume was found to correlate negatively with Full-Scale Intelligence Quotient (FSIQ) (Deboer et al., 2007) and to be associated with impairments of association and recall processes in neuropsychological tests (Debbane et al., 2006b).

2.2.2.2 Adults: In adults with 22q11.2DS and schizophrenia, overall brain volume is reduced compared to both normal controls (Chow et al., 2002) and to individuals with developmental disabilities without 22q11.2DS (van Amelsvoort et al., 2001). Reduced cerebellar volume has also been demonstrated in adult 22q11.2DS patients, both with and without psychosis (Chow et al., 1999; van Amelsvoort et al., 2001; 2004). Patients with 22q11.2 microdeletions who have schizophrenia also have enlarged lateral ventricles compared with healthy controls (Chow et al., 1999; 2002), which is one of the most replicated neuroimaging findings in patients with non-22q11.2DS-associated schizophrenia (Styner et al., 2005).

There are a number of interesting contrasts between the results of imaging studies in children and adolescents with 22q11.2DS and adult deletion carriers. Prominently, both frontal and temporal lobes were found to be reduced in adults (Gothelf et al., 2008) but enlargement of the caudal nucleus is no longer identifiable (van Amelsvoort et al., 2001). Together these data indicate that the rostro-caudal gradient is no longer present in adult patients. Van Amelsvoort et al (2004) compared regional brain volumes between patients with 22q11.2DS and schizophrenia and age-matched patients with 22q11.2DS without schizophrenia, and found generalized decrease in the gray and white matter volume and specifically more significant reduction in frontal and temporal lobes in the patients with 22q11.2DS and schizophrenia. In addition to this, longitudinal studies have demonstrated an association between reduced prefrontal gray matter volume and the development of psychosis (Gothelf et al., 2005) as well as greater longitudinal increase of both cerebral and cerebellar white matter in patients with 22q11.2DS (Gothelf et al. 2007a).

2.2.3 Cortical Thickness and Shape in Patients with 22q11.2DS—Several recent studies have focused on cortical thickness in children with 22q11.2 microdeletions compared with normal controls. Specific brain regions of children with 22q11.2DS have been demonstrated to have lateral cortical thinning, including in parieto-occipital, occipital

pole, and inferior prefrontal regions, and medial thinning in anterior cingulate, medial frontal gyrus, subgenual prefrontal, posterior cingulated gyrus, cuneus, and lingual gyrus regions. Additionally, the association between cortical thinning and increasing age was more pronounced in patients with 22q11.2DS compared with normal controls (Bearden et al., 2006; 2009). Frontal, and to a lesser extent parietal gray matter volumes have also been demonstrated to correlate with specific cognitive measures in patients with 22q11.2DS (Bearden et al., 2009). Furthermore, reduced cortical complexity was identified in the frontal and parietal cortex of patients with 22q11.2DS, overlapping with certain areas of cortical thinning. These differences in gyral complexity might be associated with altered neuronal connectivity, as it has been hypothesized that mechanical tension along developing axonal tracts can act a primary driving force for cortical folding (van Essen et al., 2006).

2.2.4 Diffusion Tensor Imaging in Patients with 22q11.2DS—Diffusion tensor imaging (DTI) is an MRI imaging technique that takes advantage of the restricted water diffusion through myelinated nerve fibers in the brain to map the white matter tracts that connect brain regions. In patients with 22q11.2DS, studies have consistently shown a diffuse disorganization of white matter in the parietal region (Barnea-Goraly et al., 2003; 2005; Simon et al., 2005a). Specifically, most of the fractional anisotropy differences (*i.e.*, changes of water diffusion in white matter tracts of the brain) were localized to parieto-parietal connections (Sun et al., 2007), in addition to fronto-frontal and fronto-temporal connections (Barnea-Goraly et al., 2003). These DTI abnormalities of the frontal and parietal area or of the corpus callosum have been associated with poorer performance on specific cognitive tasks, including arithmetic (Barnea-Goraly et al., 2005), counting ability (Machado et al., 2007), and spatial attention (Simon et al., 2008). This is in concordance with previous DTI studies that have demonstrated extensive abnormalities of frontal and temporal axonal tracts as well as abnormalities in fibers connecting the frontal and temporal lobes in patients who suffer from non-22q11.2DS-associated schizophrenia (Akbarian et al., 1996; Foong et al., 2000; Foong et al., 2001; Kubicki et al., 2002).

In summary, over the last 15 years there have been a large amount of neuroimaging studies describing children and adult patients with 22q11.2DS, both with and without psychosis, that describe a wide range of neuroanatomical abnormalities in both cortical and subcortical structures. Interestingly, a rostro-caudal gradient of alterations has been noted in children with 22q11.2DS, which appears to disappear with age, giving way to more widespread loss of brain tissue, particularly in frontal and temporal lobes. However, further well-designed imaging studies with human subjects who suffer from schizophrenia and who harbor the 22q11.2 microdeletion will be required to further identify abnormalities in neuronal structure and function that are causally related to different components of the neuropsychiatric phenotype associated with 22q11.2DS. Complimentary to this, it will be crucial to carry out neuroimaging studies in the mouse models of 22q11.2DS to help analyze the neuroanatomically relevant regions of the brain.

3 Genetic Models of the 22q11.2 Deletion Region

The above discussion of studies of human carriers of 22q11.2 microdeletions provides a framework of data regarding cognitive deficits and gross neuroanatomical abnormalities associated with this syndrome. Such studies parallel decades of work in which psychiatrists, psychologists and neuroscientists have applied similar tests to schizophrenia patients in general. The strong link between the 22q11.2 mutation and the development of schizophrenia, however, offers the opportunity to create etiologically valid mouse models of the disease (see Karayiorgou et al., 2010) with which numerous experiments impossible in humans can be conducted. This is important because the paucity in understanding the etiology of psychiatric diseases means such etiologically valid models are rare (Arguello and

Gogos, 2006). Other models of the disease are typically distinct in that they are based on assumptions about the pathophysiology of the disease such as, NMDA receptor hypofunction (Tsai and Coyle, 2002; Inta et al., 2010), decreased GABAergic interneuron function (Lewis et al., 2005; Belforte et al., 2010), developmental lesions of brain regions (*e.g.* Lodge and Grace, 2008) or alterations of dopaminergic pathways (*e.g.* Kellendonk et al., 2006). Although such models are informative in terms of determining how such manipulations can affect neural circuits and give rise to behavioral changes, the modeling of highly penetrant genetic mutations allows unbiased investigations of schizophrenia pathogenesis (Arguello and Gogos, 2006; Arguello et al., 2010).

In addition, we discuss in this section the rapidly developing field of disease modeling using induced pluripotent stem cells derived from human patients and the applicability of these techniques to modeling 22q11.2DS.

3.1 Mouse Models of 22q11.2 DS

The 22q11.2 region is syntenic with a region of mouse chromosome 16, thus allowing for an accurate representation of the human deletion in this model organism. A number of long-range deletions, as well as knockouts of individual genes in this region, have been created in the mouse (see Karayiorgou et al., 2010). Single gene knockouts are useful to assess the involvement of that gene in various behaviors, but given the complexity of 22q11.2DS, long-range deletions are necessary to capture interactions between genes in this region, and to more accurately model the syndrome.

Using chromosomal engineering, several long-range deletions have been created in this region (Kimber et al., 1999; Lindsay et al., 1999; Puech et al., 2000; Lindsay et al., 2001; Merscher et al., 2001; Stark et al., 2008). Two of these models (the *Df(16)A+/−* model (Stark et al., 2008) and the *LgDel/+* model (Merscher et al., 2001)) contain the entire minimally critical 1.5 Mb region, from *Dgcr2* to *Hira*, which is 1.3 Mb in the mouse. In the human, this region has been suggested to contain all the genes crucial to the etiology of the 22q11.2DS (Carlson et al., 1997). There is a high degree of conservation in this region: all but one of the murine orthologues of the 28 functional genes found in the 1.5 Mb human deletion are present in this region. The exception is *CLTCL1* (clathrin, heavy polypeptide-like 1). Additionally, the genes are arranged in slightly different orders in the two species and humans have two functional DGCR6 genes as a result of a duplication (DiGeorge syndrome critical region 6 (*DGCR6*) and *DGCR6*-like), whereas the mouse has just one.

3.1.1 Behavioral Characterization of Mouse Models—A number of cognitive and behavioral impairments are found in individuals with the 22q11.2DS, as discussed above. In particular, deficits in cognitive tasks associated with activity in the prefrontal cortex and hippocampus, including attention, working memory, executive function, and short-term verbal memory have been found (Woodin et al., 2001; Green et al., 2004; Sobin et al., 2004; 2005a). Similar cognitive phenotypes are seen in schizophrenia in general (Elvevag and Goldberg, 2000; Green et al., 2000) and the importance of this is compounded by the observation that poor performance in working memory tasks has predictive power in estimating functional outcome for patients with schizophrenia (Green et al., 2000). An important initial step in characterizing animal models of a psychiatric disease is to determine what cohort of behavioral deficits they display over a range of assays of specific cognitive functions. Eventually, it will be assessed if various models of schizophrenia converge on common behavioral traits and then to determine the biological correlates of observed deficits.

A number of studies have now been performed on the 22q11.2 mouse models. An overview of the behavioral studies, that are discussed below, conducted on various chromosomal

deletion models as well as investigations of mice hemizygous for genes within the critical region is given in Table 4. Interestingly, piecing together the physical phenotypes found in the various long range deletion models, as well as that from *Tbx1* knockout mice, it was determined that this gene was responsible for most of the congenital defects seen in 22q11.2DS patients and mouse models (Lindsay et al., 2001;Merscher et al., 2001;Jerome et al., 2001). *Tbx1* is a member of the T-box family of transcription factors, and had been considered a candidate gene for the defects in heart and pharyngeal development. Further confirmation of *Tbx1* as a major disease gene came when *TBX1* point mutations were found in patients who displayed the key physical symptoms of classic 22q11.2DS phenotype, but did not have a 22q11.2 deletion (Yagi et al., 2003). Carriers of these mutations were reported not to have mental retardation.

Considering the neuropsychiatric phenotypes, and based on the fact that 22q11.2DS individuals display functional deficits as well as structural pathology in hippocampal and frontal circuitry, behavioral characterizations of mouse models have primarily focused on these areas. While it is not possible to specifically model complex psychiatric conditions in mice, such as hallucinations, it is possible to examine component phenotypes (Arguello and Gogos, 2006; 2010). The analysis of the animal models in this region have thus far revealed deficits in sensorimotor gating, working memory, and fear conditioning, suggesting that genes from this region modulate these behaviors.

Sensorimotor gating is a measure of preattentive processing that is a nonspecific endophenotype of several psychiatric and neurological disorders, including schizophrenia Swerdlow et al., 2001). PPI (prepulse inhibition), which is disrupted in human 22q11.2 deletion carriers (see Section 2.1.1), can be studied in many species including mice. It is the ability of an individual to attenuate the magnitude of a startle reflex, when presented with a non-startling pre-pulse stimulus prior to the presentation of the startling stimulus. 22q11.2 deletion mouse models have been shown to have disruptions in PPI, although the exact pattern of alterations has been found to differ slightly among studies. Several of the long-range deletions have shown decreases in PPI (Paylor et al., 2001; Paylor et al., 2006; Stark et al., 2008), although one model, which contains a 7 gene deletion ranging from *Zfp520* to *Slc25a1*, had an increase in PPI (Kimber et al., 1999). These studies suggest that this region plays an important role in the modulation of PPI, and that alterations in PPI may be the result of the interplay between genes in this locus that have positive and negative influences on this behavior. At the level of single gene knockouts, it has so far been found that heterozygous deficiency of *Gnb1l* (Paylor et al., 2006), *Dgcr8* (Stark et al., 2008), and possibly *Tbx1* (Long et al., 2006; Paylor et al., 2006) decreases PPI. Conversely, heterozygous deficiency of genes within the locus that had no effect on PPI include *Comt* (Gogos et al., 1998), *Zdhhc8* (Mukai et al., 2004), *Rtn4r* (Hsu et al., 2007), and *Sept5* (Suzuki et al., 2009). To date, no individual gene hemizygous deficiency has been demonstrated to increase PPI, although Sept5 homozygous null mice did display increased inhibition (Suzuki et al., 2009). Animal models with elevations in L-proline levels due to deficiency of Prodh (Gogos et al., 1999), or deficiency of Prodh in combination with decreased Comt activity (Stark et al., 2008; Paylor et al., 2006; Long et al., 2006; Paterlini et al., 2005), also displayed decreased PPI. Further evidence for the influence of Prodh (and therefore L-proline levels) on modulating PPI was also demonstrated in BAC mouse lines, where an overexpression of Prodh led to increased PPI (Stark et al., 2009). In addition to PPI testing, at least one group has now developed electrophysiological MMN assays in mice, showing a deficit in animals lacking the neuroregulin 1 gene (Ehrlichman et al. 2009). In the future, it will be of great interest to apply such tests to mouse models of 22q11.2 DS.

Working memory (WM) deficits have become increasingly recognized as a key deficit in SZ (Elvevag and Goldberg, 2000; Green et al., 2000), and deficits in spatial WM tasks have

been found in children and adolescents with the 22q11.2DS (Sobin et al., 2004; Sobin et al., 2005a, Kates et al., 2007). The DNMP (delayed non-match to place) task assesses spatial WM-dependent performance. In this task, a food reward is located at one end of a two-arm T-maze. In each trial mice are directed down one arm of the maze initially and then trained to enter the opposite arm, after a delay, in order to retrieve a food reward. Left and right turns are pseudorandomized across trials and short delays of various lengths (typically 5–30 sec) are introduced between the forced and trial runs in both testing and training phases. For the active maintenance of information to complete the task, frontal regions of the mouse neocortex and their interaction with the hippocampus are required (Aultman et al., 2001; Lee and Kesner, 2003; Jones and Wilson, 2005).

In the *Df(16)A+/−* deletion mouse model, DNMP experiments revealed a deficit in acquiring this task in the mutant mice, compared to wild type littermates. Interestingly, it was determined that this deficit arises, at least in part, due to deficiency of *Dcgr8*, since heterozygous deficiency of *Dgcr8* alone affects acquisition of the task, without affecting associative memory (Stark et al., 2008). Together, these results suggested that deficits were due to deficiencies in miRNA biogenesis (see Section 4.2). In another study, that combined pharmacological inhibition of Comt with genetically modified Prodh deficient mice, these mice were also found to have deficits in this WM task (Paterlini et al., 2005). Mice individually deficient for either *Prodh* (Paterlini et al., 2005) or *Comt* (Gogos et al., 1998; Babovic et al., 2008; Papaleo et al., 2008) performed normally in the task, as did *Rtn4r* deficient mice (Hsu et al., 2007). Hence, the pharmacological study revealed an interaction between Comt and Prodh, leading to an exaggerated phenotype (See Section 4.5). This finding suggests that the use of long-range deletion mouse models is therefore perhaps necessary in order to capture epistatic interactions between genes in the 22q11.2 region, and to accurately model the 22q11.2DS.

Another test that has been used to assess cognition in mouse models of the 22q11.2 locus is fear conditioning. In the fear-conditioning assay, the ability to associate a neutral conditioned stimulus (CS: such as a tone or a light) with an unconditioned stimulus (US: an electric shock) is quantified. The contextual version of this test relies on both the amygdala and the hippocampus, whereas the cued version is primarily dependent on amygdala function (Fanselow and Poulos, 2005). In this assay, impaired conditioned fear memory was found in the *Df1/+* 22q11.2 deletion mouse model, which spans from *Es2el* to *Ufd1l*, and is deficient for 22 genes (Paylor et al., 2001). In one deletion model, ranging from *Zfp520* to *Slc25a1*, there was no difference found in fear conditioning (Kimber et al., 1999). In the *Df(16)A+/−* deletion mouse model, there was a robust deficit in both cued and contextual fear memory (Stark et al., 2008) in the mutants, suggesting amygdaloidal deficits and, potentially, hippocampal dysfunction. In terms of the genes involved in the fear conditioning behavior, a simple explanation would be to assume that one or more genes between *Slc25a1* and *Hira* are involved. However, as we saw with the epistatic interaction of Comt and Prodh mentioned previously, simple genotype-phenotype relationships based on phenotypic characterization of variable size deficiencies cannot always be straightforwardly inferred.

Behavioral studies in the 22q11.2 mouse models have thus provided insights into the genetic architecture of the 22q11.2DS, offering a glimpse into the genes, or overlapping sets of genes, contributing to specific behavioral abnormalities. The fact that human phenotypes, such as deficits in PPI or WM can be recapitulated in mice, suggests that 22q11.2 mouse models have strong validity, and as such, can be used to dissect out underlying abnormalities in genes and neural circuits. The fear conditioning experiments in the mice, for example, have uncovered an amygdaloidal dysfunction, which has not been previously appreciated in 22q11.2DS patients, and may have been otherwise overlooked. Future behavioral studies will continue to evaluate and assess other aspects of the human neuropsychiatric phenotypes,

such as deficits in attention and social interactions. Additionally, future mouse models using conditional knockout technology will be useful to help evaluate the developmental time course and critical time periods of a gene, or set of genes from the 22q11.2 region in contributing to the development of schizophrenia. Mouse models of the 22q11.2DS will also prove useful for testing therapeutic agents, to see if these drugs can help improve behaviors and ameliorate symptoms.

3.1.2 Brain Development in Mouse Models of 22q11.2 DS—Given the extensive literature concerned with neuroanatomical changes in human carriers of the 22q11.2 microdeletion it will be of great interest to determine which of these alterations are recapitulated in mouse models of the deletion. However, to date there are no detailed published studies of brain structure in these mice. In the future, stereological and magnetic resonance imaging of these brains should provide precise data regarding structural changes.

At the cellular level, Meechan et al (2009) have demonstrated subtle but significant changes in corticogenesis in mice carrying the microdeletion (*Lgdel/+* model). The study compared medial, lateral and dorsal aspects of the cortex between genotypes. At E13.5 it was found that the mutant mice had a ≈20% reduction in the number of basal progenitor cells. These are transit amplifying progenitors that develop in the cortical subventricular zone and give rise predominately to layer 2–4 pyramidal neurons. Looking at the mature cortex it was observed that in the medial portion there was a similar 20% reduction in layer 2–3 pyramidal neurons. Apical progenitor cells, in the ventricular zone proliferated as normal and no change in cell density was observed in layers 5–6. Analysis of the medial portion of cortex also showed a slight but significant misplacement of parvalbumin-positive GABAergic interneurons in this region. Interestingly, these structural changes were restricted to medial cortex without apparent changes in the lateral or dorsal portions. Roles for Tbx1 and ProdH in this phenotype were excluded.

Further studies of this nature will be of great importance in assessing how neural circuitry is malformed due to developmental abnormalities caused by 22q11.2 deletions.

3.1.3 Neuronal Structure and Function in Mouse Models of 22q11.2DS—In addition to assessing the generation and migration of neurons in 22q11.2 models, to understand how the deletion impacts the transfer of information in neural networks we must also determine how it affects the structure of neurons and the generation of synapses, and ultimately what the physiological consequences of the mutation are on neural activity.

To date, the investigation of neuronal structure has focused on cultured hippocampal neurons and, *in situ*, on the well-characterized pyramidal neurons of the hippocampal CA1 region. In cultured neurons the microdeletion has a striking affect on dendritic complexity and the formation of excitatory synapses. Mukai et al. (2008) demonstrated that there were approximately 40% fewer primary dendrites on *Df(16)A+/−* neurons than on wild type neurons and that there was a similar decrease in the total number of dendritic branch points. To assess the number of excitatory synapses formed on these neurons, the density of mushroom spines (the postsynaptic sites of excitatory inputs) and the number of puncta of PSD95 (a scaffolding protein at excitatory postsynaptic densities) and vGlut1 (a marker of glutamatergic presynaptic sites) immunoreactivity were quantified. For all three parameters a 30–50% decrease was observed. Finally, to confirm that these changes resulted in a reduction in the number of functional synapses, miniature excitatory postsynaptic currents (mEPSCs) were recorded from cultured pyramidal cells and a corresponding decrease in the frequency of these events was detected.

Studies of pyramidal neurons *in situ* have looked at the CA1 region using transgenic mice that express GFP (under the Thy1 promoter) in a random subset of cells (Feng et al., 2000). These analyses, which looked at the basal dendrites of pyramidal neurons in stratum oriens, found alterations qualitatively similar to, although less severe than, those seen in cultured neurons. Hence, neurons in *Df(16)A+/−* mice displayed a 24% decrease in spine density with a roughly equivalent decrease in the number of PSD95 and vGlut1 puncta, and also had significantly less complex dendritic arbors (Mukai et al., 2008). Finally, there was an observed decrease in the width of dendritic spines in *Df(16)A+/−* mice, but their length was normal.

As with the behavioral phenotypes associated with the chromosomal deletion, the question arises of which deleted gene, or combination of genes, underlies the change s that are seen. In this case, investigations of Zdhhc8 and Dgcr8 deficient mice have suggested key roles for these two proteins (see Sections 4.1 and 4.2 for further discussion of these genes). Cultured hippocampal neurons from mice lacking either one or both copies of the Zdhhc8 gene showed remarkably similar morphological changes to those from *Df(16)A+/−* mice with dramatically reduced dendritic branching and spine density. Also similar to *Df(16)A+/−* mice, analysis of CA1 pyramidal neurons in brain sections from *Zdhhc8−/−* and *Zdhhc8+/−* mice showed again smaller but significant reductions in these parameters. Of the parameters measured, the decreased width of dendritic spines in *Df(16)A+/−* mice was the only one not phenocopied in *Zdhhc8−/−* and *Zdhhc8+/−* mice. A key experiment in showing the importance of Zdhhc8 in 22q11.2DS was the demonstration that heterologous expression of this gene in cultured *Df(16)A+/−* neurons reversed the deficits in dendritic branching and spine density; an effect not seen with expression of a point mutant form of this protein that is enzymatically inert.

Morphological analysis of mushroom spines on CA1 pyramidal neurons in *Dgcr8*-deficient mice showed, in contrast, that there was a significant decrease in the width, whereas the length was unaffected, hence phenocopying the *Df(16)A+/−* mice (Stark et al., 2008). However, unlike in the *Df(16)A+/−* mice, there were no changes in mushroom spine density on the basal dendrites of CA1 pyramidal neurons from *Dgcr8+/−* mice compared to wild type mice. Additionally, analysis of neuronal structure showed that Dgcr8 deficiency caused an impairment of dendritic arborization, which is partially overlapping to the one observed in *Df(16)A+/−* and Zdhhc8 deficient mice. Specifically, Scholl analysis showed that *Dgcr8* deficiency reduced the complexity of the distal dendrites at varying distances from the soma.

Together these results point to a simplified dendritic tree of pyramidal neurons in the CA1 region of the hippocampus in *Df(16)A+/−* mice with a reduced density of dendritic spines and associated markers of excitatory synapses. It appears that the primary contributors to this phenotype are the palmitoyl transferase Zdhhc8 (Section 4.1) and miRNA dysregulation due to Dgcr8 deficiency (Section 4.2). The morphological data support the notion that there is less glutamatergic synapse formation onto CA1 pyramidal cells and thus altered synaptic transmission or plasticity. In addition, the change in dendritic structure may affect the integration of synaptic input by these neurons (Gulledge et al., 2005; Spruston, 2008).

Future experiments will probe if these structural changes are specific to CA1 or if they are a general feature of pyramidal neurons throughout the cortex. Additionally, while most work so far, has focused on pyramidal neuron structure, given the interest in interneuron dysfunction in schizophrenia, in particular possible abnormalities in parvalbumin-expressing cells (Lewis et al., 2005), it will be of interest to better characterize the structure and function of these neurons throughout the hippocampus and cortex.

3.1.4 Functional Connectivity of Brain Regions in Mouse Models of 22q11.2DS

—A major goal of neuroscience, in general, is to determine the neural correlates of behavior (Buzsaki, 2004), i.e. when an organism performs a given behavior, what is the firing pattern of neurons across the brain. Such data can then inform studies of disease states where it is anticipated that behavioral changes will correlate with altered neural activity, or potentially that overtly normal behaviors are achieved by different types of activity. It has been suggested that altered connectivity between brain regions contributes to the pathophysiology of schizophrenia. The idea was originally proposed by Wernicke in 1906 (Wernicke, 1906) (who favored the idea of a degeneration of association fibers) and has been further substantiated by functional brain imaging studies of schizophrenia patients showing alterations in distributed activity and correlation across areas (Friston et al., 1996; Ford et al., 2002; Lawrie et al., 2002; Meyer-Lindenberg et al., 2005). However, it remains unclear whether such findings represent secondary reactive responses of interconnected brain areas or whether they directly relate to etiological factors, such as genetic risk variants. Moreover, the macroscopic measurements of brain activity used in patient studies (*i.e.* electroencephalograms recorded using scalp electrodes) cannot address how abnormal connectivity is manifest at the level of individual neurons. Such resolution will be a crucial step if one is to understand the pathophysiology of schizophrenia in detail. Hence, mouse models of schizophrenia predisposition, in combination with *in vivo* electrophysiology, are likely to play an important role in directly assessing the impact of schizophrenia risk alleles on neural connectivity across multiple brain areas.

As mentioned above, *Df(16)A+/−* mice showed deficits in the initial training phase of the DNMP T-maze working task (Section 3.1.1). Such spatial working memory tasks require the PFC to generate appropriate behavioral responses by integrating goal and memory aspects of the task with spatial information encoded by the hippocampus (Jones, 2002). In support of this, Matt Wilson and colleagues, working in rats, have shown that during the choice phase of a similar maze-based task (*i.e.* when working memory is engaged) there is increased synchrony in the spike timing of hippocampal and prefrontal cortical pyramidal neurons and increased coherence in the theta (4–12 Hz) band of oscillatory field potentials (Jones and Wilson, 2005).

Recent work capitalizing on the observation of impaired WM performance in 22q11.2 animal models has helped to provide important new insights into the nature of altered brain connectivity emerging as a result of the 22q11.2 microdeletion. Specifically, Sigurdsson et al (2010) assessed neural synchrony between the dorsal hippocampus and medial prefrontal cortex (mPFC) during the learning and execution of the DNMP task by simultaneously recording local field potentials from the hippocampal CA1 region and the mPFC, as well spiking activity of individual neurons in the mPFC. Overall, mPFC neurons fired at comparable rates in the two genotypes and showed similar degrees of phase-locking to the theta rhythm recorded locally in the mPFC. However, phase-locking of PFC neurons to the theta rhythm in the dorsal hippocampus was found to be significantly reduced in *Df(16)A+/−* mice. This decrease was independent of the phase of the task and suggests a decreased influence of the hippocampus on PFC neuronal activity.

The results of this study support a functional dysconnectivity between the hippocampus and prefrontal cortex in *Df(16)A+/−* mice. Another important observation made in this study was that there was a positive correlation between degree of synchrony and the rapidity with which the T-maze task was learnt in the *Df(16)A+/−* mice. This is highly suggestive that the decreased synchrony in the mutant mice hampers their ability to learn the rule.

The mechanisms underlying the decrease in functional connectivity, as demonstrated by reduced synchrony, remain to be elucidated and will provide important clues as to the

cellular pathology in *Df(16)A+/−* mice. One possibility, that would echo the original ideas of Wernicke, is that there are alterations in the structure of fiber tracts between the two regions. Such a scenario would be consistent with the observations of decreased white matter volumes in human 22q11.2 deletion carriers (see Section 2.2.4). We await similar structural imaging studies of *Df(16)A+/−* mice brains. Other possibilities include mistargetting to postsynaptic neurons in the PFC by hippocampal axons, changes in presynaptic release properties of these long-range projections and potentially altered dopaminergic signaling in mPFC (Benchenane et al., 2010). Finally, the results of this study suggested normal hippocampal function in mutant mice (comparable theta oscillations, and theta-modulation of gamma oscillations were observed in hippocampus) but the output of this brain region in terms of neuronal spiking remains to be determined. These issues are important as the dysconnectivity theory of schizophrenia proposes that there is not a single specific site of dysfunction but rather that integration between brain regions is central to the pathophysiology. In that respect, dysconnectivity as a result of the 22q11.2 microdeletion may be a widespread phenomenon, for example, affecting connections of the prefrontal cortex with other cortical and/or subcortical areas. As such, in addition to WM impairments, it is possible that 22q11.2DS-associated psychiatric symptoms arise as a consequence of dysconnectivity that results in abnormal information flow and an inability to accurately interpret sensory input (Fletcher and Frith, 2009). *Df(16)A+/−* mice offer a tool to test these theories.

In summary, studies of mice with chromosomal deletions homologous to human mutations have shown subtle differences in corticogenesis, dendritic complexity and synaptic formation. In addition, such mice show diminished synchrony between hippocampus and mPFC activity during a task reliant on communication between these two structures. These initial observations show the power of analysis of these models and suggest that both neurodevelopmental and synaptic aberrations are associated with 22q11.2DS; a key question will be to determine which of these phenomena, either alone or in combination, are essential for behavioral changes. Below we will discuss attempts to dissect out the contributions of individual genes within the locus to the observed phenotypes.

3.2 Modeling 22q11.2DS using Human Tissue - Induced Pluripotent Stem Cells

The preceding discussion demonstrates the immense value animal models, such as the *Df(16)A+/−* mouse, have as tools for the study of disease mechanisms. However, because of distinctions in anatomy and physiology between species, animal models do not always faithfully recapitulate human pathologies (Davies and Morris, 1993). To study human brain structure and function, we currently rely on neuropathological studies and non-invasive techniques such as scalp electroencephalogram recordings or neuroimaging, but their resolution is currently fairly poor and not informative of function at the cellular level. There are many caveats in studying cellular mechanisms of neuropathologies on *post-mortem* human brain tissue, particularly as it is often obtained at the late stages of the disease and therefore rarely provides insights into mechanisms of disease development.

An alternative approach to studying human neural tissue has recently become available due to the pioneering work of Yamanaka and colleagues who demonstrated that terminally differentiated cells can be reverted to a pluripotent state (Takahashi and Yamanaka, 2006). Their initial study showed that mouse fibroblasts could be reprogrammed to an embryonic-like state, termed induced pluripotent stem cells (iPSCs), by exogenous expression of a combination of transcription factors. These cells can then be differentiated to generate unlimited amounts of different somatic cell types (Cantz et al., 2008; Schenke-Layland et al., 2008; Kuzmenkin et al., 2009). Reprogramming technology informed by knowledge acquired from the studies of embryogenesis and of embryonic stem cell (ESC) differentiation, now allows the generation of several neuronal and glial populations (Dimos

et al., 2008; Wernig et al., 2008; Soldner et al., 2009; Wu et al., 2010). Furthermore, this technology has been applied to reprogramming human dermal fibroblasts, showing that disease-causing mutations are maintained throughout the process (Takahashi et al., 2007; Dimos et al., 2008). Conservation of genetic background renders iPSC reprogramming highly valuable for the study of disease mechanisms, particularly in the absence of animal models (e.g. for some multigenic or sporadic pathologies), when causal genetic alterations are unknown, or when the affected cell type is not easily accessible (e.g. neural tissue). Another advantage of the reprogramming method is that neuronal differentiation of iPSCs recapitulates physiological neuronal development, an aspect of particular importance when studying a neurodevelopmental disorder such as schizophrenia.

In recent years a number of studies have shown successful reprogramming of somatic cells from patients affected by various nervous system diseases, including Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA) and Rett syndrome (Park et al., 2008; Dimos et al., 2008; Ebert et al., 2009; Hotta et al., 2009). The potential for disease modeling of iPSC reprogramming is highlighted by studies showing recapitulation of disease phenotype in cells of interest (Lee et al., 2009; Ebert et al., 2009). These studies have applied knowledge of molecular cues for tissue specification from developmental studies to derive a number of neuronal populations from both murine and human ESCs and iPSCs. These included cortical neurons that expressed specific molecular markers, had electrophysiological properties characteristic of this population and that sent appropriate projections upon transplantation into the mouse cerebral cortex (Zhang et al., 2001; Eiraku et al., 2008; Gaspard et al., 2008; Ideguchi et al., 2010).

This review has summarized the deficits that have been observed in the *Df(16)A+/−* mouse model, including cellular deficits exhibited by HPC and PFC neurons. Although this model has solid etiological validity (based upon robust genetic causality), it remains necessary to determine how well it recapitulates aspects of the human disease. The aforementioned studies demonstrate that iPSC reprogramming offers the possibility of generating human neurons that can be tested for the impact of disease-causing mutations. One could envisage using iPSC reprogramming to generate cortical neurons from *Df(16)A+/−* mice and their wild type littermates. Similarities in cellular properties of primary and iPSC-derived cortical neurons from the mouse model would strengthen confidence in the use of reprogramming to model the 22q11.2 microdeletion. Subsequently, reprogramming could be applied to humans carrying the 22q11.2 microdeletion and their unaffected relatives. For a disease such as schizophrenia where the pathophysiology may be restricted to subtle changes in synaptic function, this will likely prove invaluable and will allow the probing of human neural function at the cellular and subcellular level during the course of disease development in unprecedented ways.

The possibility of screening drugs on reprogrammed neural tissue has been recently highlighted in a study on SMA, in which mutations in the survival motor neuron (SMN) 1 gene cause reduced levels of SMN protein and result in selective death of lower α-motor neurons (Ebert et al., 2009). iPSC-derived motor neurons from a SMA patient had reduced numbers of SMN protein nuclear aggregates compared to the patient's unaffected mother, a deficiency that was partially reversed by treatment with valproic acid. The presence of a disease phenotype in iPSC-derived cortical neurons from 22q11.2 microdeletion carriers compared to unaffected controls would similarly allow the testing of drugs for their potential to correct observed deficits, and may generate new avenues for the development of effective treatment strategies (Hockemeyer et al., 2009).

iPSC reprogramming however, is not devoid of limitations and several points should be considered when using this technology for disease modeling. Recent studies on ALS and PD

have failed to detect phenotypic differences between iPSC-derived neurons from affected individuals and from controls (Soldner et al., 2009). Reprogramming ablates environmental influences that a cell is subjected to, both during the course of development, and during the life of an individual, an aspect of particular importance when studying schizophrenia, which can be triggered by psychological stress (Brown et al., 1968; Day et al., 1987). Also, despite the earlier onset exhibited by schizophrenia compared to neurodegenerative disorders, properties of iPSC-derived neurons would be assessed at a much earlier point in their lifespan than age of neurons at disease onset. For these reasons, it might require subjecting iPSC-derived cortical neurons to cellular stress (e.g. oxidative stress) to uncover cellular deficits. However this is unlikely, due to the developmental nature of schizophrenia. Another possible impediment in the use of iPSC reprogramming for modeling of schizophrenia relies in discrepancies in patterns of DNA methylation and miRNA and mRNA expression between ESCs and iPSCs (Deng et al., 2009; Wilson et al., 2009; Stadtfeld et al., 2010). These differences between ESCs that are considered the gold standard of pluripotency, and iPSCs, might indicate incomplete reprogramming of iPSCs, and might impede their developmental potential. Despite these technical challenges, which will have to be overcome, iPSC reprogramming holds great promises on the road to elucidating the mechanisms underlying psychiatric disorders such as schizophrenia.

4 Dissecting the Genetic Complexity of 22q11.2DS

The considerable number of genes contained within the critical 1.5 Mb region of 22q11.2 creates a significant challenge in dissecting the molecular mechanisms that lead to the observed cellular, physiological and behavioral phenotypes of humans and mice deficient for this region. The majority of these genes are expressed in the brain (Maynard et al., 2003) but with the exception of COMT, and perhaps RTN4R (the NOGO receptor), most of them are, or until recently, were, of poorly defined neural function. Hence, it is necessary to first assess the roles of these genes in the nervous system and then to determine how loss of ≈50% of protein levels for each affects neurobiology.

Tables 1 and 2 list all the genes in the 1.5 Mb critical region (Table 1) and those, in the neighboring region, that are typically lost in 3 Mb deletions (Table 2). The known (or putative) functions of the genes are shown where known, and in Table 1 we summarize any relevant (i.e. neurobiological or behavioral) analyses of mice lacking one or two copies of the genes in the 1.5 Mb deletion region.

It is thought that a small number of genes will contribute most of the phenotypic effects of the deletion but that at some level some form of synergistic interaction between these elements is occurring to substantially increase disease risk (Karayiorgou et al., 2010). Although one or a few loci may have a greater phenotypic impact, it is the imbalance of several deleted genes that determines the overall phenotype. The presence of additional trans- or cis- acting genetic modifiers may also contribute to the variability of the cognitive and psychiatric phenotypes of the syndrome.

Below we discuss work focused on 4 genes for which there is good evidence for them being involved in the pathogenic process. For each, the evidence implicating them in the increased risk of developing schizophrenia comes from a combination of human genetic studies and animal modeling work, and we seek to give an overview of both data sets. The genes we focus on are: COMT, an important enzyme in monoamine catabolism, PRODH, an enzyme required for proline metabolism, ZDHHC8, a palmitoyl transferase enzyme, and DGCR8, a key enzyme in the microRNA synthetic pathway.

For the first two of these enzymes their substrates are well defined but for the latter two it is anticipated that their multiple substrates (potentially involved in many aspects of neuronal

biology) could give rise to widely divergent effects on brain function. Determining these targets is both a challenging and an exciting part of better understanding 22q11.2DS and its association with schizophrenia.

4.1 ZDHHC8

The *Zdhhc8* gene encodes a palmitoyl transferase enzyme (Mukai et al., 2008). Protein palmitoylation is the addition of the saturated 16-carbon palmitate lipid at specific cysteine residues by a liable thioester bond (El-Husseini and Bredt, 2002). This posttranslational modification of proteins has been known for decades, but the biochemical machinery involved has only recently been identified. In fact, the research defining these mechanisms was roughly concurrent with the observation of direct genetic evidence for a role of ZDHHC8 in schizophrenia susceptibility (see below). Forward genetic screens in yeast identified two palmitoyl transferases that contained cysteine rich DHHC domains (Linder and Deschenes, 2004; Smotrys and Linder, 2004). Subsequent sequence analysis of the mouse and human genomes demonstrated the presence of 23 DHHC proteins in these species (Fukata et al., 2004). In this study, four of the identified DHHC proteins (DHHC 2, 3, 7 and 15) were shown to palmitoylate PSD-95 at cysteine residues 3 and 5 although evidence for PSD-95 palmitoylation by DHHC8 was apparent in the presented data.

There is some suggestive evidence from genetic association studies supporting the notion that single nucleotide polymorphisms (SNPs) in ZDHHC8 may contribute to susceptibility for schizophrenia independently of 22q11.2 deletions. In one study, (Liu et al., 2002a), a *ZDHHC8* SNP, rs175174, located in intron 4, showed the highest significance of 72 SNPs examined from the entire 22q11.2 locus (including the SNPs from the *PRODH* gene). It was shown that the genotype at SNP rs175174 affects the rate of intron 4 retention of the *ZDHHC8* gene and therefore the ratio of an intron 4-containing unspliced form over the fully spliced form (Mukai et al., 2004). Specifically, the presence of the risk allele rs175174-A resulted in the production of relatively higher levels (~1.7fold) of the unspliced form that is predicted to encode for an inactive truncated protein. There have been both positive and negative follow up studies (http://www.schizophreniaforum.org/res/sczgene/default.asp), and the effect of the *ZDHHC8* gene is predicted to be much stronger in individuals with 22q11.2 deletions and schizophrenia where at least 50% decrease in ZDHHC8 activity levels is predicted. The outcomes of haploinsufficiency of Zdhhc8 remain for the most part unknown. Many neuronal proteins are palmitoylated including postsynaptic density proteins, ion channel subunits, G protein-coupled receptors and non-receptor tyrosine kinases (reviewed in El-Husseini Ael and Bredt, 2002; Fukata and Fukata, 2010). The addition of the palmitate lipid group to neuronal proteins is a reversible modification that increases their hydrophobicity and so affects their interaction with cell membranes. Together these observations suggest that palmitoylation functions dynamically in many aspects of neural signaling.

To date, it has been demonstrated that ZDHHC8 palmitoylates PSD-95 in cell lines and hippocampal neurons (Mukai et al., 2008), which may account for deficits in synaptogenesis in 22q11.2DS models (see Section 3.1.3). Moreover, other substrates have been identified that are likely to impact neuronal cell biology in other ways (JM and JAG, unpublished data). Together, dysregulation of target proteins by hemizygous deficiency of ZDHHC8 is likely to significantly disrupt neural development and function (See Fig. 1). The functional impact of reductions in Zdhhc8 expression was shown in analysis of morphological analysis of dendrites and dendritic spines of neurons from mice homo- and heterozygous for the gene, as discussed in Section 3.1.3.

Together, the genetic and functional data, adding substantial support to the idea that the *ZDHHC8* gene is a schizophrenia susceptibility gene from this locus, the broad importance

of protein palmitoylation in neurons and the analysis of Zdhh8 null mice all strongly support the notion that *ZDHHC8* contributes to the behavioral deficits associated with the 22q11.2DS.

4.2 DGCR8 and miRNAs in 22q11.2DS

The schizophrenia critical region contains the DGCR8 gene, which encodes an essential component of the synthetic pathway for microRNAs (miRNAs), as well as two genes that themselves encode miRNAs. miRNAs are a class of small non-coding RNAs typically 21–22 nucleotides in length, produced through a series of cleavage steps from precursor RNA transcripts. miRNAs function to regulate gene expression by complementary base-pairing to target mRNAs, which results in either degradation or translational repression of the target. More than half of miRNAs are highly or exclusively expressed in the brain, suggesting prevalent miRNA-mediated genetic regulation in aspects of neuronal development and function (Cao et al., 2006).

Several properties of miRNAs make them an ideal means for intricate regulation of gene expression. For example, miRNAs can regulate mRNA expression in various intracellular compartments and therefore achieve local regulation. For example, a number of miRNAs have been shown to control protein synthesis at synaptic sites which is critical for synaptic plasticity (Schratt, 2009). Additionally, regulation of gene expression by miRNAs is a dynamic, reversible and combinatorial process. Each miRNA controls multiple mRNA targets while one mRNA target can be synergistically modulated by multiple miRNAs (Sathyan et al., 2007; Didiano et al., 2008). This property allows miRNAs to simultaneously integrate different intracellular signals and coordinate different signaling pathways (Johnston et al., 2003; Choi et al., 2007). Finally, the expression of miRNAs can itself be controlled at various levels (transcriptional and posttranscriptional) and at several cellular locations, offering a further layer of regulatory flexibility (Hutvagner et al., 2001; Obernosterer et al., 2006; Thomson et al., 2006; Wu and Xie, 2006; Lugli et al., 2008; Ramachandran et al., 2008; Han et al., 2009; Kadener et al., 2009; Triboulet et al., 2009;). It is therefore not surprising that the regulation of miRNA function in the central nervous system has drawn enormous interest and is an area of intensive research.

Our recent studies in the *Df(16)A+/−* mouse strain provided compelling evidence that miRNA dysregulation is involved in the pathobiology of 22q11.2DS (Stark et al., 2008). The deleted region of human chromosome 22 and mouse chromosome 16 contains at least two miRNA genes (*mir-185* and *mir-649*) but more importantly also contains *Dgcr8*, a key component of the “microprocessor” complex essential for miRNA production (Tomari et al., 2005). Therefore, the 22q11.2 microdeletion leads to an approximate halving of *Dgcr8* expression and as subsequent investigation showed, an impairment of miRNA biogenesis and so haploinsufficiency at this locus (see Fig. 2).

In *Df(16)A+/−* mice, initial evidence for miRNA dysregulation was provided by expression profiling in the PFC and HPC, which revealed that probe sets overlapping with known miRNA locations were among the affected targets. More thorough investigation showed that decreased *Dgcr8* expression results in the down-regulation (by ~20–70%) of a specific subset of mature miRNAs. It is still unclear why only a subset of miRNAs was more affected by *Dgcr8* hemizygosity. It may reflect differences in processing efficiency of individual pri-miRNAs to pre-miRNAs via the “bottleneck” step by the microprocessor. Other possibilities include that the key control of some miRNAs occurs at the microprocessor step while for others it occurs at downstream steps, or that the biogenesis of some miRNAs proceeds through a non-canonical pathway, or yet unidentified compensations have taken place.

To determine if haploinsufficiency at the *Dgcr8* locus and the consequent alterations in miRNA biogenesis contribute to the neuronal abnormalities observed in *Df(16)A+/−* mice, Stark et al generated *Dgcr8*-deficient mice (i.e. heterozygous *Dgcr8* knock-out mice) and compared them to the *Df(16)A+/−* mice at several levels of investigation. At the molecular level, *Dgcr8*-deficient mice demonstrated a similar miRNA alteration pattern as *Df(16)A+/−* mice in the miRNAs examined, including several miRNAs within the largest microRNA cluster region around the *Mirg* gene (miRNA containing gene) on distal chromosome 12. Partial reduction in miRNA levels may have a widespread impact on gene expression considering the number of potential target mRNAs that can be epigenetically regulated simultaneously. On the other hand, since target mRNAs can have binding sites for different miRNA species, the level of repression achieved may be highly sensitive to the amount of available miRNA complexes and the number of affected miRNAs (Hobert, 2008; Stark et al., 2008). Consistent with this reasoning, it was estimated that impaired miRNA biogenesis accounts for at least a portion of the transcript upregulation observed in the PFC and HPC of the *Df(16)A+/−* mice. Specifically, genes that had increased expression in *Df(16)A+/−* mice were more likely than genes with reduced expression to have 2 or more seed sites for miRNAs that were decreased in the mutant mice. This suggests a potential cooperative action of the altered miRNAs on the expression of individual transcripts. Finally, it is worth considering that the effect of a reduction in several miRNAs may converge on a few key or hub target mRNAs and result in a substantial upregulation of these hub targets.

In conclusion, the potential of miRNAs to contribute to the regulation of expression of multiple genes, as well as their possible convergent influence on several key targets, could be an important component of the genetically complex architecture of psychiatric and cognitive phenotypes associated with the 22q11.2DS (Xu et al., 2009). In addition, miRNAs have been suggested to function in phenotypic robustness and so alterations in this regulatory network could also contribute to the phenotypic variability of 22q11.2DS (Hornstein et al., 2006). Identifying the targets affected by this miRNA dysregulation will be the future challenge and promises to offer a more comprehensive understanding of how alteration in miRNA-regulated genetic network can contribute to the pathology of 22q11.2DS.

At the cellular level, it is of great importance to dissect and study the contribution of hemizygosity for *DGCR8* and the consequent changes in miRNA abundance to deficits in synaptic development and maturation (Kosik, 2006; Schratt et al., 2006; Fiore et al., 2009). The fact that miRNAs may contribute to synaptic development and maturation, specifically the finding that *Dgcr8+/−* mice have narrower mushroom spine heads (see Section 3.1.3), suggests that morphological impairment may be part of the underlying cause of the cognitive and behavioral deficits observed in the *Dgcr8+/−* mice (as discussed in Section 3.1.1).

In a broader context, a number of recently emerging connections between miRNAs and psychiatric disorders have been made. Alteration in miRNA expression in the brains of schizophrenia patients has been discovered in recent human postmortem studies, providing further support to the notion that altered miRNA expression could be a significant, if not critical, factor in the dysregulation of gene expression in the brain of schizophrenia patients (Xu et al., 2009). For example, Perkins et al (2007) conducted an expression profiling study using postmortem PFC samples from individuals with schizophrenia and unaffected controls. Of 264 human miRNAs monitored, 15 showed significantly decreased expression and one showed higher expression in schizophrenia patients compared to controls (Perkins et al., 2007). Considering the high incidence of schizophrenia among 22q11.2DS patients, it will be of great interest to determine how integral miRNA dysregulation is to the complex genetic architecture of 22q11.2DS and what role miRNA-dependent regulation of neuronal

connectivity contributes to the pathogenesis of schizophrenia and other psychiatric disorders.

4.3 COMT

Approximately midway within the critical 1.5 Mb 22q11.2DS microdeletion is the *COMT* gene that encodes the cytosolic enzyme catechol-O-methyltransferase that, in addition to mitochondrially localized monoamine oxidase (MAO), acts as a key degradative enzyme of numerous neuroactive monoamines including dopamine (DA) (Axelrod and Tomchick, 1958). While COMT can metabolize a number of neuroactive catecholamines, a possible preferential impact upon DA metabolism at the systems level is suggested by a number of clinical findings in the literature (Chong and Mersfelder 2000).

In the context of the dopamine hypothesis that continues to heavily influence – for better or for worse – the basic science and clinical research of psychosis and schizophrenia, this apparent preferential impact of COMT upon DA metabolism makes it an attractive candidate gene for schizophrenia (Howes and Kapur, 2009; Montcrieff, 2009).

COMT is a cytosolic enzyme that exists in either a soluble cytosolic form or is membrane bound with its active site likely directed towards the cytosolic compartment (Lundstrom et al., 1995). COMT is expressed throughout the neurons of the CNS, is largely absent from glia, and shows notable high expression in neurons within layers II, III, and IV of the pre-frontal cortex, postsynaptic to dopaminergic inputs (Matsumoto et al., 2003).

The mechanistic role of COMT in regulating DA metabolism can begin to be appreciated from results of neurobiological studies carried out in the *Comt*−/− mouse. Although the *Comt* knockout mouse showed normal basal levels of DA within the PFC, clearance of DA from the extracellular space of the PFC was two-fold slower following evoked release (Huotari et al., 2002; Yavich et al., 2007). In considering the balance of tonic versus phasic dopamine receptor (DR) stimulation by DA, these results taken together suggest the likelihood that a reduction in COMT activity would lead to prolonged phasic activation of DRs while having potentially less of an impact upon tonic activation of DRs.

In neurobehavioral theories of cognition, one hypothesis of dopamine function proposes that tonic DA signaling in the PFC is involved in stabilizing signaling and thought processes, and when this tonic dopamine signaling becomes excessive, perseverative and inflexible cognitive processing results (Vijayraghavan et al., 2007; Dickinson and Elveag, 2009). Conversely, phasic DA signaling has been interpreted as a destabilizing force that can disrupt established neuronal patterns of activity, allow new patterns to be established, and thus lead to more flexibility in cognitive processing.

This perseverative, inflexible cognitive performance proposed to be due to excessive tonic DA signaling in the PFC is one of the hallmark findings of the cognitive style associated with schizophrenia and schizoaffective disorder (Dickinson and Elveag, 2009). In this regard, much attention has focused on the role of a COMT polymorphism at residue 158 that alters enzymatic activity; the Val158 variant encodes a higher activity isoform of COMT, while Met158 generates a low activity isoform. These isoforms have been shown to influence cortical DA levels and cognitive performance in both humans and transgenic mouse models. Despite a number of independent studies confirming a role of COMT polymorphisms in affecting cognitive performance, another cadre of similarly designed studies have failed to replicate these findings leaving the influence of the Val158Met COMT polymorphism's impact upon the flexibility of cognition in question (reviewed in Meyer-Lindenberg and Weinberger, 2006; Dickinson and Elveag, 2009).

4.4 PRODH

At the far centromeric end of the 22Q11.2 microdeletion is the *PRODH* gene, which encodes the mitochondrially expressed proline dehydrogenase that serves as the rate-limiting enzyme in proline degradation (Phang et al., 2001). Homozygous deletion of the *PRODH* gene reliably leads to hyperprolinemia (as assessed by serum levels) and significantly elevated proline levels within the CSF as well as to significant neuropsychiatric dysfunction (Phang et al. 2001). Similarly, hemizygous deletion of *PRODH*, either in isolation or in the context of 22q11.2 DS, also leads to elevated proline levels and about half of all 22q11.2DS patients show elevated proline levels (Jacquet et al., 2002; Bender et al., 2005; Raux et al., 2007). Although the exact neurobiological substrates upon which proline acts have yet to be resolved, a number of candidate targets within the nervous system have been explored and these are discussed below.

The *PRODH* gene was first implicated by genetic association studies in cohorts of affected families. In one study, common SNPs in the *PRODH* gene were shown to be associated with schizophrenia in karyotypically normal patients (Liu et al., 2002b). Importantly, the same study uncovered an unusual pattern of *PRODH* gene variation that mimics the sequence of a linked pseudogene located in one of the DNA repeat elements (copy-number polymorphic duplications) in the 22q11.2 region. Several of the pseudogene-like variants resulted in missense changes at conserved residues that prevent synthesis of a fully functional enzyme and some of them were over-represented in patients. Thus, *PRODH* is one of several hundred genes in the human genome that maps to a region of copy-number polymorphic duplications, that is highly variable among individuals, and is one of the first examples of the contribution of such highly variable genes in the genetic makeup of common complex disorders (Liu et al., 2002b).

In contrast to the case of COMT, where there exists a clear molecular mechanism capable of directly impacting a neurotransmitter system strongly implicated in schizophrenia but where the evidence of COMT dysfunction impacting cognition is inconsistent, the opposite scenario exists for the case of PRODH and related hyperprolinemic states.

Two forms of hyperprolinemia (defined by 2–10 fold increased serum levels of proline), caused by distinct genetic lesions are well characterized clinically. They are type 1 hyperprolinemia (HPI, caused by deletion of the *PRODH* gene) and in type 2 hyperprolinemia (HPII, a result of deletion of the P5C-dehydrogenase gene). In both diseases neuropsychiatric symptoms dominate the condition (Phang et al., 2001). Both diseases are characterized by seizures, mental retardation, psychiatric, and behavioral problems with the severity of these symptoms, as well as the hyperprolinemia, being more extreme in HPII (Phang et al., 2001; Jacquet et al. 2002; Willis et al., 2008). Despite the clear neurological impact of elevated proline upon brain function, a mechanistic explanation for the dysfunction or a discrete target of proline's actions has remained largely elusive.

Results from a number of studies suggest that proline itself may function as a *bona fide* neurotransmitter, however, follow up studies have failed to provide convincing evidence in support of this role (Fremeau et al., 1992). An alternative explanation for proline's pathological neuroactivity is that proline acts to interfere with the normal function of other neurotransmitter systems. Enzymatically, proline can be converted to glutamate, the major excitatory neurotransmitter, and glutamate can be further enzymatically converted to GABA, the major inhibitory neurotransmitter. Thus, while yet to be established, hyperprolinemia within the CNS might serve to upset the normal metabolic balance between glutamate and GABA and, thus, lead to neuronal dysfunction.

In addition to its possible role in influencing the production of these neurotransmitters, a number of studies have shown that millimolar levels of proline – although at concentrations significantly above hyperprolinemic CSF levels of proline – can activate glutamate responsive ion channels and can even exert neurotoxic effects through activation of these channels (Henzi et al., 1992; Nadler et al., 1988).

Thus, while the exact mechanism of proline's action that causes neuronal dysfunction has yet to be firmly established, promising candidates as sites of action have been described. In addition to its possible impact upon neurotransmitter systems, alterations in a number of mitochondrially expressed genes involved in cellular energetics in the Prodh-deficient mouse suggested a possible important metabolic role of proline (Paterlini et al, 2005). In support of this, it has long been appreciated that proline and its ox-redox congener P5C undergo a cyclical transformation between the mitochondrial matrix and the cytosol and, in so doing, transfer reductive potential between these compartments (reviewed in Phang et al., 2010).

In initial steps towards understanding the mechanistic impact of hyperprolinemia upon neuronal function, *Prodh* mutant mice were produced and characterized. These mice have a very low activity form of this enzyme and have drastically increased serum and brain levels of proline, *i.e.* pronounced hyperprolinemia. In an initial study, these mutant mice were shown to have attenuation deficits in sensorimotor gating as assessed by PPI of the acoustic startle reflex (Gogos et al., 1999). In further characterization of these Prodh-deficient mice, extracellular recordings from hippocampal slices demonstrated altered glutamatergic synaptic transmission with enhanced basal transmission at the Schaffer collateral to CA1 synapse (putatively due to increased release probability) and reduced synaptic plasticity as evidenced by deficits in long-term potentiation as compared to wild type controls (Paterlini et al., 2005).

While neither of these studies specifically addressed the discrete causal role of hyperprolinemia in neuronal dysfunction, they have provided the experimental substrate upon which more mechanistically focused studies can be used to elucidate the exact molecular targets of proline.

4.5 Epistatic Interaction between COMT and PRODH

As discussed in the introduction to Section 4, 22q11.2DS is a complex syndrome resulting from hemizygosity at multiple loci. To understand why increased susceptibility to schizophrenia arises the interactions between these different deleted genes must be investigated. In this final section, we consider the best characterized interaction in the region; that between COMT and PRODH. Both human studies, as well as studies from mouse models, have begun to provide significant support for the idea of an epistatic interaction between these two genes as they both functionally converge upon the dopaminergic system by providing a dual negative regulation of dopaminergic tone (Paterlini et al., 2005; Raux et al., 2007).

Thus, in the context of the 22q11.2 microdeletion, the simultaneous reduction in the activity of both COMT and PRODH may lead to a synergistic dysregulation – in the form of disinhibition – of dopaminergic systems resulting in a hyper-dopaminergic state that may predispose to psychosis and schizophrenia (Paterlini et al. 2005). Although the exact mechanisms by which deletion of *PRODH* leads to over-activation of the dopaminergic system remains an important mechanism to be determined, the potential role of COMT deletion in fostering excessive DA appears more mechanistically straightforward.

Despite the equivocal data for a role of COMT alone in neuronal dysfunction or the deficits of cognitive flexibility associated with schizophrenia spectrum disorders, recent studies have suggested that a COMT deficiency may play a significant role in neuronal dysfunction in the context of a simultaneous PRODH deletion.

Initial evidence supporting an epistatic interaction between COMT and PRODH came from a study of Prodh-deficient mouse (Gogos et al., 1999) in which gene chip analysis revealed that *Comt* was the most upregulated neuronal-specific transcript, suggesting increased *Comt* expression as a significant compensatory response to Prodh knockdown (Paterlini et al., 2005). Significant support for increased dopaminergic tone and a compensatory response to this came from analysis of other signaling molecules in this pathway; expression of D1 receptors and the dopamine- and cAMP-regulated phosphoprotein DARPP-32 were down-regulated by around one third and there was increased expression of calcineurin.

At the functional level, when Prodh deficient mice were challenged with amphetamine, extracellular levels of dopamine (assayed using *in vivo* microdialysis) in the PFC were significantly higher than in wild type controls but no difference was observed in dorsal striatum. In further support of this notion, this same study tested a variety of dopaminergic-dependent behavioral parameters and found that Prodh-deficient mice were hyper-responsive in these assays when compared to wild type mice. In particular, the mutant mice were substantially more sensitive to amphetamine-induced hyperlocomotion. Finally, in another series of experiments pharmacological inhibition of Comt in Prodh knockdown mice produced stronger effects than it did in wild type mice. Overall, these hyper-dopaminergic responses of Prodh-deficient mice coupled with the apparently compensatory upregulation of Comt – which should serve to reduce dopaminergic tone – strongly suggests that hyperprolinemia facilitates dopaminergic transmission, with potentially the largest effects in the PFC (see Fig. 3). The cellular or circuit level mechanisms by which this occurs remain to be determined.

Further support for an epistatic interaction between PRODH and COMT came from a comprehensive study of patients with 22q11.2DS (Raux et al., 2006). This study not only showed hyperprolinemia as a likely risk factor for schizophrenia spectrum disorders but also showed that when the remaining COMT copy was the low activity Met158 allele, hyperprolinemia and COMT-Met158 synergistically increased the risk for the development of schizophrenia.

Both of these studies provide support for the possibility of a hyperdopaminergic state secondary to the hyperprolinemia when one copy of PRODH is deleted. Further, these studies also suggest that elevated COMT function might serve to partially overcome this hyperdopaminergic state and that the low activity Met158 COMT allele is less effective in providing adequate functional compensation (Paterlini et al., 2005; Raux et al., 2006).

5 Conclusions: The Next Fifteen Years

In conclusion, 22q11.2DS offers an immense opportunity to probe the genetics and biology of schizophrenia. In terms of genetic insights, work on the 22q11.2DS highlighted four important aspects of the genetic architecture of common complex psychiatric and neurocognitive disorders.

First, it provided the original evidence supporting the importance of rare recurrent mutations in schizophrenia vulnerability and highlighted the pressing need to characterize the contribution of CNVs (especially *de novo* CNVs) to the genetic and neural complexity of psychiatric disorders. Second, it strongly suggested that CNVs increase disease risk via the cumulative effect of under- (or over-) expression of a number of gene products rather than

via a single dosage-sensitive gene. Third, it highlighted the contribution of genetic variation in genes (such as *PRODH*) located in diverse, repeated and unstable regions of the genome, which are more likely to be subject to recurrent mutations (i.e. via gene-conversion). Fourth, it provided compelling evidence for a potentially pervasive role of miRNAs in regulatory networks mediating the effects of genetic variation on gene expression. The challenge in the coming years will be to attribute key phenotypic deficits to haploinsufficiency at single loci or to interactions of a subset of loci, and to elucidate how reduction in dosage of a subset of neighboring genes affects the properties of neural networks.

The relatively high incidence of the 22q11.2 microdeletion means that there is a sizable cohort of human subjects on which longitudinal studies of cognitive and neural development can be conducted. Some such studies have been initiated (Gothelf et al., 2007a), and expansion of these will allow the development of psychotic illness to be more fully comprehended. Key questions are whether or not specific cognitive phenotypes or changes in brain structure will have predictive power for psychiatric illness (see Section 2). Additionally, large-scale analysis of the interaction of the deletion with other risk factors, be they environmental, genetic or metabolic will be highly informative. Current examples include the increased risk associated with hyperprolinemia and the genotype at the remaining copy of COMT (Jacquet et al., 2002). Other factors that interact with the genetic lesion are likely to be uncovered, however, the possibility that stochastic factors, perhaps related to diminished developmental robustness (due to altered miRNA biogenesis, Hornstein et al. (2006)) contribute heavily to disease development remains to be determined. Finally, whether factors such as altered immune function and calcium homeostasis in 22q11.2 deletion carriers that can indirectly affect behavioral and cognitive performance can be recapitulated in mouse models remains to be determined and characterized.

Biologically, major steps forward in terms of altered gene expression, neuronal morphology, synapse formation and altered neurophysiology have been made (Section 3). Clearly many questions remain; some key issues include whether or not inhibitory synapse formation is disrupted or if interneuron function is compromised. The observation of decreased functional connectivity/synchrony between the PFC and hippocampus raises the issue of how widespread such deficits are between cortical areas or between cortex and subcortical structures. Given the robust reductions in white matter tracts in 22q11.2DS patients (Section 2) testing whether long-range anatomical connectivity is disrupted, either by altered axonal growth or problems with myelination, in *Df(16)A+/−* mice is imperative. Other mechanisms that could account for altered functional connectivity and synchronization include altered synaptic formation by long-range projections, altered targeting of postsynaptic neurons and/or altered short-term synaptic dynamics that could affect information transfer (Abbott and Regehr, 2004). Finally, how the observed structural and functional changes in glutamatergic pyramidal neurons interact with putative alterations in dopaminergic neuromodulation requires further characterization.

Continued characterization of mice lacking one or both copies of candidate genes will continue and the generation of mice heterozygous for two genes in the deleted region (by crossing individual gene knockout animals) is straightforward. Another approach will be to breed *Df(16)A+/−* mice with transgenic mice that have additional copies of a single candidate gene, via BAC engineering, to determine if phenotypes are rescued. Finally, to distinguish between neurodevelopmental effects and maintained deficits in synaptic biology, inducible and tissue specific knockouts will be valuable. Currently, efficient conditional manipulation of large chromosomal deletions is not possible.

Overall, the definitive link between deletions at the 22q11.2 locus and greatly heightened incidence of schizophrenia has allowed the generation of specific hypotheses of what

cellular and circuit level changes underlie this disease. With the observation that CNVs likely contribute to schizophrenia pathogenesis substantially, analogous etiologically valid mouse models will become available in which these hypotheses can be further tested. Ultimately, it is hoped that the outcomes of this work will provide novel and effective therapeutic targets for the development of a new generation of rationally designed antipsychotic medication.

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Figure 1. Function of protein palmitoylation in neurons affected by hemizygosity of the palmitoyl transferase ZDHHC8

Palmitoyl transferases are primarily localized to the Golgi apparatus although there is evidence that ZDHHC8 is also found in dendrites. Such enzymes modify proteins by addition of the 16-carbon palmitate lipid group at cysteine residues. This modification increases protein hydrophobicity and, hence, increases their interaction with the plasma membrane and with intracellular membranes, such a sorting vesicles. Shown is the modification of PSD-95, which has been shown to be a substrate of ZDDHC8 – decreased PSD-95 localization at synapses may be due to 1) decreased palmitoylation at the Golgi apparatus (*left*) or 2) reduced local palmitoylation at the synapse (*right*).

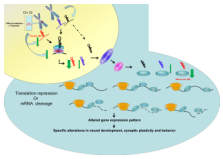

Figure 2. Dysregulation of miRNA biogenesis and alteration of gene expression patterns in 22q11.2 animal models

Both the *Dgcr8* and *mir-185* genes are located within the 1.5-Mb microdeletion region within chromosome 22q11.2 and the equivalent region of mouse chromosome 16. Hence, they are hemizygous in the microdeletion syndrome. This deficit results in a reduction in the levels of a subset of mature miRNAs (*red*, *blue*) in both PFC and HPC in *Df(16)A+/−* mice. *mir-185* expression is particularly affected due to the combination of hemizygosity and reduced synthesis due to *Dgcr8* hemizygosity, hence its down-regulation is stronger than other miRNAs (indicated by a thicker arrow). Partial reduction in miRNA levels can affect mRNA and protein levels of target genes. Moreover, since target mRNAs can be regulated by the combinational control of more than one miRNA, the level of repression achieved may be highly sensitive to the amount and types of available miRNA complexes. Therefore, miRNA dysregulation is a molecular pathway strongly affected in 22q11.2DS. For example, target genes may be upregulated due to reduction of miRNAs affected by the *Dgcr8* deficiency (*bottom left*), due to reduction of miRNA genes removed by the 22q11.2 microdeletion (*mir-185* within the 1.5-MB minimal region is shown as an example here, *bottom right*), or due to a combination of both (*bottom middle*). The alteration of target gene expression patterns could result in a number of phenotypic abnormalities controlled by these target genes.

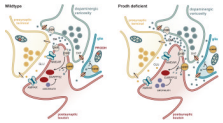

Figure 3. Shown is a schematic of a cortical synapse in Prodh deficient mice (*right*) compared to a wild type synapse (*left*)

There is evidence of increased dopaminergic tone in the cortex of in Prodh deficient mice. Such mice show increased expression of Comt as a presumed compensatory mechanism to this change, in addition to decreased expression of D1 receptors and Darpp32 and increased expression of calcineurin. Increased dopaminergic tone is likely to underlie hypersensitivity to amphetamine challenge and potentially other behavioral changes in these mice.

Table 1

Genes located in the 1.5Mb Critical Region of 22q11.2DS

Gene Symbol	Name	Function (/Hypothesized Function)	Expression in HPC or forebrain?	Abnormal brain-related phenotypes of knockout mice
DGCR6	diGeorge Syndrome Critical Region Gene 6	Unknown function. Protein has homology with D. melanogaster gonadal protein and the gamma-1 subunit of human laminin.	Yes	
PRODH	Proline Dehydrogenase (Oxidase) 1	Mitochondrial enzyme proline dehydrogenase that catalyzes the first step in the degradation of L-proline, a putative neuromodulator. Defects in this gene cause hyperprolinemia type 1.	Yes	Prodh-deficient mice: Altered synaptic transmission, reduced PPI, impaired fear conditioning, altered cortical dopaminergic transmission.
DGCR5	diGeorge Syndrome Critical Region Gene 5 (non-coding)	N/A		
DGCR9	diGeorge Syndrome Critical Region Gene 9	Unknown.		
DGCR1	diGeorge Syndrome Critical Region Gene 1	Unknown		
DGCR2	kiaa163 Gene Product	Predicted novel adhesion receptor protein.	Yes	
DGCR11	diGeorge Syndrome Critical Region Gene 11	Unknown.		
DGCR14	diGeorge Syndrome Critical Region Gene 14	Protein may be a component of spliceosomes. Mouse orthologue has nuclear localization.	Yes	
TSSK2	Testis-Specific Serine Kinase 2	Member of a family of serine/threonine kinases; highly expressed in testis	Yes	
GSC2	Goosecoid-like	A homeodomain-containing gene, expressed in a limited number of adult tissues and in early development.	Yes	
SLC25A1	Solute Carrier Family 25, Member 1	Mitochondrial carrier, citrate transporter.	Yes	
CLTCL1	Clathrin, Heavy Polypeptide-like 1	A major protein of the polyhedral coat of coated pits and vesicles. NB. Not present in mouse syntenic region.		
HIRA	Hir Histone Cell Cycle Regulation Defective Homolog A (S. cerevisiae)	A histone chaperone that preferentially places the variant histone H3.3 in nucleosomes. May play an important role in the formation of transcriptionally silent heterochomatin.	Yes	Knockout: Embryonic lethal; abnormal morphology of head, forebrain, neural tube, embryonic neuroepithelium, neural plate and altered hindbrain development.
MRPL4	Nuclear Localization Signal Deleted in Velocardiofacial Syndrome	A mammalian mitochondrial ribosomal protein; controls protein synthesis in mitochondria.	Yes	
C22orf39	Hypothetical Protein loc128977	Unknown.		
UFD1L	Ubiquitin Fusion Degradation 1-like (S. cerevisiae)	Protein necessary for the degradation of ubiquitinated proteins. Regulates the disassembly of the mitotic spindle and the formation of a closed nuclear envelope after mitosis.	Yes	
CDC45L	cdc45 Cell Division Cycle 45-like (S. cerevisiae)	Highly homologous to the S. cerevisiae Cdc45, a protein required to the initiation of DNA replication.	Yes	
CLDN5	Claudin 5 (Transmembrane Protein Deleted in Velocardiofacial Syndrome)	A major cell adhesion molecule of tight junctions in brain endothelial cells.	Yes	Knockout: Neonatally lethal; blood-brain barrier against small molecules (<8 D) was selectively affected.
LOC15185		Unknown.		

Gene Symbol	Name	Function (/Hypothesized Function)	Expression in HPC or forebrain?	Abnormal brain-related phenotypes of knockout mice
SEPT5	Septin 5	Member of the septin gene family; regulation of cytoskeleton.	Yes	Knockout + Heterozygote: Decreased anxiety-related response, Abnormal spatial learning. Knockout: Abnormal social/conspecific interaction, increased PPI.
GP1BB	Glycoprotein ib (platelet), Beta Polypeptide	A heterodimeric transmembrane protein that constitutes the receptor for von Willebrand factor, and mediates platelet adhesion. Mutations in the gene have been associated with Bernard-Soulier syndrome and giant platelet disorder.	Yes	
TBX1	t-box 1	Member of a family of transcription factors that share a common DNA-binding domain, the T-box. Responsible for many physical symptoms of 22q11.2 DS.	Yes	Heterozygote: Decreased PPI
GNB1L	Guanine Nucleotide Binding Protein (G- Protein), Beta Polypeptide 1-like	Encodes a G-protein beta-subunit-like polypeptide containing WD repeats.	Yes	Heterozygote: Decreased PPI.
C22orf29	Hypothetical Protein flj21125	Unknown.		
TXNRD2	Thioredoxin Reductase 2	Member of a family of pyridine nucleotide-disulfide oxidoreductases; regulation of the intracellular redox environment.	Yes	
COMT	Catechol-o-Methyltransferase	Catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines. One of the major degradative pathways of the catecholamine transmitters.	Yes	Heterozygote: Reduced clearance of cortical dopamine, increased aggression towards males, altered anxiety-related response, cognitive deficits.
ARVCF	Armadillo Repeat Gene Deletes in Velocardiofacial Syndrome	A member of the catenin family; encodes a protein containing a coiled coil domain and an armadillo repeat sequence as well as a predicted nuclear-targeting sequence.	Yes	
C22orf25	Chromosome 22 Open Reading Frame 25	Unknown	Yes	
DGCR8	diGeorge Syndrome Critical Region Gene 8	Encodes for a component of the “microprocessor”, a nuclear complex involved in microRNA processing.	Yes	Knockout is embryonic lethality before somite formation. Heterozygote: Abnormal dendrite morphology, abnormal spatial working memory, decreased prepulse inhibition.
TRMT2A			Yes	
RANBP1	Ran Binding Protein 1	Protein binds to RAN complexed with GTP but not GDP and markedly increases GTP hydrolysis by the RanGTPase-activating protein.	Yes	
ZDHHC8	Zinc Finger, DHHC-type Containing 8	Member of the DHHC family of palmitoyl-transferases	Yes	Female knockout and heterozygote: decreased exploration in new environment, reduced PPI. Knockout and heterozygote: Abnormal dendrite and spine morphology.
LOC15197		Unknown.		
RTN4R	Reticulon 4 Receptor / Nogo receptor 1	Receptor for reticulon 4 (Nogo receptor 1); mediation of axonal growth inhibition and may play a role in regulating axonal plasticity in the adult CNS.	Yes	Heterozygote: Hypoactivity, impaired coordination, decreased exploration in new environment, abnormal central

Gene Symbol	Name	Function (/Hypothesized Function)	Expression in HPC or forebrain?	Abnormal brain-related phenotypes of knockout mice
				nervous system regeneration, axon outgrowth and plasticity.
DGCR6L	diGeorge Syndrome Critical Region Gene 6-like	One of two functional genes encoding nearly identical proteins that have similar expression patterns. NB. Not present in mouse syntenic region.		

Genes listed according to the RefSeq genes (DGCR6-DGCR6L). Evidence for expression patterns was taken from http://www.informatics.jax.org/expression.shtml. Where phenotypic data is available for heterozygous knockout animals it is given, where it is of interest data from knockout studies are summarized.

Table 2

Genes located in 3 Mb deletions but outside of the 1.5Mb critical region of 22q11.2DS

Gene Symbol	Name	Function (/Hypothesized Function)	Expression in HPC or forebrain?
PI4KAP1		Unknown.	
RIMBP3	RIMS binding protein 3	Unknown.	
ZNF74	Zinc Finger Protein 74 (cos52)	Putative trancription factor with 12 zinc finger motifs of the Kruppel/TFIIIA type.	
SCARF2	Scavenger Receptor Class F, Member 2	Similar to SCARF1/SREC-I, a scavenger receptor protein that mediates the binding and degradation of acetylated low-density lipoprotein (Ac-LDL).	
KLHL22	Kelch-like 22 (D. melanogaster)	Unknown.	
MED15	Trinucleotide Repeat Containing 7	May function as a transcriptional coactivator in RNA polymerase II transcription.	Yes
POM121L4P	Pom121 Membrane Glycoprotein-like 4 Pseudogene (rat)	Unknown.	
TMEM191A	Hypothetical Protein dkfzp434n35	Unknown.	
PI4KA	Phosphatidylinositol 4-Kinase, Catalytic, Alpha Polypeptide	A phosphatidylinositol (PI) 4-kinase that catalyzes the first committed step in the biosynthesis of phosphatidylinositol 4,5-bisphosphate.	
SERPIND1	Serpin Peptidase Inhibitor, Clade D (Heparin Co-factor), Member 1	Serine proteinase inhibitor. Mutations in this gene are associated with heparin cofactor II deficiency.	
SNAP29	Synaptosomal-associated Protein, 29kDa	A member of the SNAP25 gene family, encodes a protein involved in multiple membrane trafficking steps. Loss of functional SNAP29 asssociated with CEDNIK syndrome	
CRKL	v-crk Sarcoma Virus ct1 Oncogene Homolog (Avian)-like	Protein kinase containing SH2 and SH3 (src homology) domains; activates the RAS and JUN kinase signaling pathways. May be involved in the Reelin pathway.	Yes
AIFM3	Apoptosis-Inducing Factor-like		
LZTR1	Leucine-Zipper-like Transcription Regulator 1	Member of the BTB-kelch superfamily, localized to Golgi network.	Yes
THAP7	thap Domain-containing 7		
FLJ39582	Hypothetical Protein flj39582		
MGC1673		Unknown.	
P2RX6	Purinergic receptor P2X6	An ATP-gated ionotropic receptor.	
SLC7A4	Solute Carrier Family 7 (Cationic Amino Acid Transporter, y+ System), Member 4		
P2RX6P		Unknown.	
LOC4891	Similar to Chromosome 14 Open Reading Frame 166b	Unknown.	
POM121L8P		Unknown.	
RIMBP3C	RIMS binding protein 3C	Unknown.	
RIMBP3B	RIMS binding protein 3B		

Patients with “typical” 3-Mb deletions that are indistinguishable by FISH analysis may exhibit differences of up to 200 Kb on each side of the breakpoint that can be determined by higher resolution approaches. Since these differences can affect one or more known and predicted genes at each end, the exact complement of deleted genes may be different in each carrier.

Table 3

Neuroanatomical Abnormalities Associated with 22Q11.2DS

Neuroanatomical abnormalities	**Reference**
Qualitative abnormalities in children and adults	
Cavum septum pellucidum cavum vergae	Chow et al. 1999 van Amelsvoort et al., 2001 Shashi et al., 2004
Polymicrogyria	Sztriha et al., 2004 Robin et al. 2006
White matter hyperintensities	Chow et al., 1999 van Amelsvoort et al., 2001
Volumetric abnormalities	
Children and adolescents	
Enlarged ventricles	Eliez et al., 2000 Kates et al., 2001
Decrease in total brain volume (white matter > gray matter)	Eliez et al., 2000 Kates et al., 2001
Volumetric reduction of parietal lobe with relative sparing of frontal lobe	Eliez et al., 2000 Kates et al., 2001 van Amelsvoort et al. 2001
Reduced cortical thickness – lateral cortical thinning in parieto-occipital, occipital pole, and inferior prefrontal regions and medial thinning in the anterior cingulated, medial frontal gyrus, subgenual prefrontal, posterior cingulate gyrus, cuneus, and lingual gyrus regions.	Bearden et al., 2006 Bearden et al., 2009
Volumetric reduction of cerebellum – vermis, anterior lobes, and neocerebellum	Chow et al., 1999 Shashi et al., 2010 Eliez et al., 2001b Bish et al., 2006 Kates et al., 2006
Volumetric reduction of posterior regions with relative sparing or enlargement of anterior regions of specific subcortical structures, including caudate nucleus, thalamus, and corpus callosum	Eliez et al., 2002 Kates et al., 2004 Campbell et al., 2006 Bish et al., 2004 Antshel et al., 2005 Machado et al., 2007 Gothelf et al., 2008
Reduction of hippocampal volume	Debbané et al., 2006b Deboer et al., 2007
Adults	
Decrease in total brain volume	Chow et al. 2002 van Amelsvoort et al., 2001
Reduced volume of frontal and temporal lobes	Gothelf et al., 2008 Gothelf et al., 2005 van Amelsvoort et al., 2004
Enlarged ventricles	Chow et al., 1999 Chow et al., 2002
Reduced cerebellar volume	Chow et al., 1999 van Amelsvoort et al., 2001 van Amelsvoort et al., 2004
Diffuse disorganization of white matter of parieto-parietal, fronto-frontal, and fronto-temproal connections	Barnea-Goraly et al., 2003 Barnea-Goraly et al., 2005 Simon et al., 2005a Sun et al., 2007

Table 4

Behavioral Phenotypes of Mouse Models of 22q11.2DS

A. Deletion Mouse Models Involving Genes Within the 22q11.2DS Region	Size,	Bkgrd Strain	Sex tested	Behavioral Phenotypes Relevant to Neuropsychiatric Disorders				
Strain Name*	**# of Genes**	**(backcross generation)**		**PPI**	**Startle Response**	**FC**	**Anxiety/Depression****	**Other**
Del(16Dgcr2-Hira)2Aam. AKA *Del(16)2Aam* or *Df(16)A/+*	1.3 Mb, 27	C57BL/6J(N3)[1], C57BL/6J(>N10)[2]	(M,F)[1];M[2]	D(M,F)[1]	= (M,F)[1]	cd: D (M,F)[1], cxt: D(M,F)[1]	OF: I anxiety (M only)[1]; L/D: I anxiety (M, F nt)[1]	OF: hyperactive (M,F)[1], Difficulty acquiring DNMP task (working memory)(M[1,2],F[1])
Del(16Dgcr2-Hira)1Rak. AKA *Del1Rak* or *1+Del/+*	1.3 Mb, 27	C57BL/6J(>N5)[3]	(M,F)[3]	D(M,F)[3]	I (M)[3], = (F)[3]	cd: = (M,F)[3], cxt: = (M,F)[3]	OF: = (M,F)[3]	Thermal pain hypersensitivity (hot plate) (M,F)[3]
Del(16Es2el-Ufd1l)217Bld. AKA *Del217Bld* or *Df1/+*	1.2 Mb, 22	C57/129mix [4], C57BL/6 $^{c-/c-}$(N5–N6) [5]	(M,F) [4, 5]	D(M,F) [4],D(M,F) [5]	I (M only) [4], =(M,F) [5]	cd: = (M,F) [4], cxt (24h): D (M,F) [4], cxt (1hr): = (M,F) [4]	OF: =(M,F) [4], L/D: =(M,F) [4]	
Del(16T10-Hira)4Bld. AKA *Del4Bld*, *Df(16)4*, or *Df4/+*	1.0 Mb, 13	C57BL/6$^{c-/c-}$(N5–N6)[5]	(M,F)[5]	D(M,F)[5]	= (M,F)[5]	N/R	N/R	
Del(16Es2el-Sept5)3Bld. AKA *Del3Bld*, *Df(16)3*, or *Df3/+*	700 Kb, 19	C57BL/6$^{c-/c-}$(N5–N6)[5]	(M,F)[5]	D(M,F)[5]	I (M,F)[5]	N/R	N/R	
Del(16Dgcr2-Arvcf)1Ais. AKA *Del1Ais*	550 Kb, 16	C57BL/6J × 129Sv × SJL mix*	N/R	N/R	N/R	N/R	N/R	
Del(16Es2el-T10)2Bld. AKA *Del2Bld*, *Df(16)2*, *or Df2/+*	500 Kb, 12	C57BL/6$^{c-/c-}$(N5–N6)[5]	(M,F)[5]	= (M,F)[5]	= (M,F)[5]	N/R	N/R	
Del(16Zfp520-Slc25a1)1Awb. AKA *Del1Awb*	150 Kb, 7	129S6 (pure)[8],(129S6 × Black Swiss)[8]	(M,F)[8]**	I(M,F)[8]	= (M)[8]	cd: = (M)[8], cxt: = (M)[8]	OF: = (M)[8], L/D: = (M)[8]	
Del(16Cldn5-Hira)5Bld. AKA *Del5Bld*, *Del(16)(Cld5-Hira)*, or *Df5/+*	150 Kb, 5	C57BL/6$^{c-/c-}$(N5–N6)[5]	(M,F)[5]	= (M,F)[5]	= (M,F)[5]	N/R	N/R	

B.

Genes Located Within the 22q11.2DS Region, with Behaviorally Characterized Heterozygous Mouse Models							
Rtn4r (Reticulon 4 receptor)	C57BL/6J × 129Sv mix[9]	(M,F)[9]	= (M,F)[9]	= (M,F)[9]	cd: = (M,F)[9], cxt: = (M,F)[9]	OF: = (M,F)[9], L/D: = (M,F)[9]	OF: hypoactive (M,F)[9], Working memory (DNMP): =(M,F)[9]
Zdhhc8 (Zinc finger, DHHC-type containing 8)	C57BL/6J × 129Sv mix[10]	(M,F)[10]	= (M,F)[10]	= (M,F)[10]	N/R	OF: I anxiety (Fs only)[10]	OF: hypoactive (Fs only)[10]
Dgcr8 (DiGeorge syndrome critical region gene 8)	C57BL/6J(N3)[1]	M[1]	D(M)[1]	= (M)[1]	cd:= (M)[1], cxt: = (M)[1]	OF: =[1]L/D: =(M,F)[11], EPM: I	Difficulty acquiring DNMP task (working memory)[1]
Comt (Catechol-O-methyltransferase)	C57/129mix[11],C57BL/6J(>N10)[12,13]	(M,F)[11,12],M[13]	= (M,F)[11,13]	= (M)[13]	N/R	anxiety(M)[13], NO: I anxiety(M)[13]	Increased aggression (M, F nt)[11], increased working memory performance (T maze)[13]
Gnb1l (Guanine-nucleotide-binding protein (G protein) β-polypeptide 1-like)	C57BL/6$^{c-/c}$-(N5–N6)[5]	(M,F)[5]	D (M,F)[5]	= (M,F)[5]	N/R	N/R	N/R
Tbx1 (T-box1)	C57BL/6$^{c-/c}$-(N5–N6)[5], C57/129mix[3]	(M,F)[3,5]	D (M,F)[5], = (M,F)[3]	I (M,F)[5], = (M,F)[3]	= (M,F)[3]	OF: = (M,F)[3]	Mild muscle weakness[3]
Sept5 (Septin 5) AKA *Cdcrel1*	C57BL/6$^{c-/c}$-(N5–N6)[5], Mix[14], ***, 129E[14] ***	(M,F)[5],M[14]	= (M,F)[5], = (M)[14]	I (M,F)[5], = (M)[14]	N/R	EPM: D anxiety (M)[14], OF: =(M)[14]	

Behavior performed on adult mice (>2 months), unless otherwise noted.

* Information obtained from the Mouse Genome Database (MGD).

Abbreviations/Key: AKA: Also known as; N/R: Not reported, Bkgrd: background, M: males, F: females, nt= not tested FC: fear conditioning, cd:cued, cxt :contextual, = : No difference from wild-type, I: Increased compared to wild-type, D: Decreased compared to wild-type OF: open field, EPM: elevated plus maze, L/D: light/dark test, NO: novel object task.

** For Ref. [8], female data was only reported for PPI, on the 129S6 × Black Swiss mixed bkgrd.

*** See ref. [14] for more thorough description of background strains used.

**** In the OF, "anxiety"as assessed by decreased center measures (time or distance in the center).

References

[1] Stark et al. (2008),

[2] Sigurdsson et al. (2010),

[3] Long et al. (2006),

[4]Paylor et al. (2001),
[5]Paylor et al. (2006)
[6]Lindsay et al. (2001),
[7]Puech et al. (2000),
[8]Kimber et al. (1999),
[9]Hsu et al. (2007),
[10]Mukai et al. (2004),
[11]Gogos et al. (1998),
[12]Babovic et al. (2008),
[13]Papaleo et al. (2008),
[14]Suzuki et al. (2009)